Curtin Medical School

Purine isosters in design and development of novel xanthin oxidase inhibitors

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which have been accepted for the award of any other degree or diploma in any university.

Signature:

Date:
23 July 2022

Acknowledgements

Firstly, I would like to thank my family for their support and patience during this long journey of my research project.

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Abbreviations

AHPP: 4-amino-6-hydroxypyrazolo[3,4-*d*]pyrimidine

DHNB: 3,4-dihydroxy-5-nitrobenzaldehyde

DMSO: dimethylsulphoxide

EWG: electron withdrawing group

HPLC: high pressure liquid chromatography

NMR: nuclear magnetic resonance

PDA: photo diode array detector

TLC: thin layer chromatography

XOR: xanthine oxidoreductase

XDH: xanthine dehydrogenase

XO: xanthine oxidase

Abstract

Uric acid is formed during the purine catabolism process, specifically the last two steps when hypoxanthine is oxidised to xanthine and then to uric acid. Both oxidations are catalysed by both forms of the enzyme xanthine oxidoreductase (XOR) known as xanthine dehydrogenase (XDH) and xanthine oxidase (XO). XDH is easily converted into XO by oxidation of sulfhydryl residues or by proteolysis. High levels of uric acid in blood, referred to as hyperuricemia, can develop into arthritic gout in some people, due to the formation of deposits of monosodium urate crystals in joints and other tissues. Hyperuricemia has also been shown to be associated with a series of other conditions like chronic kidney disease, hypertension, cardiac disease and type 2 diabetes.

There are currently three ways to treat hyperuricemia, either using inhibitors of the enzyme XO to block the formation of uric acid, or using the enzyme uricase to convert uric acid into a more water soluble compound (allantoin), or using available treatments, uricosuric agents, to stimulate the excretion of uric acid in the urine. Inhibition of the enzyme XO is the preferred first line of intervention as it is the only approach that stops the production of uric acid. There are only two drugs approved in Australia for the treatment of hyperuricemia and gout. These are both inhibitors of XO: Allopurinol and Febuxostat. Both drugs come with some risks of side effects that could, in some cases, result in death. There is a clear need to develop new therapeutic approaches for the treatment of these diseases.

This research aimed to identify novel purine analogue inhibitors of XO. Facile methods were developed for the synthesis of four libraries of these molecules. Reaction conditions were optimized by using a microwave reactor, which allowed very short reaction times. A total of 95 compounds were synthesised and tested in vitro as inhibitors of XO. The majority of these molecules exhibited either comparable or higher inhibitory potency than the established drug Allopurinol. The inhibitory mechanism of the most active compounds was determined using inhibition kinetics studies. Molecular modelling was also employed to identify interactions between the inhibitors and the active site of the enzyme. The most potent inhibitor demonstrated to be more than 900 times more potent than Allopurinol. Future investigation of the most active purine analogue may potentially lead to other, far more effective, treatments for hyperuricemia and gout.

List of publications by the candidate included in the thesis

1. **Luna G**, Dolzhenko AV, Mancera RL. Inhibitors of Xanthine Oxidase: Scaffold Diversity and Structure-Based Drug Design. ChemMedChem. 2019; 14(7): 714-43

Relevant publications to the thesis but not forming a part of it

1. Lim, FPL.; **Luna G.**; Dolzhenko AV. A new, one pot, multicomponent synthesis of 5-aza-9-deaza-adenines under microwave irradiation. Tetrahedron Lett. 2014; 55(37): 5159-5163.
2. Lim, FPL.; **Luna, G.**; Dolzhenko, AV. A one-pot, three-component aminotriazine annulation onto 5-aminopyrazole-4-carbonitriles under microwave irradiation. Tetrahedron Letters. **2015**, *56*(3), 521-524.
3. Lim, FPL.; **Luna, G.**; Dolzhenko, AV. A one pot, three-component, microwave-assisted synthesis of novel 7-amino-substituted 4-aminopyrazolo[1,5-a][1,3,5]triazine-8-carbonitriles. Tetrahedron Letters. 2015; 56: 7016-7019.
4. Lim, FPL.; **Luna, G.**; Dolzhenko, AV. A new, one pot, multicomponent synthesis of bioactive *N*-pyrazolylformamidines under microwave irradiation. Synthesis. 2016; 48(15): 2423-2428.
5. Lim, FPL.; Tan, KC.; **Luna, G.**; Tiekink, ERT.; Dolzhenko, AV. A new practical synthesis of 3-amino-substituted 5-aminopyrazoles and their tautomerism. Tetrahedron **2019**, *75*(15), 2314-2321.
6. Lim, FPL.; Tan, KC.; **Luna, G.**; Tiekink, ERT.; Dolzhenko, AV. A synthesis of new 7-amino-substituted 4-aminopyrazolo[1,5-*a*][1,3,5]triazines via a selective three-component triazine ring annulation. Tetrahedron **2019**, *75*(15), 2322-2329.

Declaration of attribution by the candidate

I, Giuseppe Luna, as the first author of the publication entitled “Inhibitors of Xanthine Oxidase: Scaffold Diversity and Structure-Based Drug Design” and constituting a major part of the Chapter 2, declare that this work was primarily conducted and written by the first author of this manuscript.

Name	Manuscript preparation	Manuscript editing	Final approval
Giuseppe Luna	X	X	X
Prof. Ricardo Mancera		X	X
Prof. Anton Dolzhenko		X	X

Signature: 

Date: 17 Oct 2022

Co-authors:

Prof. Ricardo Mancera

Signature: 

Date: 17 October 2022

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Date: 17 October 2022

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Chapter 1

1 Introduction

Hyperuricemia is a condition characterised by high levels of uric acid in the blood, and it can degenerate into gout when monosodium urate crystals precipitate in joints and other tissues. The prevalence of hyperuricemia and gout is steadily increasing worldwide with higher incidence in developed countries compared to developing countries. During the decade 2011-2020 it is estimated that 1.5-6.8% of the Australian population aged 25 years and above was affected by gout, resulting in approximately $200 million in annual health care costs[1].

Hyperuricemia has been shown to be associated with a series of other conditions like chronic kidney disease, hypertension, cardiac disease[2] and type 2 diabetes[3]. However it is still unclear if hyperuricemia is a contributing factor of these diseases or if it rather contributes to their worsening. For this reason, debate continues on the need for prophylactic treatment to reduce uric acid levels in asymptomatic hyperuricemic patients.

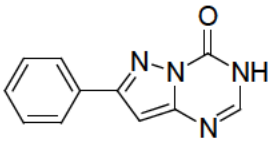
Uric acid is the final product of the last two steps of purine catabolism. Hypoxanthine is oxidised to xanthine and then to uric acid, and both steps are catalysed by the enzyme xanthine oxidase (XO). The source of purine is both endogenous, from the degradation of both guanine nucleotide and adenine nucleotide, and exogenous through the intake of purine rich food: red meat, alcohol and sea food. More recently, fructose intake, via fruit juices and soft drinks, has been shown to contribute to the increase in uric acid levels[4]. One of the key problems with uric acid is that it has a very poor solubility in serum, and its saturation point has been estimated to be 6.4 mg/dL. Normal levels of uric acid in the blood are considered to be lower than 6 mg/dL in men and 5 mg/dL in women. Hyperuricemia is diagnosed when uric acid concentration is above 7 mg/dL in men and 6 mg/dL in women.

It has been shown that for 30% of asymptomatic hyperuricemic patients there is continuous accumulation of uric acid in hyaline cartilage, joints and tendons[5, 6]. Moreover studies have shown that there is an increase of blood flow around the deposit of monosodium urate crystals indicating an ongoing inflammation status[6]. Mechanical friction caused by the solid deposition in joints could be responsible of initiating and contributing to degenerative arthritis[3].

There are currently three ways to treat hyperuricemia, either using inhibitors of the enzyme XO to block the formation of uric acid, or using the enzyme uricase to convert uric acid into a more water soluble compound (allantoin), or using available treatments, uricosuric agents, to stimulate the excretion of uric acid in the urine. Inhibition of the enzyme XO is the preferred first line intervention as it is the only approach that stops the production of uric acid.

There are only two drugs approved in Australia for the treatment of hyperuricemia and gout. These are both inhibitors of XO: Allopurinol and Febuxostat. Both drugs come with some risks of side effects that could, in some cases, result in death. Previous studies have shown that Allopurinol at a dosage of 300 mg/day is less than 50% efficacious in lowering uric acid level to less than 6 mg/dL and Febuxostat at a dosage licensed in USA is 48-67% efficacious in lowering uric acid to the same target level[7].

The research described in this thesis aimed to identify novel purine analogue inhibitors of XO to potentially assist the treatment of the serious health conditions of hyperuricemia and gout. The hypothesis for this research originated from the review of the work published in 1985 by Robins et al.[8] The authors synthesised and tested *in vitro* the activity of more than 100 purine analogues. One of the most active compounds was 7-phenyl-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (**1**), which demonstrated to be 126 times more potent than Allopurinol. In the same publication, the authors reported six structural derivatives of compound **2** and five structural derivatives of compound **3** with substituents only in position 5.



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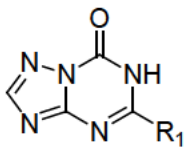

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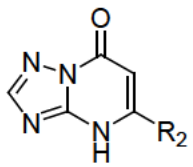
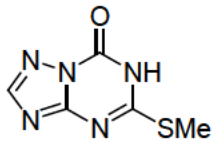


3

R_1 = H, HO, HS, MeO, MeS, Ph.

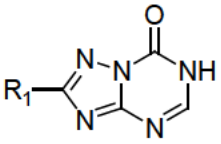
R_2 = H, HO, Ph, H_2N, Cl.

Amongst the compounds of series **2** and **3**, only one analogue **2a** was reported to be more potent than Allopurinol, with an IC_{50} value four times lower than Allopurinol. All the other compounds showed IC_{50} values that were between four and 25 times worse than the control drug.



2a

None of the structures reported containing a scaffold of type **2** or **3** had a substituent in position 2 (**4** and **5**).



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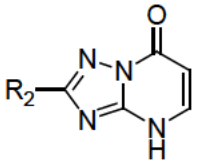


5

The hypothesis for this research was that compounds of types **4** and **5** would demonstrate better inhibitory activity than compounds of types **2** and **3** because the substituents in position 2 could mimic the substituent in position 7 of one of the most potent inhibitor reported by Robins et al.

1.1 Aim of the project

The aim of this project was to identify novel purine analogue inhibitors of XO to inhibit the formation of uric acid and assist in the treatment of hyperuricemia and gout.

1.1.1 Objective 1

To identify purine analogue scaffolds suitable to interact with XO active site by reviewing the literature and design new potential XO inhibitors.

1.1.2 Objective 2

To develop efficient synthetic methods for the preparation of the designed structures and synthesise representative libraries.

1.1.3 Objective 3

To test *in vitro* the synthesised molecules for their inhibitory activity against XO using hypoxanthine as substrate.

1.1.4 Objective 4

To identify interactions between the inhibitors and the residues inside the enzyme active site by docking studies.

1.1.5 Objective 5

To identify the type of enzyme inhibition by the most active compounds using enzyme kinetics studies.

1.1.6 Objective 6

To evaluate the results obtained and propose directions for future research.

1.2 Structure of the thesis

The remainder of the thesis is structured into different chapters as follows:
Chapter 2: Literature review. This chapter discusses the role of the enzyme XO in the human body and its mechanism of action. It also includes a comprehensive review of all the chemical structures that have been reported as XO inhibitors. The review encompasses the period between the discovery of the first XO inhibitor (allopurinol) in 1956 and 2021.

Chapter 3: Synthesis, in-vitro testing and molecular modelling of 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine derivatives. This chapter discusses the method development for the synthesis of 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine derivatives. It also reports the *in vitro* biological efficacy of the compounds as XO inhibitors and the molecular modelling prediction of the interactions of key compounds with the amino acid residues in the active site.

Chapter 4: Synthesis, in-vitro testing and molecular modelling of 2-substituted-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one derivatives. This chapter discusses the synthetic procedures for the synthesis of 2-substituted-

1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one derivatives. It also reports the in vitro biological efficacy of the compounds as XO inhibitors and the molecular modelling prediction of the interactions of key compounds with the amino acid residues in the active site.

Chapter 5: Conclusions and future directions. This chapter summarises the findings of this research, discusses its limitations and identifies directions for future research in this area.

Chapter 2

2 Literature review

2.1 Introduction

Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) are two forms of the enzyme xanthine oxidoreductase (XOR). Both forms are involved in the last two steps of the catabolism of purines. Specifically they catalyse the oxidation of hypoxanthine to xanthine and then to uric acid (Fig 1). While XDH transfers two electrons generated during the oxidation of the substrate in each reaction step to one molecule of NAD^+, XO transfers the electrons to oxygen molecules to form either hydrogen peroxide or superoxide anion.

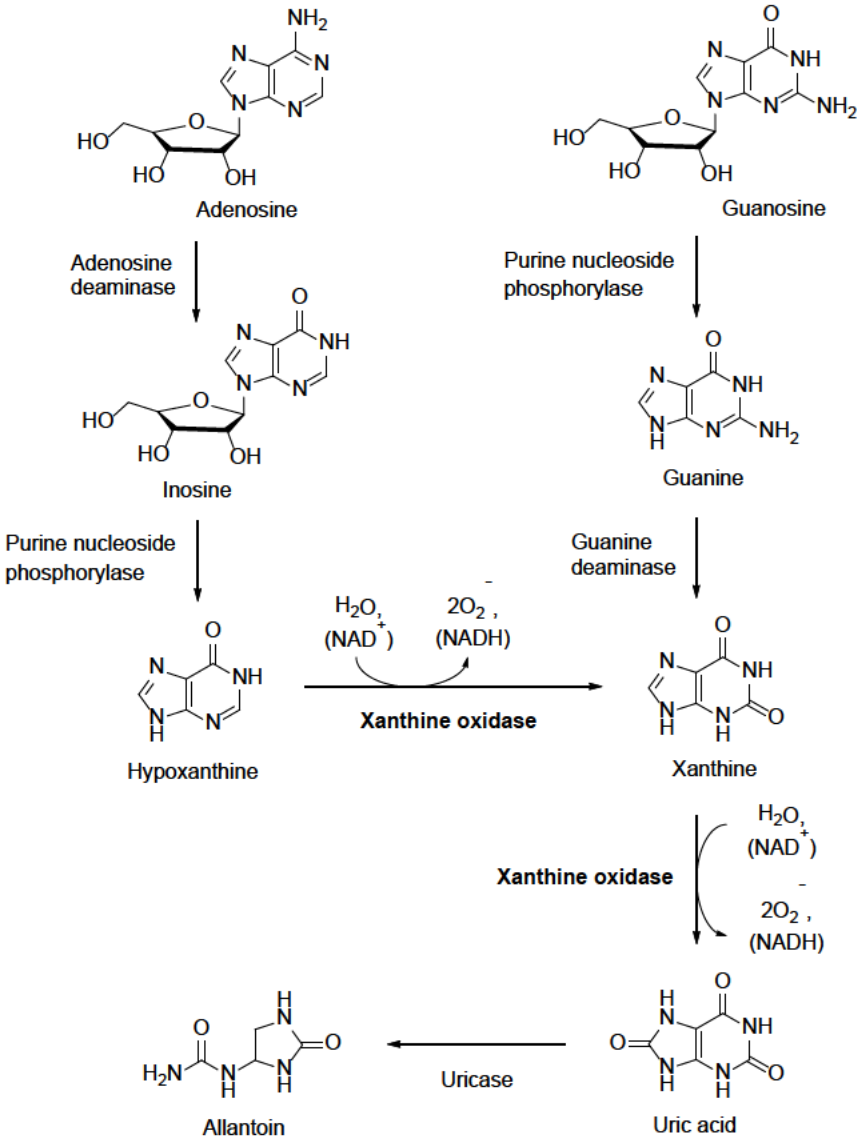


Fig 1: Purine catabolism

The human body accumulates purines externally through the intake of food and internally through the degradation of DNA and RNA molecules. Uric acid is the final product of purine catabolism in humans and higher primates and is excreted via the intestinal tract but mainly through the kidneys. Uric acid can also be re-cycled in the kidneys by the urate anion transporter (URAT1)[9] and adenosine 5'-triphosphate-binding cassette subfamily G transporters (ABCG2)[10-12], but only by ABCG2 transporter at intestinal level. For most other mammals uric acid is further oxidised by the enzyme uricase to the more-water soluble compound allantoin[13].

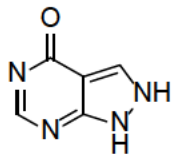
Uric acid has a pKa of approximately 5.4, meaning that at physiological pH it is predominantly present in its ionized form as urate. Uric acid is characterized by its very poor solubility in water, and its saturation concentration in serum has been determined to be 6.8 mg/dL[14].

Hyperuricemia is the presence of a high concentration of uric acid in serum, which may affect up to 18% of some populations and, in many cases, it manifests as gout due to the deposition of urate crystals in joints and other tissues[15]. Hyperuricemia has also been found to be an independent cardiovascular risk factor[16-19]. The prevalence of hyperuricaemia and gout have been steadily increasing during the last few decades, resulting in a significant impact on the global health system. Gout affects 1-2% of the adult population in developed countries. It is the most prevalent form of inflammatory arthritis in men older than 40 years[20]. A continuous increase in chronic gout prevalence is expected as a consequence of lifestyle changes[21], increasing obesity and ageing of the population[22].

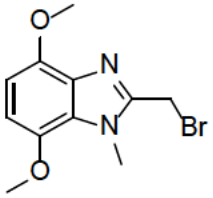
High levels of serum uric acid do not always escalate to gout. It has been observed that for high uric acid level (greater than 10 mg/dL) there is only a 7% risk per year of developing the first episode of gout attack[23, 24]. Hyperuricemia has also been associated with high serum levels of chemokine ligand 2 (CCL2) and increased numbers of leukocytes; their combined presence could explain the higher risk of cardiovascular disease found in hyperuricemic patients[25]. It is still much debated the necessity of prophylactic therapy to reduce serum uric acid levels in asymptomatic hyperuricemic patients to prevent gout attacks as well as any other possible condition caused by high serum uric acid levels[26]. Current approaches for reducing serum uric acid levels include[27, 28]:

1. Preventing formation of uric (i.e. inhibitors of enzymes of the uric acid-forming biochemical pathway, mainly XO),
2. Converting uric acid to a more soluble and readily excreted product, *i.e.* allantoin (by uricase),
3. Stimulating excretion of uric acid (i.e. uricosuric agents).

The latter two approaches are less commonly used for hyperuricaemia monotherapy as they do not halt the oxidation of hypoxanthine and xanthine to uric acid. The inhibition of XO is the most desirable as it interferes with the catabolism of natural purines (hypoxanthine and xanthine), directly preventing uric acid biosynthesis. Inhibition of XO activity can occur either by the interaction with its molybdenum (Mo) centre, as in the case of allopurinol[29], or with the binding site of its flavin adenine dinucleotide (FAD) cofactor, as in the case of 1-methyl-2-bromomethyl-4,7-dimethoxybenzimidazole (**1**)[30].



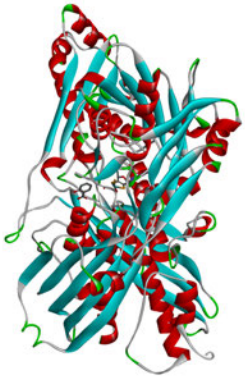
allopurinol



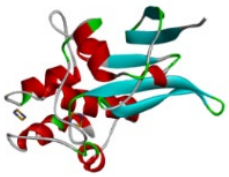
1

Bovine milk XO is a homodimer of 290 KDa and until 2004 it was thought that each subunit worked independently. Subsequently Tai and Hwang showed the existence of cooperative interactions between the two monomers: binding of the substrate to the active site of one subunit affects the catalytic rate of the other subunit[31]. This cooperative effect may explain the presence of mixed-type inhibition of XO reported in the literature, which previously could not be explained because there was no evidence of an allosteric site in the enzyme. Each subunit (Fig 2) contains a molibdopterin unit in the C-terminal domain (with a molecular mass of 85 KDa) (2b), two iron-sulphur clusters in the N-terminal domain (with a molecular mass of 20 KDa) (2c), and one FAD central domain (with a molecular mass of 40 KDa) (2d)[18, 32] . The amino acid sequence in bovine milk XO has 90% sequence identity to the human form of the enzyme. This has made bovine milk XO an enzyme suitable for *in vitro* biological assessment of enzyme inhibitors.

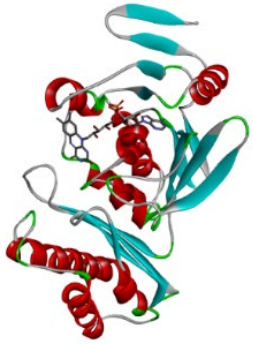
(a) Single subunit of XO **2a**



(b) C-terminal domain **2b**



(c) N-terminal domain **2c**



(d) FAD central domain **2d**

Fig 2: (a) Bovine milk Xanthine Oxidase subunit, (b) C-terminal domain containing the molibdopterin unit, (c) N-terminal domain containing two iron-sulphur clusters, (d) Central domain containing the FAD cofactor.

The first form of the enzyme produced is a dehydrogenase (XDH), which can be easily converted into the oxidase (XO) by oxidation of sulphydryl residues or by proteolysis[33]. It has been hypothesised that the conversion of XDH to XO is triggered during ischemic tissue injuries[34]. During the initial reduction in blood perfusion there is a decrease in ATP formation due to the reduced amount of oxygen available. This drop in ATP translates into a reduction of charge inside the cell, causing disequilibrium of ion gradient across the cell membrane. More calcium ions are transported inside the cell and their high concentration activates the protease responsible for the conversion of XDH into XO. At the same time, there is also an accumulation of hypoxanthine within the cell due to the catabolism of the excess AMP that has not been used to form ATP. XO and hypoxanthine formed during the above process are then responsible for the

formation of reactive oxygen species (ROS) as soon as oxygen flows back to the tissue during the reperfusion process.

XDH and XO do not show any significant difference around or within their active sites, but they possess conformational differences in the FAD binding site[32]. The reaction mechanism catalysed by the enzyme has been elucidated with the help of X-ray diffraction studies of the complex of the bovine milk enzyme with hypoxanthine[35].

During the substrate oxidation, two electrons are transferred from the substrate to the molibdopterin unit, reducing Mo(VI) to Mo(IV)[36]. The re-oxidation of the Mo centre and re-activation of the enzyme occurs by transfer of the two electrons to NAD^+ in the case of XDH and molecular oxygen in the case of XO, as per Fig 3:

$$XO\ [Mo(IV)] + 2O_2 \longrightarrow XO[Mo(VI)] + 2O_2^-$$

$$2O_2^- + 2H_+ \longrightarrow H_2O_2 + O_2$$

Fig 3: Transfer of electrons for the re-oxidation of the Molibdenum atom

The two electrons are transferred from the Mo centre to the first iron sulphur cluster called Fe/S I and, subsequently, to the second iron sulphur cluster called Fe/S II. The electrons are then transferred to the FAD unit before they are used to reduce oxygen molecules, in the case of XO, generating superoxide anion and hydrogen peroxide as final products of the re-oxidation of the molibdopterin unit in the enzyme[37, 38]. XO is therefore responsible for the increase in the level of free radicals that seem to be involved in ageing and many pathological states like atherosclerosis, diabetes, stroke and cancer[39-42]. Free radical levels exceeding the amount that can be controlled by the antioxidant defence system cause oxidative stress.

The active site of the enzyme and the mode of interaction with the substrate during the oxidation process have been extensively studied[43-45]. X-ray diffraction studies conducted using a slow substrate (FYX-051) on XOR elucidated the orientation of the ligands with respect to the Mo metal centre, showing that double-bonded molecular oxygen is on the apical position of the Mo centre, bringing the sulphur atom to a suitable position to interact during the oxidation of the substrate (Fig 4)[44]. Glu1261 in bovine milk XO has been identified as the amino acid residue that initiates the reaction by extracting the hydrogen from the hydroxyl group bound to the Mo centre[43].

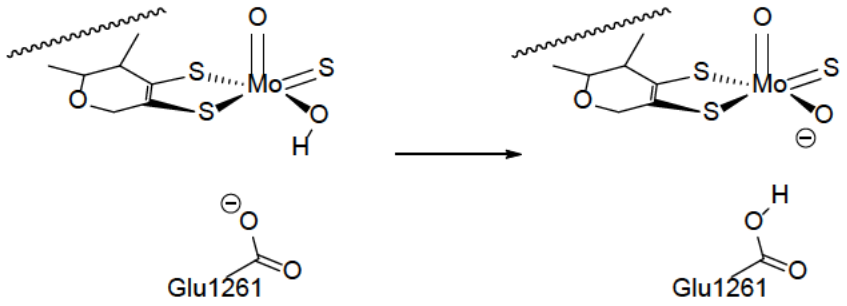


Fig 4: Initial step for the enzyme catalysis reaction

In 2007 Pauff et al. studied *Rhodobacter capsulatus* XDH and identified the function of Arg310 (corresponding to Arg880 in bovine milk XO) during purine oxidation[46]. It was proposed that the guanidino group stabilizes the delocalized negative charge formed in the purine structure during oxidation of xanthine to uric acid. Two XDH mutations were investigated: R310K and R310M. Substitution of Arg310 with a Lys (R310K) resulted in an enzyme with a slower catalytic rate of oxidation for xanthine than the wild type. Substitution of Arg310 with Met (R310M) reduced the oxidation rate by more than 10^4-fold compared to the wild type, making it almost inactive. These mutations demonstrated the essential role of the positively charged guanidino groups in Arg310 in stabilizing the reaction intermediate with a newly formed negative charge, as the substrate oxidation rate diminished when there was a less basic amino group (R310K) or was almost halted when there was no basic group available in this position (R310M). Furthermore, two different orientations of purines inside the active site of the enzyme were identified: “good” and “poor”. Purines with either a carbonyl or thiocarbonyl on the C6 carbon (Fig 5a) were considered to adopt the “good” orientation as they could stabilize the negative charge formed during the oxidation reaction due to their orientation towards Arg310. On the other hand, substrates with either an amino group or a methyl on the C6 (2,6-diaminopurine and 2-hydroxy-6-methylpurine) (Fig 5b) could not benefit from the stabilization offered by Arg310 were oriented with the oxygen atom on the C2 towards Arg310.

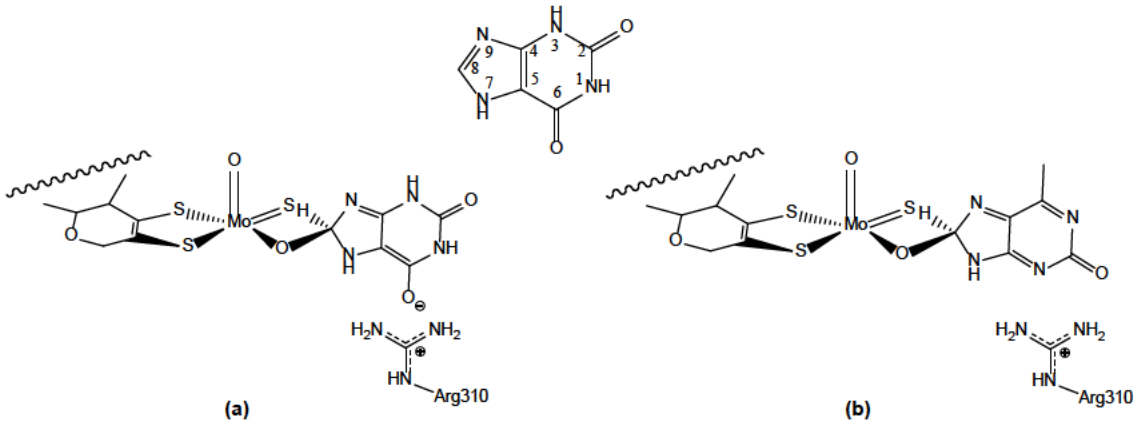


Fig 5: Orientation of purines inside the enzyme active site as identified by Pauff et al.[46]; (a) "good" orientation, (b) "poor" orientation

The above findings were also confirmed by subsequent works by the same authors published in 2008 and 2009, where they described X-ray diffraction studies of the complexes of bovine milk XO with 2-hydroxy-6-methylpurine, xanthine and lumazine[47, 48].

The X-ray structures revealed that the preferred orientation of lumazine and xanthine, both of which have a carbonyl group in a similar position (C6 for xanthine and C4 for lumazine) within the active site, pointing towards Arg310 (Arg880 in bovine milk XO). On the other hand, 6-methylpurine-2-one (**2**) had an opposite orientation, with the carbonyl in C2 pointing towards Arg310 due to the replacement of the carbonyl group at position 6 with a methyl group, which cannot benefit from the stabilization by Arg of the newly formed negative charge during the oxidation step. The carbonyl group at position C2 is then oriented towards Arg310 but with reduced stabilization due to the larger distance to Arg310.

lumazine **2**

In 2010 Okamoto et al. reported the crystal structures of urate in complex with the demolibdo form of rat XOR and in complex with bovine milk XO[49]. Their findings were in contrast with Pauff et al. hypothesis of "good" and "poor" substrates, since they concluded that the oxygen atom on the C2 of the urate is oriented towards Arg880 while the oxygen on C6 and N7 both interact via hydrogen bonds with Glu802. This hydrogen bond network, together with the interaction of the oxygen on C2 with Arg880, were proposed to stabilize the

complex more than it is possible in the opposite orientation. During catalysis, five amino acid residues were deemed to play an important role. Glu1261 (in the bovine milk XO sequence) is negatively charged and it initiates the reaction by deprotonating the hydroxyl group in the equatorial position of the Mo centre. The newly-formed charged oxygen undertakes a nucleophilic attack of the carbon of the substrate that is undergoing oxidation while, at the same time, the hydrogen on the carbon atom is abstracted by the sulphur in the equatorial position of the Mo centre, as described in Fig 6. As a result, the Mo centre reduces its oxidation state from +6 to +4.

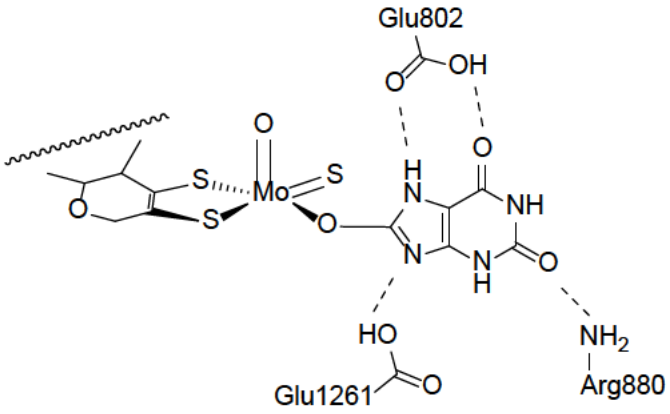


Fig 6: Okamoto et al.[49] hypothesis of interactions between the enzyme and the substrate inside the active site.

Glu802 and Glu1261 help stabilize the resulting reaction intermediate through hydrogen bonding, as well as through hydrogen bonding between Arg880 and the carbonyl at position 2 of the substrate. Phe914 and Phe1009 place their phenyl rings above and below the aromatic ring of the substrate and stabilise the intermediate by π-π interactions, as shown in Fig 7.

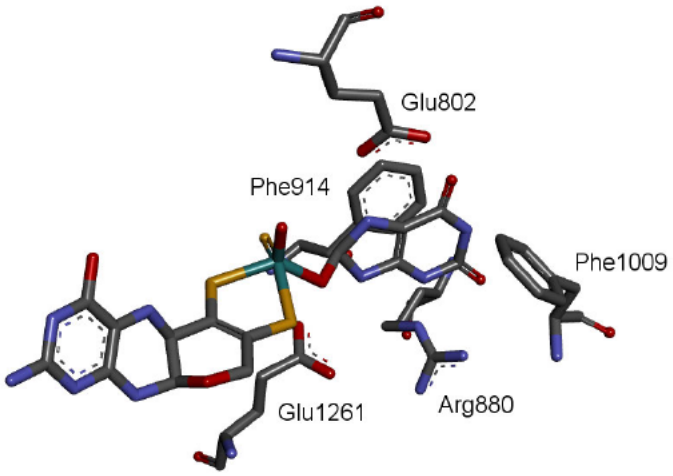


Fig 7: Extract of the crystal structure of bovine milk xanthine oxidoreductase bound to urate (3amz)

The roles of Glu803 (Glu802 in bovine milk XO) and Arg881 (Arg880 in bovine milk XO) in human XO during oxidation of the substrate were investigated by Yamaguchi et al. on mutant human XO[45]. The replacements of Glu803 with Val and Arg881 with Met generated two mutants that were much less effective in oxidising xanthine and hypoxanthine. The authors also noted that the E803V mutation resulted in the loss of activity towards hypoxanthine but less so towards xanthine. The R881M mutation had the opposite effect. Fig 8 illustrates the proposed hydrogen bonding interactions between the substrate and the enzyme during the oxidation reaction. Xanthine has hydrogen bonds with Glu803, Glu1262 and Arg881, while hypoxanthine has hydrogen bonds only with Glu803 and Glu1262. Replacing Glu803 with Val had a major effect on the oxidation of hypoxanthine, with a less prominent effect on the oxidation of xanthine, as the latter still had interactions with Arg881. Replacing Arg881 with Met instead had a major effect on the oxidation of xanthine, with a lesser effect on the oxidation of hypoxanthine, as Arg881 is not involved in the stabilization of hypoxanthine.

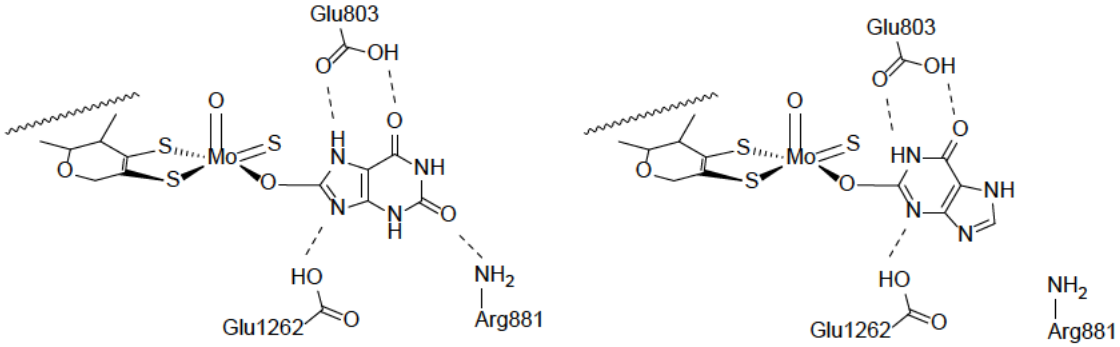


Fig 8: Yamaguchi et al.[45] hypothesis of interactions between the enzyme active site and the substrates xanthine and hypoxanthine

It has also been demonstrated that although purine-6,8-dione (**3**) can be metabolized to uric acid, the same compound is not formed from hypoxanthine oxidation, as summarized in Fig 9[35]. This finding demonstrates that the oxidation of hypoxanthine to uric acid proceeds only to xanthine.

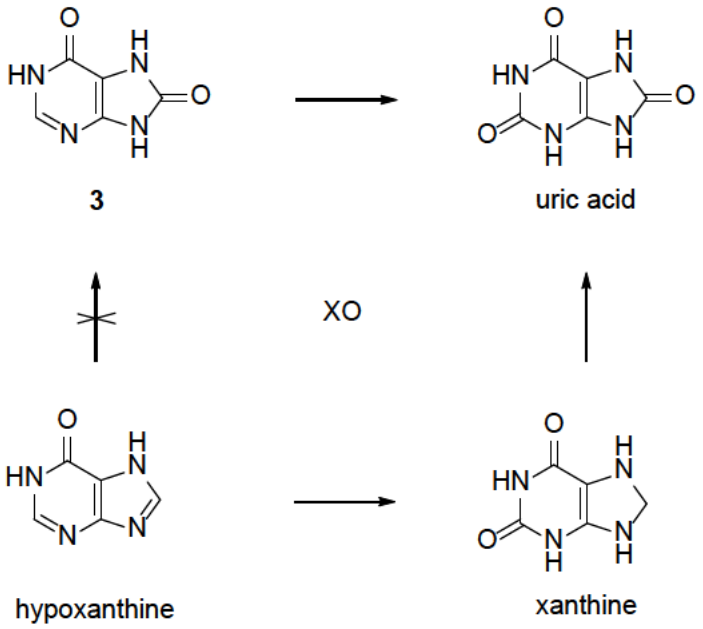


Fig 9: Oxidation of hypoxanthine to uric acid proceeds only via xanthine

The mechanism of action of allopurinol has been also extensively studied[50, 51]. The resulting oxidation product oxypurinol was shown to be tightly attached to the enzyme, but spontaneously dissociated from it during re-oxidation of Mo(IV) to Mo(VI). X-ray diffraction studies demonstrated also that the amino acids interacting with oxypurinol within the active site are the same ones that interact with the substrate during the oxidation of xanthine to uric acid[52], as shown in Fig 10 for xanthine as the substrate.

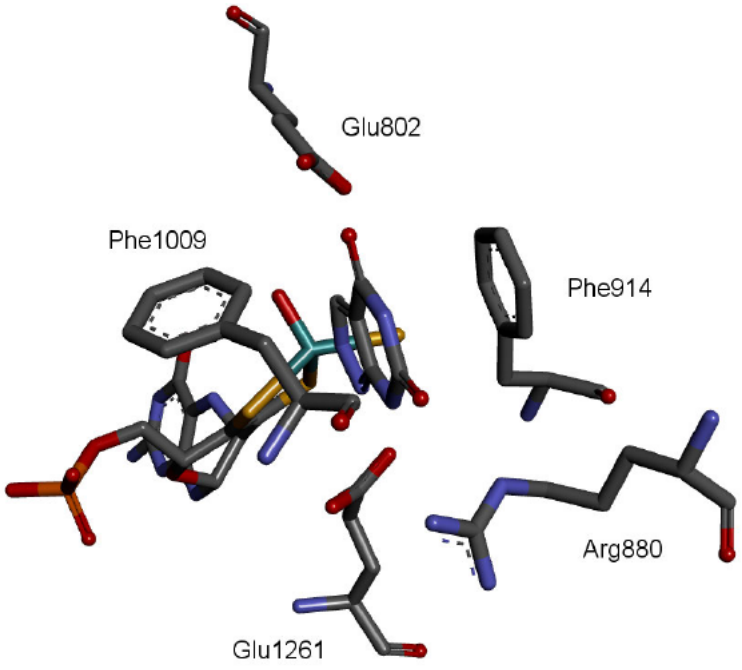


Fig 10: Extract of the crystal structure of bovine milk xanthine dehydrogenase with oxypurinol (3bdj)

As mentioned above, ROS are generated during the oxidation of hypoxanthine to xanthine and then to uric acid, primarily in the form of hydrogen peroxide (H_2O_2). Oxidative stress is the condition where there are high levels of ROS in the body. It is known to be a complication of type 1 diabetes. Diabetic rats demonstrated an increased activity of XO in both liver and plasma compared to non-diabetic rats, resulting in a larger production of free radicals leading to oxidative stress[53]. Desco et al. found that the XO inhibitor allopurinol helps to prevent oxidative stress and, therefore, helps to avoid further complications in patients with type 1 diabetes[53].

XO has also become a target in some anti-cancer therapies[54] and in the treatment of hypertension[55]. In the first case, its inhibition prevents the inactivation of the cancer drug 6-mercaptopurine by the enzyme[54]. In the second case, as indicated earlier, XO is one of the major producers of ROS O_2^- and H_2O_2 in the body and is overexpressed during ischemia and inflammatory conditions. It has high affinity for the internal surface of blood vessels, where its production of ROS negatively affects the bioavailability of nitric oxide (NO). NO-mediated decrease in vascular pressure is therefore reduced, leading to a negative contribution to hypertension.

The preliminary test to evaluate the efficacy to inhibit the enzyme is usually performed *in vitro* using bovine milk XO. The enzyme was extracted from cow's milk for the first time more than 80 years ago and it is now one of the most studied enzymes[56]. Bovine milk is the main and cheapest source of XO, although it could be obtained also from the extraction of other tissues. Laboratory *in vitro* tests are commonly performed by using xanthine as substrate instead of hypoxanthine and by monitoring the formation of uric acid. Both hypoxanthine and xanthine are substrates of XO, but xanthine is preferred to hypoxanthine, in *in vitro* tests, as it oxidises directly to uric acid while hypoxanthine is converted to xanthine first, before producing uric acid. The reaction product is monitored by UV absorbance at 295 nm.

The efficacy of the inhibitor is commonly measured by two methods: either measuring the ratio of the speed of conversion of xanthine to uric acid, obtained when the inhibitor is used, against the speed of conversion when no inhibitor is present, or by comparing uric acid absorbance obtained from the solution with the inhibitor with the absorbance of the solution without inhibitor after a specific

incubation time. Significant comparison of the data could be achieved only among results obtained from the same method.

In this review we focus only on the inhibition of XO as an approach for the treatment of hyperuricemia. Describing chemical diversity of XO inhibitors, we classify them as two main groups *viz*. (1) purine-based and purine-like inhibitors and (2) non-purine inhibitors.

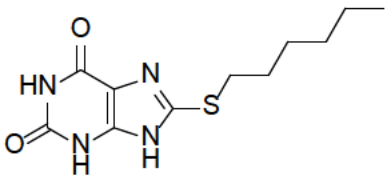
2.2 Purine-based and purine-like inhibitors of XO

A common method used to develop new drugs is to make small changes to the structure of the natural substrate of an enzyme to obtain structurally similar analogues.

2.2.1 Xanthine analogues

Xanthine (purine-2,6-dione), the natural substrate of XO, is a purine base that has been used for a long time by many researchers as the starting point to produce purine-like analogues. The introduction of new substituents to a natural substrate is often used to increase the number of substrate-enzyme interactions, resulting in a better affinity towards the enzyme.

In a work published in 2001 by Biagi et Al[57] it was reported that the newly synthesised 8-(*n*-hexylthio)xanthine (**4**) showed more than 6 times better potency than Allopurinol. Three years later the xanthine derivative 1,3-dipropylxanthine substituted benzenesulfonic acid **5**, already known as an adenosine receptor antagonist, was reported to be a good inhibitor of XO by Sousa et al.[58], with an IC_{50} value in the micromolar range. *In vivo* studies conducted in rats showed that this compound was able to reduce both the level of uric acid and the generation of superoxide radical.



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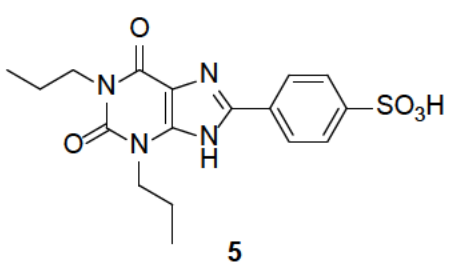

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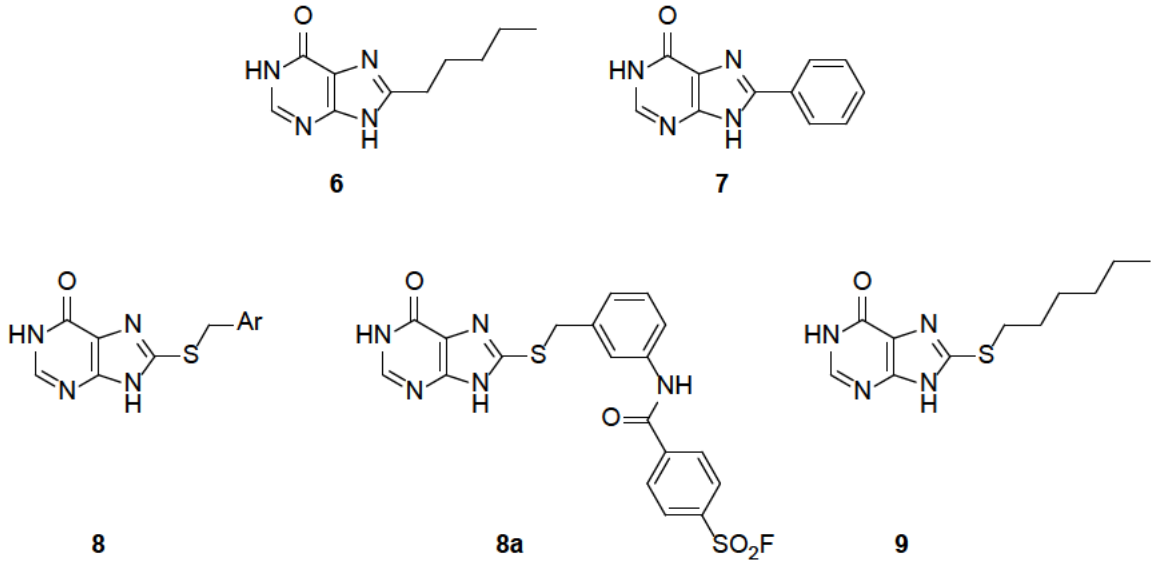
2.2.2 Hypoxanthine analogues

The orientation of hypoxanthine derivatives within the active site of XO was investigated by Biagi et al.[59]. The 8-alkyl hypoxanthine derivatives were found to act as substrate for the enzyme producing 8-alkyl xanthines while the 2-alkyl hypoxanthines were not. This finding demonstrated that the orientation of these compounds within the active site is dictated by the alkyl chain. The most active compound of the series was 8-*n*-pentylhypoxanthine (**6**). On the opposite side of the ring, 2-alkylhypoxanthines did not show significant inhibitory efficacy. Interestingly, 2,8-di(*n*-pentyl)hypoxanthine was also found to be inactive against the enzyme.

The presence of a phenyl group instead of the *n*-alkyl chain in position 8 (compound **7**) (IC_{50} = 0.062 μM) of hypoxanthine was found to result in a very potent XO inhibitor as reported by Robins et al[60]. On the other hand, either a methoxy, bromo or benzylthio substituent in this position dramatically decreased inhibitory activity.

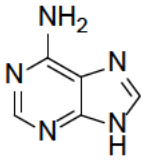
In 1968 Baker et al. reported series of derivatives of 8-(benzylthio)hypoxanthine (**8**) with the aim of finding good irreversible XO inhibitors[61]. All the compounds had a sulphonyl fluoride moiety on a phenyl ring as the best reacting group to achieve irreversibility in the inhibition mechanism, as identified in their previous work[62]. The authors defined this mechanism of inhibition as “exo”, where the covalent bond between the inhibitor and the enzyme is formed outside of the active side. One of the best irreversible inhibitor was 8-(m-(p-fluorosulfonylbenzamido)benzylthio)hypoxanthine (**8a**) that inhibited 50% of the enzyme after 7 min of incubation at a concentration of 0.46 μM.

Similar 8-(alkylthio)hypoxanthine derivatives were synthesised by Biagi et al[57] in order to further investigate the interactions between the inhibitors and the amino acid residues in the active site. The authors synthesised and tested compound **9** (IC_{50} = 0.56 μM) and **4** (IC_{50} = 0.66 μM). Both molecules showed better activity than allopurinol and it was also reported that 8-(*n*-hexylthio)hypoxanthine (**9**) was a substrate for XO as it was converted to 8-(*n*-hexylthio)xanthine (**4**).



2.2.3 Adenine analogues

The natural purine adenine (**10**) (IC_{50} = 75.61 μM) has been found to exhibit comparable inhibitory activity to allopurinol against XO with a competitive mechanism[63-65]. The molecule is oxidised by the enzyme and its product is more active than the parent compound[66]. In 1968 Baker et al.[61] identified XO irreversible inhibitors in derivatives of 8-(benzylthio)adenine (**11**). One of the most potent inhibitor was adenine analogue **11a**. Substituents on the exocyclic amino group produced active purine analogues, with **12** (IC_{50} = 0.45 μM) being more than 150-fold more potent than adenine itself[65]. The novel compound **12** was also tested with respect to the mechanism of electron transfer in the enzyme, and was found to perform better than allopurinol as it did not generate ROS. Allopurinol produced hydrogen peroxide by transferring two electrons to the enzyme, while **12** did not react in this manner.



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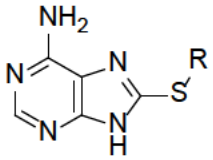

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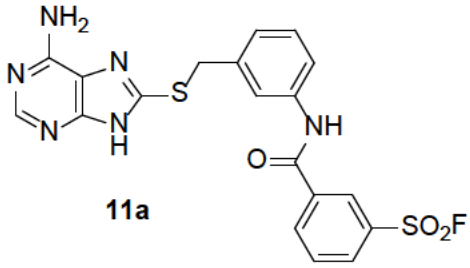
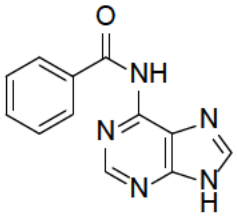


11a



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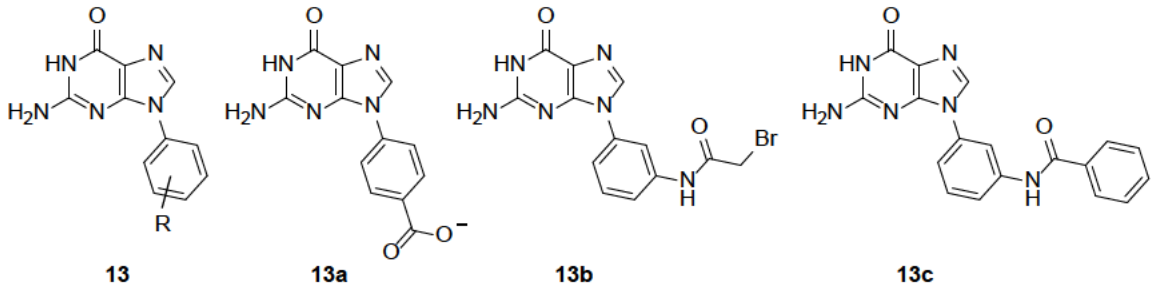
2.2.4 Guanine analogues

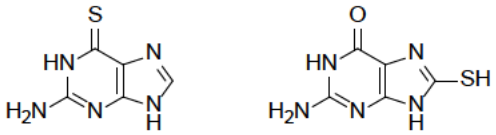
In 1967 Baker and Wood[67] reported the inhibitory effects of a series of 9-phenylguanines (**13**) substituted diversely on the phenyl ring. Introduction of a carboxylate into *para*-position of the phenyl ring gave rise to the most active compound (**13a**), with an IC_{50} of 0.12 μM. Subsequent studies conducted in 1968 by Baker et al.[68] identified the need for the phenyl ring at position 9 to be coplanar to the purine ring to ensure maximum activity among the structures studied. An increase in inhibitory activity was also obtained by replacing the *para*-carboxylate with either a bromoacetamido (compound **13b**) (IC_{50} of 0.071 μM) or a benzamido group (**13c**) (IC_{50} of 0.072 μM) in the *meta*-position.

The replacement of the oxygen atom in the carbonyl with a sulphur in guanine molecules resulted in a competitive inhibitor **14**, but its activity was lower than that of allopurinol[69]. When a sulphydryl substituent was placed at position 8 of guanine, the mechanism of XO inhibition for the resulting **15** was of a mixed type but with lower activity than allopurinol.

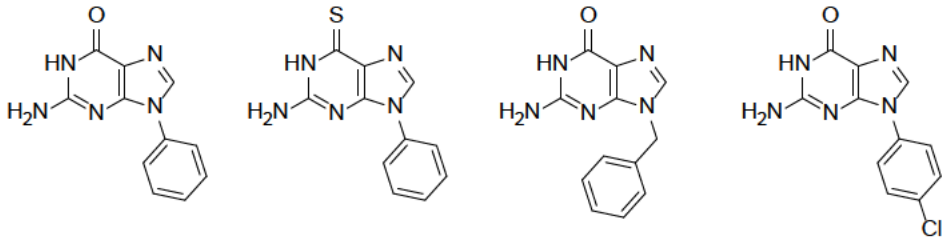
9-Arylguanines were also investigated by Robins et al.[60]. They reported that compound **16** (IC_{50} = 0.41 μM) showed better activity than allopurinol (IC_{50} = 5.9 μM). Its activity was reduced by replacing the carbonyl group with a thiocarbonyl group (**17**) (IC_{50} = 1.1 μM), but was still better than allopurinol. Either extending the distance to the phenyl group, using a benzyl substituent (**18**) (IC_{50} = 23 μM), or introducing chlorine at the *para*-position of the phenyl group (**19**) (IC_{50} = 1.8 μM) significantly reduced the efficacy.



13 13a 13b 13c



14 15

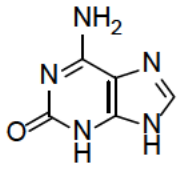


16 17 18 19

2.2.5 Isoguanine analogues

Isoguanine (**20**) was reported to show very good inhibitory activity against XO[70]. It almost completely inhibited the enzyme at a concentration of 22 nM. Interestingly, substitution of the amino group (except phenylamino and 5-phenylpentylamino) was found to significantly decrease activity level.

In 1999 Nagamatsu et al.[71] identified a new class of XO inhibitors. Hydrazone-substituted 2-oxopurines were demonstrated to be very potent inhibitors. The series tested exhibited IC_{50} values ranging from 0.025 to 1.184 μM (with allopurinol possessing in the assay an IC_{50} value of 24.3 μM). The chloro substituent at the *para*-position of the phenyl ring was shown to be the most effective substituent producing the most potent in the series compound **21**. The activity was reduced if the chlorine atom was replaced by fluorine, methoxy, nitro or a hydroxyl group. Furthermore, the carbonyl at position 2 of the purine scaffold was shown to be essential for retaining the best inhibitory activity as its substitution with a thiol, phenyl, chlorine or amino group resulted in lower inhibitory activity.



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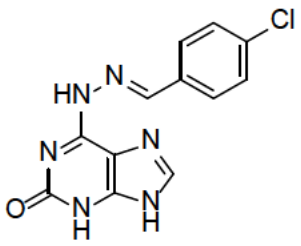

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2.2.6 Other purines

Derivatives of hypoxanthine obtained by the introduction of a thioalkyl group in position 2 have been investigated by various research groups.

In their search of irreversible inhibitors of XO Baker et al[61] reported in 1968 a series of 2-benzylthiohypoxanthines (**22**). One of the most potent representative of this class of compounds was 2-(*m*-(*p*-fluorosulfonylbenzamido) benzylthio) hypoxanthine (**22a**), which inhibited 53% of the enzyme activity after 5 min of incubation at a concentration corresponding to its IC_{50} value of 0.092 μM. After 60 min of the incubation, the inhibition increase to 58%.

A benzylthio group at position 2 of hypoxanthine resulted in compound **23** (IC_{50} = 0.75 μM), which was more than 7 times stronger inhibitor than allopurinol (which has IC_{50} = 5.9 μM) while a simple methyl group at the same position resulted in an inactive compound. [60]

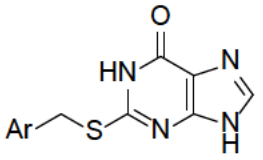
Thioalkyl groups at position 2 of hypoxanthine were also investigated by Biagi et al.[72]. They reported that analogues with either a butylthio or *n*-pentylthio group demonstrated comparable activity to allopurinol (IC_{50} = 4.2 μM), while the analogue with the *n*-hexylthio group (compound **24**) (IC_{50} = 0.115 μM) in the same position conferred better activity than allopurinol. The introduction of a phenyl group at position 8 of compound **24** decreased the activity of the parent compound confirming the previous findings of the same authors[59] that double lipophilic substitution in position 2 and 8 is detrimental for the inhibitory activity.

In an effort to identify the characteristics of the narrow lipophilic channel close to the Mo centre in the active side of the enzyme, Biagi et al.[57] studied various 2-(alkyloxyalkylthio)hypoxanthines in 2001. Among the molecules tested, it was found that, while keeping the length of the side chain constant (6 atoms), the longer the distance between the sulphur and oxygen atoms in the lipophilic chain the better the inhibitory activity exhibited towards the enzyme. The lowest IC_{50}

value was obtained for 2-(4-methoxybutylthio)hypoxanthine (**25**) (IC_{50} = 0.028 μM compared to IC_{50} = 4.2 μM for allopurinol).

In 1964 Gilbert et al.[73] reported that 6-formylpurine (**28**) interacted strongly and in a competitive manner at the active site of XO similarly to hypoxanthine.

The amino group at position 2 of purine is not favourable for the inhibition of XO as demonstrated by the very poor activity observed for 2-aminopurine (**27**) (IC_{50} > 200 μM) and 2,6-diaminopurine (**28**) (IC_{50} > 200 μM) compared to allopurinol with IC_{50} = 7.82 μM and adenine (**10**) (IC_{50} = 10.89 μM)[63, 74].



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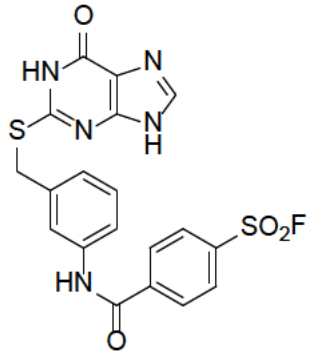
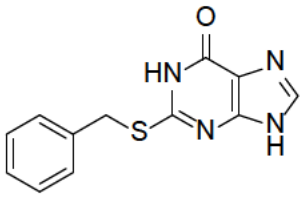


22a



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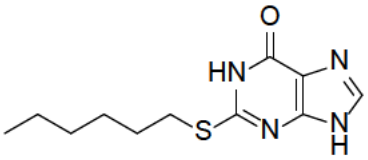

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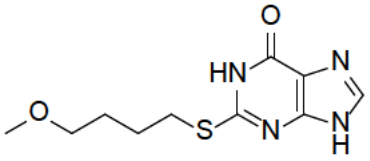

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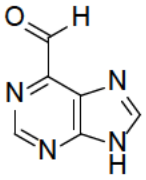

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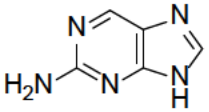

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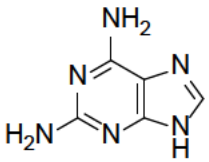

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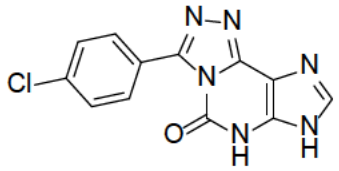
2.2.7 Fused purines

A tricyclic system with a triazole ring fused to the purine scaffold was reported by Nagamatsu et al.[71] In the prepared series, 3-(4-chlorophenyl)-1,2,4-triazolo[3,4-*i*]purine (**29**) was reported to possess an IC_{50} value of 0.066 μM (while allopurinol had an IC_{50} value of 24.30 μM) being the most active among the library of compounds synthesised. The *p*-chlorophenyl substituent was shown to be the best as its replacement with *p*-methoxyphenyl or even just a phenyl group significantly reduced the inhibitory activity of the resulting compounds.



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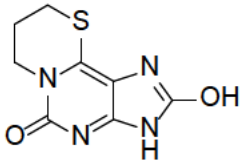
Tricyclic systems with a third ring fused to the purine scaffold were also studied by Biagi et al.[72]. Compounds **30** and **31**, which have hydrated thiazine or thiazole ring fused to purine, respectively, showed *in vitro* activity in the micromolar concentration range.



K_i = 0.33 μM

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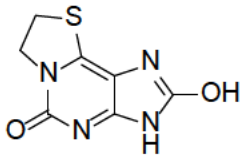


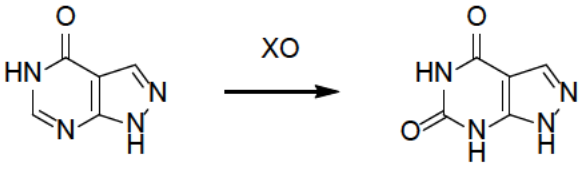
K_i = 1.26 μM

31

2.2.8 Pyrazolo[3,4-*d*]pyrimidines

The first drug that was approved for the treatment of hyperuricemia was the purine analogue allopurinol (**32**), which was first synthesised in 1956 by Roland K. Robins. The drug was studied during the investigation of novel compounds for the treatment of leukaemia in children[75]. The half-life of 6-mercaptopurine (**34**), identified as a good anti-leukemic drug, was affected by its degradation due to the enzyme XO. The compound was converted into 6-thiouric acid and excreted in the urine[54]. The use of allopurinol as an inhibitor of XO was aimed at increasing the half-life of the anti-leukemic drug; however, it was found that the combination therapy of mercaptopurine/allopurinol did not improve the efficacy of the anti-leukemic drug. Allopurinol was subsequently investigated for its inhibitory activity against XO and was approved by the FDA in 1966 for the treatment of gout, and is currently still the drug of first choice in the treatment of hyperuricemia as advised by the 2016 European League Against Rheumatism (EULAR) guideline[76].

Allopurinol (**32**), 1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one, is a hypoxanthine isostere that is converted by XO into its more active metabolite oxypurinol (**33**), also known as alloxanthine (Fig 11). As a substrate of the enzyme, allopurinol does not halt the production of ROS[77].



32 **33**

Fig 11: Oxidation of allopurinol to oxypurinol

Allopurinol is both a substrate and a competitive inhibitor of XO. It effectively reduces serum urate levels both by inhibiting XO and by generating a negative feedback regulation process of earlier steps in the biosynthesis of purines[78]. The conversion of allopurinol to the non-competitive inhibitor oxypurinol *in vivo* is completed within two hours. Oxypurinol binds to the active site of the reduced enzyme and dissociates from it slowly, with an estimated half-life of 18-33 hours. The *in vitro* IC_{50} for allopurinol and oxypurinol was shown to be always better than that of xanthine, and this drove the focus of research to further develop new allopurinol analogues.

Allopurinol was the first and only drug used for the treatment of hyperuricemia until 2009, when febuxostat was approved by the FDA[79]. The interaction of both allopurinol and oxypurinol with XO has been extensively investigated[29, 80]. It was proposed that the mechanism of inhibition by oxypurinol involved the formation of a coordinate bond between the nitrogen at position 2 of oxypurinol and the Mo centre of the reduced enzyme cofactor (Mo IV). The coordination was irreversible and, therefore, impeded the enzymatic production of uric acid. The inhibition is time dependent and oxypurinol dissociates from the enzyme subsequently to the spontaneous oxidation of the Mo centre[51, 81]. Oxypurinol shows better activity *in vivo* than allopurinol due to its longer half-life and lower excretion rate, but it is not administered directly because its average absorption in the body is only 40% and would thus require higher dosages than allopurinol, which has a better absorption (80%).

A similar mode of action and inhibition activity was observed with the allopurinol isostere thiopurinol, 1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-thione (**34**) with an IC_{50} of 8.4 µM compared to allopurinol with an IC_{50} of 5.9µM[60, 82-85].

A group of four analogues of structure **34** with various substituents at position 6 (**35**) were reported by Baker in 1967[86]. The presence of a methyl group at position 6 was detrimental for *in vitro* inhibitory efficacy, while either *p*-nitrophenyl

or *p*-methoxyphenyl groups in the same position led to an approximately 30-fold increase in binding to the enzyme compared to hypoxanthine.

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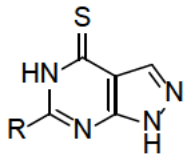

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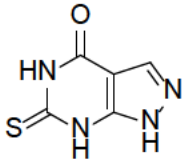
The thiopurinol isostere 6-mercapto-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*,7*H*)-one (**36**) showed better inhibitory activity than oxypurinol *in vitro*[83, 87]. The same efficacy was not observed *in vivo*, and it was thought that binding to serum protein was the main cause of the reduction in inhibitory activity. Both a methylthio group (**37**) and a chlorine atom (**38**) at position 6 were also shown to affect negatively the activity of these compounds[83].



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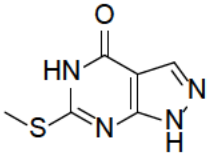

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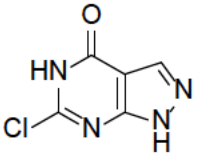

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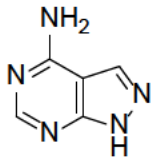
When the carbonyl of allopurinol was replaced by an amino group to give 4-amino-pyrazolo[3,4-*d*]pyrimidine (**39**), the activity decreased almost 10 times from that of allopurinol[85]. Aryl substituents on the amino group increased significantly the potency of compound **39,** with 4-methoxyphenylmethyl (**40**) and 3,4-dimethoxyphenylmethyl (**41**) substituents at the amino group being the most potent derivatives[88].



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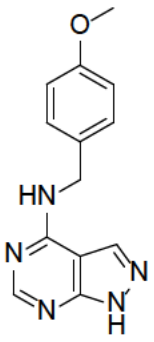

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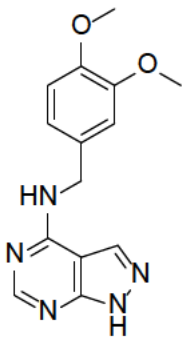

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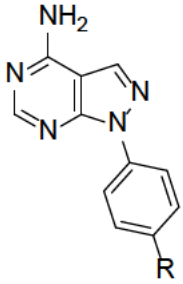
Aromatic substituents on the nitrogen at position 1 of compound **39** were also investigated for their capacity to enhance the activity of the unsubstituted parent compound. Gupta et al. reported the inhibitory activity of a series of 4-amino-1-aryl-pyrazolo[3,4-*d*]pyrimidines (**42**), as well as their corresponding precursors *N*-aryl-5-amino-4-cyanopyrazoles (**43**)[89]. Most of the pyrazolo derivatives had an

IC_{50} higher than 100 μM, with the exception of the derivatives that had either a carboxylic group at position 4 of the phenyl ring or the carboxylic group amidated with a glycine methyl ester. Much better IC_{50} values were obtained for the pyrazolo[3,4-*d*]pyrimidine series, ranging from 0.08 to 80.97 μM. The lowest reported IC_{50} value was observed for methyl *N*-[4'-(4"-aminopyrazolo[3,4-*d*]pyrimidin-1"-yl)-benzoylamino]acetate (**42a**) (compared to allopurinol with IC_{50} = 24.40 μM).



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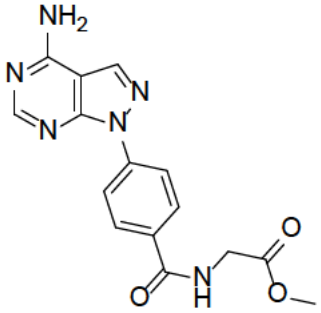

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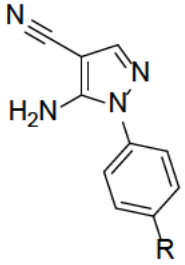

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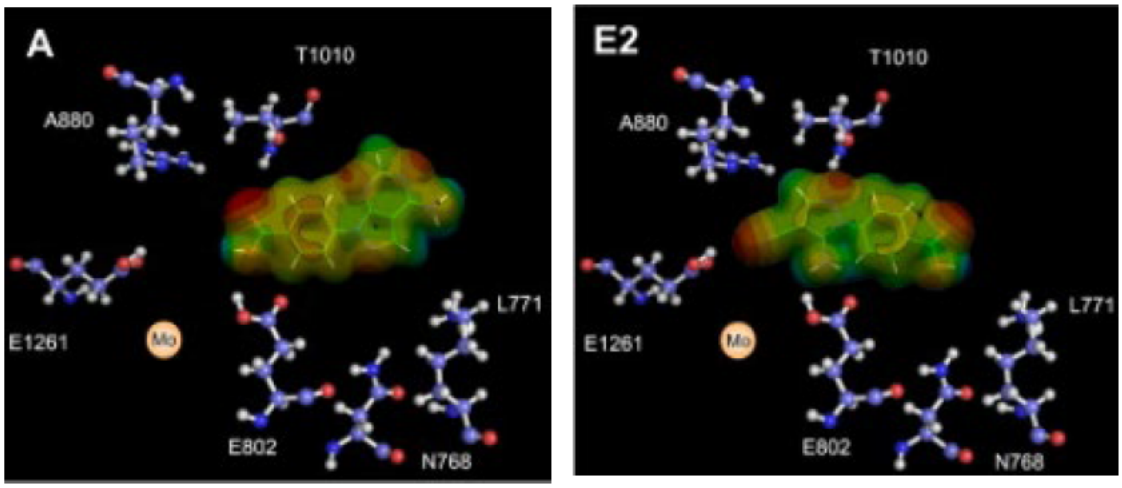
It was noted that the amidation of the carboxylic acid group with glycine methyl ester in the pyrazole series did not affect the inhibitory activity of the parent derivative carrying the free carboxylic acid group. By contrast, the same substitution in the pyrazolo[3,4-*d*]pyrimidine series increased the activity by about 1000-fold. A similar behaviour was observed upon addition of glucose to the free amino group of the 4-amino-7-(4'-carboxyphenyl)-pyrazolo[3,4-*d*]pyrimidine and corresponding 5-amino-4-cyanopyrazole. In the pyrazole series, the addition caused a loss of inhibitory activity of the compound, while in the pyrazolo[3,4-*d*]pyrimidine series the addition resulted in a higher activity. The authors explained this interesting trend through molecular docking studies and the identification of 2 specific regions in the active site that were responsible for hydrogen bonding with the substrate and, therefore, were deemed to be critical for the interactions experienced by the two series of compounds. The predicted orientation of N-(4'-carboxyphenyl)-1*H*-aminopyrazolo[3,4-*d*]pyrimidine (**12a**) and 5-amino-1-(4'-carboxyphenyl)-1*H*-pyrazolo-4-carbonitrile (**12b**) within the active site are shown in Fig 12. In the case of the pyrazolo[3,4-*d*]pyrimidine series, the phenyl ring and its substituents point towards the Mo centre, while in the pyrazole series the pyrazole ring itself is closer to the Mo centre. The authors explained that in the first case there was a stronger interaction between the

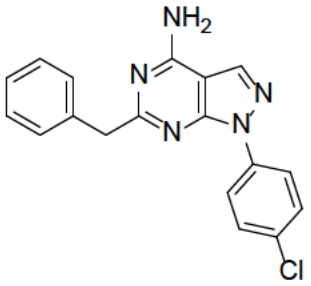
inhibitor and the active site residues, compared to a weaker interaction in the case of the pyrazole series.



(a) (b)

Fig 12: Predicted orientation within the active site for N-(4'-carboxyphenyl)-1*H*-aminopyrazolo[3,4-*d*] pyrimidine (**12a**) (a) and 5-amino-1-(4'-carboxyphenyl)-1*H*-pyrazolo-4-carbonitrile (**12b**) (b)

Double substitution at positions 1 and 6 in compound **39** did not increase the activity of the parent un-substituted compound. Compounds 4-amino-1-(4-chlorophenyl)-6-(phenylmethyl)-pyrazolo[3,4-*d*]pyrimidine (**44**) (IC_{50} = 76.6 µM) and 4-amino-1-(4-bromophenyl)-6-(4-pyridinyl)-pyrazolo[3,4-*d*]pyrimidine (**45**) (IC_{50} = 78.1 µM) were the most effective, but were still much weaker inhibitors than both the un-substituted parent compound **39** and allopurinol (IC_{50} = 24.4 µM)[90].



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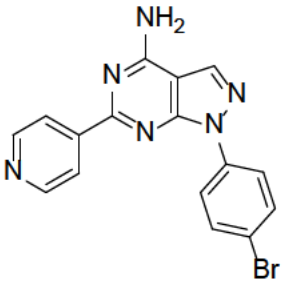

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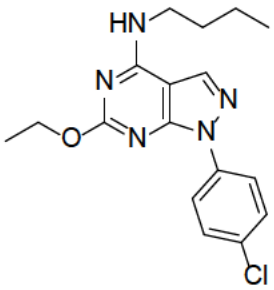
Active XO inhibitors, though less potent than allopurinol, were obtained from a library of 1-(4-chlorophenyl)-pyrazolo[3,4-*d*]pyrimidines with various substituents at positions 4 and 6[91]. At a concentration of 13 µM the most potent compound was *N*-butyl-1-(4-chlorophenyl)-6-ethoxy-pyrazolo[3,4-*d*]pyrimidin-4-amine (**46**), which was reported to exhibit only 29% inhibition vs. the 80% inhibition displayed by allopurinol at the same concentration.



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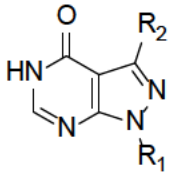
A single bromine atom at position 3 of allopurinol reduced its activity by more than 17 times, as reported by Chu et al.[92]. These authors also showed that methyl, cyclohexyl and aryl substituents at position 1, together with either nitro, amino, bromine or chlorine substituents at position 3 (**47**) of allopurinol resulted in a drastic loss of activity compared to allopurinol.



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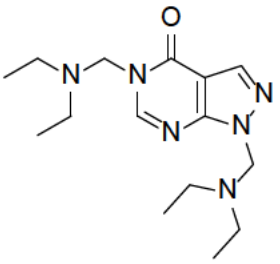
Vartanyan et al. found that 1,5-bis[(diethylamino)methyl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**48**) and 1,5-bis[(4-methyl-1-piperazinyl)methyl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**49**) exhibited higher activity than allopurinol[93]. These authors also reported the negative influence of a substituent at position 3 of the pyrazolo[3,4-*d*]pyrimidine core on the XO inhibition.



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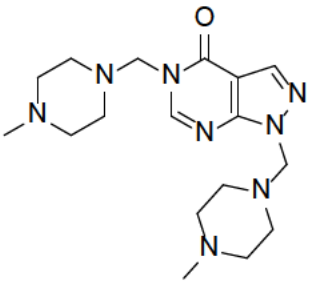

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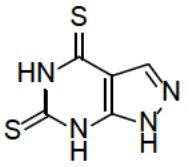
These same authors reported also the activity of various oxypurinol analogues. The replacement of the carbonyl groups of allopurinol with thiocarbonyls produced 1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dithione (**50**) with activity comparable to allopurinol. Further modifications of this compound (**50**) demonstrated that introduction of several electron-withdrawing groups at position 3 reduced the XO inhibitory activity. It was also reported that the activity was

further reduced if both thiocarbonyl groups were replaced by methylthio groups in the same series of compounds.

In the search for potent inhibitors of XO among a series of pyrazolo[3,4-*d*]pyrimidines able to avoid the generation of ROS, Tamta et al. found isoguanine isostere **51** (IC_{50} = 1.56 μM) and its thio-analogue **52** (IC_{50} = 0.60 μM) to possess activity comparable to allopurinol (IC_{50} = 0.78 μM)[85].



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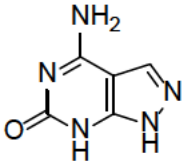

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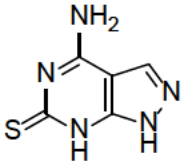


52

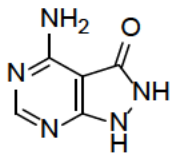
These compounds were shown to be competitive inhibitors of the enzyme. They were also tested in two different electron transfer experiments to determine their capacity to participate in the enzyme-mediated electron transfer reactions: reduction of 2,6-dichlorophenolindophenol (DCPIP) (a two-electron acceptor) and reduction of cytochrome c (a one-electron acceptor). The single electron transfer mechanism is responsible for the formation of ROS. Compound **52** behaved as allopurinol and did not produce any ROS by inhibiting the one-electron transfer mechanism, but they both participated in the enzyme-mediated reduction of DCPIP. Compound **51** inhibited both electron transfer mechanisms, demonstrating to have a mechanism of inhibition of the enzyme different from that of allopurinol, and to inhibit the formation of ROS thus reducing the oxidative stress damage.

The good *in vitro* activity of 4-aminopyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one, also known as 4-amino-6-hydroxypyrazolo[3,4-*d*]pyrimidine (AHPP) (**51**)[60, 69, 94] (Ki = 0.17 μM) compared to allopurinol (Ki = 0.50 μM) and oxypurinol (Ki = 3.54 μM) made it a good candidate for *in vivo* tests[95-97]. Following promising *in vivo* results, further studies were conducted for the treatment of gout, hypertension, cardiac allograft rejection, ROS modulation, ischaemia-reperfusion injury and inflammatory bowel disease[95-101]. It was found that **51** had anti-hypertensive activity by regulating nitric oxide (NO) level as well as a cardio-protective effect by modulating superoxide production.

A *p*-chlorophenyl substituent at position 3 of structure **51** reduced the *in vitro* inhibitory activity of the unsubstituted compound[60], while at the concentrations

used by Kobayashi both *p*-chlorophenyl and phenyl substituents at position 3 of AHPP slightly increased the potency of the parent unsubstituted compound[83].

In 1995 Izumida et al. reported the identification and isolation of 4-aminopyrazolo[3,4-*d*]pyrimidin-3-one (**53**) (IC_{50} = 16.9 μM) from the marine bacterium *Agrobacterium aurantiacum*[102]. This compound, also known as akalone, was found to be an inhibitor of XO but with lower activity than allopurinol. The same authors published a subsequent work reporting the identification of a derivative of akalone, 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,6-dione (hydroxyakalone, **54**) (IC_{50} = 4.6 μM), obtained under different cultivation conditions of Agrobacterium[103]. Hydroxyakalone (**54**) was reported to possess comparable to allopurinol potency (IC_{50} = 4.0 μM).



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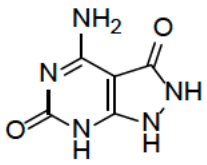

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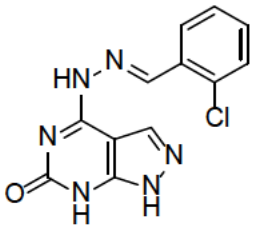
Substituents on the amino group at position 4 of compound **51** resulted in very potent inhibitors of XO with hydrazone **55** being the most active in the series[104]. SAR studies conducted on hydrazones **56** showed that the planar orientation of the pyrazolopyrimidine moiety inside the narrow channel of the enzyme active site allowed for van der Waals interactions with the phenylalanine Phe914 and Phe1009, in addition to the numerous hydrogen bonds between the inhibitor and the active site of the enzyme[105].



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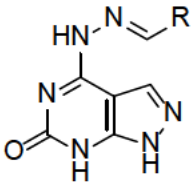

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The interactions between inhibitors and the enzyme were also investigated by molecular docking studies using two crystal structures already available (PDB codes 1n5x and 1v97). Fig 13 shows the predicted hydrogen bonds between **56** (R=Ph) and both Glu802 and Thr1010 in the active site.

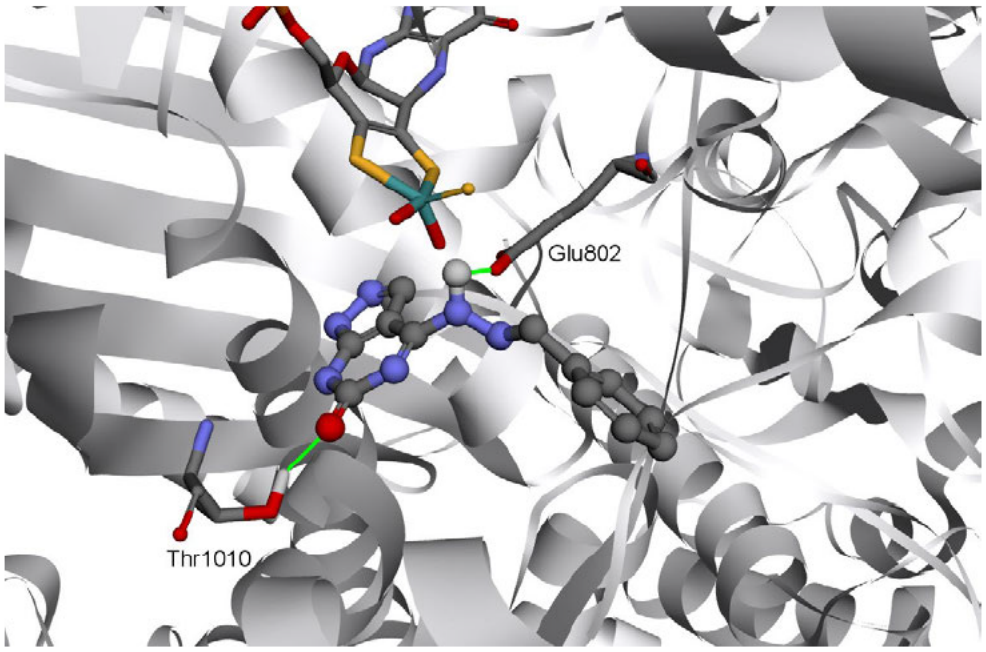
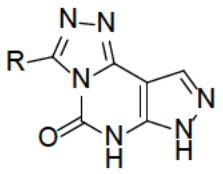


Fig 13: Predicted hydrogen bonds between 2-(6-oxo-pyrazolo[3,4-*d*]pyrimidin-4-yl)hydrazone benzaldehyde and both Glu802 and Thr1010 in the enzyme active site

2.2.9 Fused pyrazolo[3,4-*d*]pyrimidines

Pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5-ones (**57**) represent a tricyclic system with a triazole ring fused to an allopurinol-type scaffold[104-106]. These compounds were found to be more potent XO inhibitors than allopurinol. Aryl substituents (R) on the triazole ring of **57** generated more active compounds than alkyl substituted analogues. The planar orientation of the fused tricyclic ring system was shown to be sandwiched between Phe914 and Phe1009 residues in the active site of the enzyme allowing for aromatic/aromatic interactions between the inhibitor and the enzyme. Introduction of a plane phenyl ring as substituent R resulted in good inhibitory activity against XO. The enzyme inhibitory activity tolerated various substituents in the *ortho*, *meta* and *para*-positions of this phenyl ring. The most potent compound of this series was 3-(4-chlorophenyl)-pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5-one (**58**), with an IC_{50} of 0.032 µM (approximately 760 times more potent than allopurinol, with IC_{50} = 24.3 µM).



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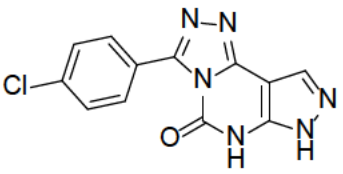

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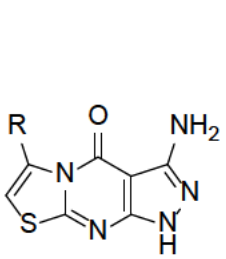
In 2010 Khobragade et al. reported a series of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one derivatives of a tricyclic system containing a thiazole ring fused

to an allopurinol structure with an amino group on the pyrazole ring (**59**)[107]. Compounds with various substituents on a phenyl ring at position 6 were synthesised and tested, with the most potent being 3-amino-6-(2-hydroxyphenyl)-pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one (**60**) (K_i = 7.8 μM) and 3-amino-6-(4-chloro-2-hydoxy-5-methylphenyl)-pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one (**61**) (K_i = 10.2 μM) compared to allopurinol, which had a K_i value of 13.3 μM.



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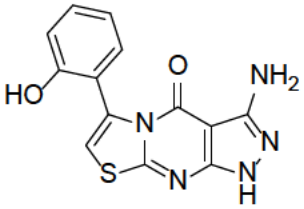

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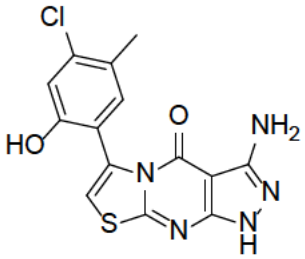
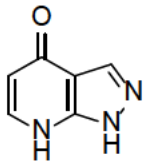


61

2.2.10 Pyrrolo[2,3-*d*]pyrimidines and pyrazolo[3,4-*b*]pyridines

Some other heterocyclic isosteres of hypoxanthine were reported to be less potent for the XO inhibition compared to allopurinol. For example, 1*H*-pyrazolo[3,4-*d*]pyridin-4(7*H*)-one (**62**) and 1*H*-pyrrolo[2,3-*d*]pyrimidin-4(5*H*)-one (**63**) possessed IC_{50} values of 130 and 110 μM, respectively (allopurinol had IC_{50} = 5.9 μM)[60].



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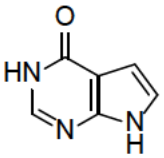

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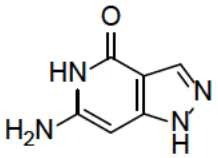
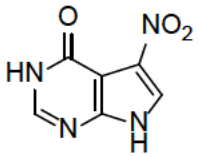


64

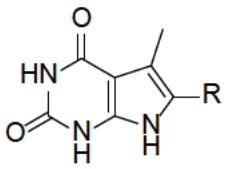
Addition of a *p*-chlorophenyl substituent on the nitrogen at position 1 of structure **62** slightly increased activity (IC_{50} = 108 μM), while addition of an amino group at position 6 maintained the activity of the parent compound (IC_{50} > 120 μM). Interestingly, if the pyridine nitrogen was changed to position 5, the resulting compound **64** showed better activity (IC_{50} = 84 μM).

Addition of a methyl group at position 6 increased the activity of **63** by almost 3 times, but the best result was obtained when a nitro group was placed at position 5, giving rise to compound **65** (IC_{50} = 0.40 μM) with better activity than **63**.



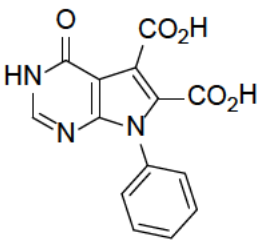
65

A series of 5-methyl-6-substituted-pyrrolo[2,3-*d*]pyrimidin-2,4-diones (**66**) was reported to be weak XO inhibitors[108]. With a phenyl group as a substituent (R = Ph), the inhibitory activity was found to be weaker than that of allopurinol. Methyl, benzyl, ethyl, isobutyl and *p*-hydroxyphenyl groups at the same position resulted in even less active XO inhibitors.



66

The introduction of carboxylic groups at positions 5 and 6 and a phenyl substituent on the nitrogen at position 7 of the pyrrolo[2,3-*d*]pyrimidin-4-one scaffold produced a very potent inhibitor of XO 7-phenyl-4-oxo-3*H*-pyrrolo[2,3-*d*]pyrimidine-5,6-dicarboxylic acid (**67**) possessing an IC_{50} value of 7.4 nM[109]. Very strong activity was also reported for 4-amino-7-phenyl-3*H*-pyrrolo[2,3-*d*]pyrimidine-5,6-dicarboxylic acid (**68**) (IC_{50} = 47 nM) and 5-formyl-6-methyl-7-phenyl-pyrrolo[2,3-*d*]pyrimidin-4-one (**69**) (IC_{50} = 69 nM).



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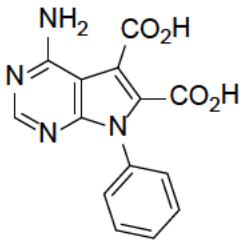

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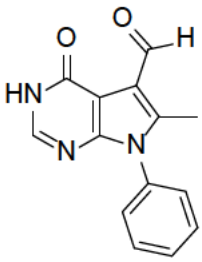
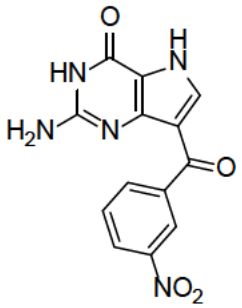


69

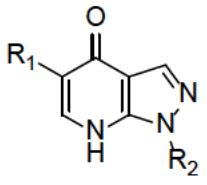
Inhibitory activity in the nanomolar range was also reported for a library of 9-benzoyl-9-deazaguanines by Rodrigues et al. in 2016[110]. The most active compound had a nitro group at the *meta*-position of the phenyl ring (**70**). The other analogues in the library had either none or 1, 2 or three halogen substituents on the phenyl ring, and they all showed lower activity compared to the nitro-substituted compound. It was also reported that the lead compound 2-amino-3,5-dihydro-7-(3-nitrobenzoyl)pyrrolo[3,2-*d*]pyrimidin-4-one (**70**) (K_i = 55.1 nM compared to K_i = 1.55 μM for allopurinol) exhibited good selectivity towards

XO, with very weak activity towards purine nucleoside phosphorylase (PNP). This specificity is important in studies targeting PNP as the enzyme is often used in combination with XO. In the PNP assay, xanthine oxidase is used to convert the product obtained from the reaction catalysed by PNP into uric acid, which can be detected spectrophotometrically. Any non-specific inhibitor would interact with both PNP and XO, giving a false positive results when the target enzyme is PNP.



70

Derivatives of the allopurinol analogue 4-oxo-pyrazolo[3,4-*b*]pyridine (**71**) showed inhibitory activity worse than allopurinol[92]. These analogues differ from allopurinol in that they do not have the nitrogen at position 5, but have either a carboxylic acid, ethoxycarbonyl or methyl group instead, and various substituents on the nitrogen at position 1. The most active compound was 1-(4-chlorophenyl)-4-oxo-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (**72**), with an IC_{50} of 7.3 μM (with allopurinol having an IC_{50} of 1.3 μM in the assay). A loss of activity was experienced when the aryl substituent at position 1 was replaced by a methyl group, as well as when the carboxylic acid group was replaced by either a methyl or an ethoxycarbonyl group.



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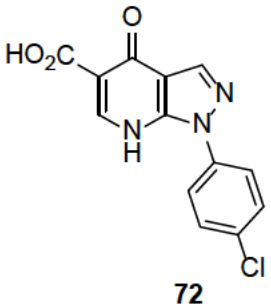
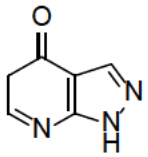
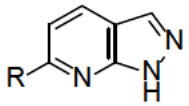


72

Vartanyan et al. reported that 4-hydroxypyrazolo[3,4-*b*]pyridine (**73**) did not show any activity against XO, although it was similar to allopurinol[93]. Other pyrazolo[3,4-*b*]pyridine analogues (**74**) were also found to be less active than allopurinol.





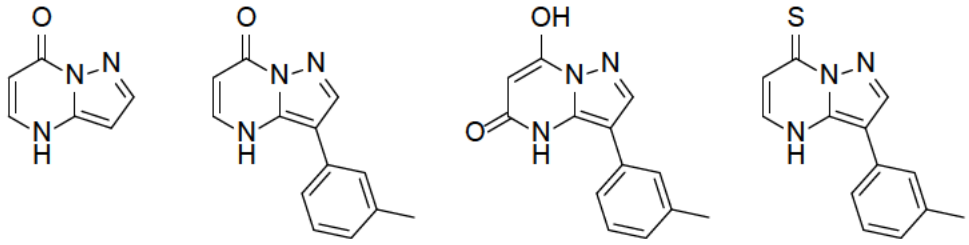
R = OH, SH, Cl, SCH_3

73 **74**

The most active compound among the series was 6-thiolpyrazolo[3,4-*b*]pyridine and, interestingly, its methylthio derivative showed a complete lack of activity against XO.

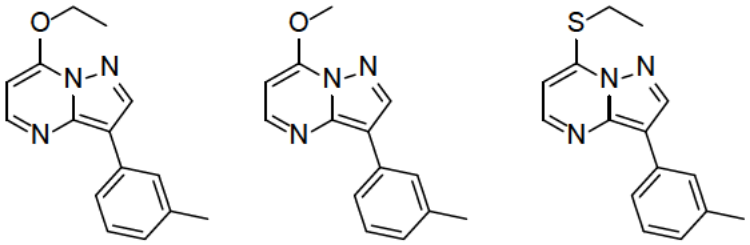
2.2.11 Azolo[*a*]pyrimidines

Pyrazolo[1,5-*a*]pyrimidines have one nitrogen atom in common between the six and five membered rings. The simplest compound of this family, pyrazolo[1,5-*a*]pyrimidin-7-one (**75**) (IC_{50} = 11 µM), is an isostere of allopurinol which was measured to be approximately 2 times less potent than allopurinol (IC_{50} = 5.9 µM)[60]. Activity improved significantly when an aryl group was added at either position 3 or 5, with the most active compound being 3-(3-methylphenyl)-pyrazolo[1,5-*a*]pyrimidin-7-one (**76**) (IC_{50} = 0.06 µM). Activity increased even further with the oxypurinol isostere 3-(3-methylphenyl)-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**77**) (IC_{50} = 0.025 µM).



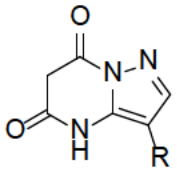
75 **76** **77** **78**

Very good activity was also observed after replacing the carbonyl function with a thiocarbonyl group as shown by compound **78** (IC_{50} = 0.40 µM). The carbonyl and thiocarbonyl groups seem to have an important role in the enzyme-inhibitor interaction as a dramatic loss of activity was observed when those functions were replaced by either ether (**79,** IC_{50} > 140 µM and **80**, IC_{50} = 150 µM) or thioether (**81**) (IC_{50} = 110 µM) functional groups.



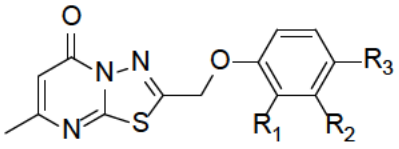
79 **80** **81**

The required presence of an aryl group at position 9 of a purine scaffold in order to retain good inhibitory activity was also confirmed by Springer et al. in 1975 in their work on 5,7-dihydroxypyrazolo[1,5-*a*]pyrimidines (**82**)[111]. Position 3 was thought to be spatially equivalent to position 9 of the purine derivatives studied by Baker and Wood[62, 67].



82

A series of 20 derivatives of 7-methyl-2-(phenoxymethyl)-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-one (**83**) with various substituents on the phenyl ring were synthesised and tested against XO obtained from three different sources (rat liver, bovine milk and microbial source)[112]. A group of four compounds showed better inhibitory activity than allopurinol as well as consistent activity against XO from the three sources (Table 1).



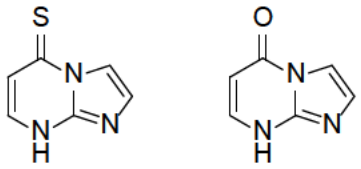
83

Table 1. Inhibitory activity of selected 7-methyl-2-(phenoxymethyl)-5*H*-[1,3,4]thiadiazolo [3,2-*a*]pyrimidin-5-ones against different XO forms.

Compound	Rat liver XO IC_{50}	Bovine milk XO IC_{50}	Microbial XO IC_{50}
R_1=R_2=H, R_3=OCH_3	289 nM	269 nM	269 nM
R_1=R_2=H, R_3=Cl	449 nM	461 nM	423 nM
R_1=CH_3, R_2=R_3=H	362 nM	326 nM	346 nM
R_1=H, R_2=CH_3, R_3=Cl	623 nM	634 nM	596 nM
allopurinol	753 nM	730 nM	730 nM

Good response was also obtained from imidazo[1,2-*a*]pyrimidine-5-thione (**84**) (IC_{50} = 5.4 μM), which was shown to be more potent than the corresponding

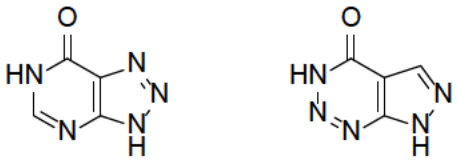
carbonyl form (**85**) (IC_{50} = 120 µM)[60], but only of comparable activity to allopurinol (IC_{50} = 5.9 µM).



84 **85**

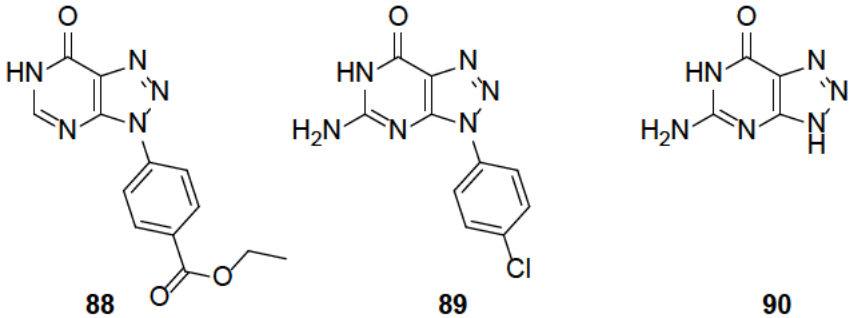
2.2.12 1,2,3-Triazolo[4,5-*d*]pyrimidines and pyrazolo[3,4-*d*][1,2,3]triazines

Addition of a nitrogen atom to either the six-membered ring or the five-membered ring of allopurinol produced 3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**86**) (IC_{50} = 8.2 µM) and 4*H*-pyrazolo[3,4-*d*]1,2,3-triazin-4(3*H*,7*H*)-one (**87**) (IC_{50} = 7.4 µM), which inhibitory activity comparable to that of allopurinol (IC_{50} = 5.9 µM)[60].



86 **87**

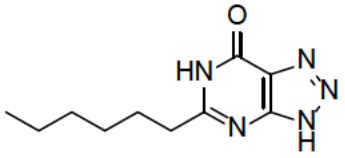
The *p*-ethoxycarbonyl phenyl (**88**) (IC_{50} = 3 µM) substituent on the 1,2,3-triazolo[4,5-*d*]pyrimidin-7-one core (**86**) increased activity by almost 3 times. Substitution with a chlorine atom on the phenyl group (**89**) (IC_{50} = 0.25 µM) instead of the ethoxycarbonyl, and an amino group introduced at position 5 led to an increase in activity of more than 30 times. The requirement of the aromatic ring to retain good activity was proven by the reduced activity exhibited by 5-amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**90**) (IC_{50} = 7.5 µM).



88 **89** **90**

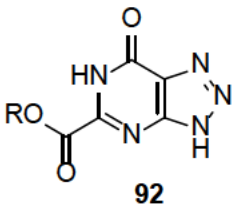
The effect on the inhibitory activity of purine derivatives carrying a lipophilic group at position 2 was analysed by Biagi et al. by synthesizing and testing 2-alkyl-8-azahypoxanthines[113]. The alkyl group at position 2 was a linear carbon chain up to 8 carbons long. Only 2-*n*-hexyl-8-azahypoxanthine (**91**) showed a similar level of activity as allopurinol. The heptyl derivative demonstrated to be half as active

as allopurinol and the unsubstituted 8-azahypoxanthine was shown to be less active than allopurinol[60, 113].



91

Following the study on 2-alkyl-8-azahypoxanthine, Biagi et al. investigated the effect of having an ester group at position 2 of the same scaffold (**92**) on inhibition activity[114]. Derivatives with linear esters were found to be more active than derivatives with ramified esters, especially if the ramification was on the first atom connected to the heterocyclic ring. *n*-Pentyl and *n*-hexyl derivatives were the most potent inhibitors, showing IC_{50} values of 0.25 μM and 0.24 μM, respectively (with allopurinol having IC_{50} = 1.88 μM). Introduction of a methylene at position 2 as link between 8-azahypoxanthine and a phenyl ring was found to decrease the potency with respect to the compound with the phenyl group attached directly to position 2 of the heterocycle. These observations made the authors conclude that the active site of XO had a well-defined lipophilic region and that there could be a positive charge present, as the ester derivatives showed better activity than the *n*-alkyl derivatives.



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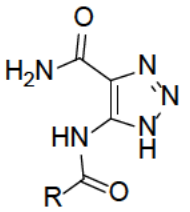
One year later the same research group tested for XO inhibition the intermediates isolated during the optimization of the synthetic method to obtain 8-azahypoxanthine derivatives differently substituted at position 2[115]. The majority of the isolated intermediates were triazoles with a carboxy amido group at position 4 and either an acylamino (**93**) or an alkyl oxalylamino (**94**) group at position 5. In general, these compounds showed better activity than the 8-azahypoxanthine derivatives. The authors arguing that this was because these molecules were characterized by higher conformational freedom of their *n*-alkyl chains. It was thought that the orientation of the triazolo ring mimicked the orientation of the same ring in 8-azahypoxanthines inside the active site of the enzyme.



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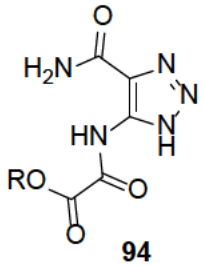

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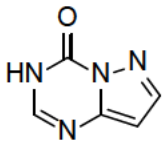
2.2.13 Pyrazolo[1,5-*a*]triazines

Reorganization of nitrogen atoms within the structure of allopurinol to give pyrazolo[1,5-*a*]triazin-4(3*H*)-one (**95**) (IC_{50} = 84 μM) reduced activity by approximately 14 times compared to allopurinol (IC_{50} = 5.9 μM)[60]. However, addition of a phenyl group at position 7 resulted instead in a compound (**96**) (IC_{50} = 0.047 μM) more than 1700 times more potent than the parent molecule.



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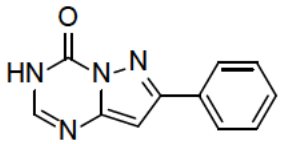
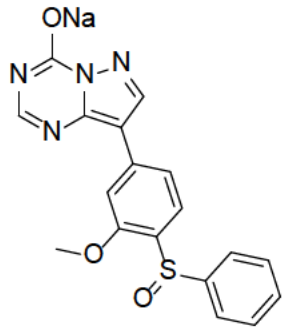


96

Thiomethyl, methyl and thiocarbonyl groups at position 2 of either compound **95** or **96** decreased the activity of the derivatives, as well as ethyl, phenyl and carbonyl groups at position 2 of compound **96** lowered the activity of the parent compound.

The two enantiomers (S-(-) and R (+)) of sodium 8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo[1,5-*a*]-1,3,5-triazine-4-olate monohydrate (BOF-4272) (**97**) demonstrated mixed type inhibitory activity towards XO, with K_i = 1.2 nM and K_i = 300 nM, respectively, and a longer period of inhibition than allopurinol in *in vivo* studies on rat liver XDH[116]. The S-(-)-enantiomer showed higher plasma concentration in rat after either oral or intravenous administration of the racemic drug compared to the R-(+)-enantiomer[117].



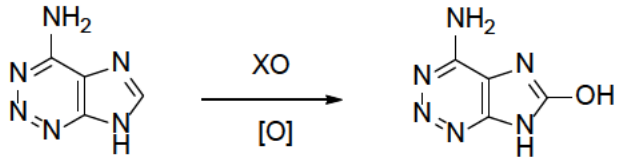
BOF-4272

97

BOF-4272 has also been evaluated for its potential for the treatment of various diseases where XO was thought to play an important role. It was found that BOF-4272 helped to restore blood pressure to normal levels in spontaneously hypertensive rats by inhibiting XO and, therefore, the production of radicals in the proximity of microvascular endothelium[118]. Inhibition of XO by BOF-4272 was shown to be also an effective way of reducing the transformation of 2-nitrofluorene into its carcinogenic amino derivative, reducing the incidence of cancer in rat skin[119]. Oral administration of BOF-4272 to a small group of healthy male volunteers revealed that the drug was well tolerated while reducing serum uric acid concentration[120].

2.2.14 Imidazo[4,5-*d*][1,2,3]triazines

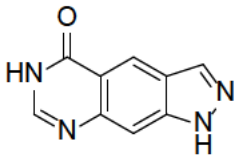
Imidazo[4,5-d][1,2,3]triazin-4-amine (**98**), also called 2-azaadenine, was shown to be a substrate and competitive inhibitor of XO[121]. It was converted by the enzyme to 8-hydroxy-2-azaadenine (**99**), which was also an inhibitor of the enzyme. When 2-azaadenine was administered together with hypoxanthine, the enzyme showed some selectivity as it converted 2-azaadenine approximately 5 times faster than the natural substrate. The observed enzyme inhibition was therefore due to the unavailability of the enzyme to oxidise hypoxanthine. When 2-azaadenine was instead administered together with xanthine, the enzyme did not show any selectivity and oxidised both substrates at the same rate.



98 **99**

2.2.15 Pyrazoloquinazolines

By inserting a phenyl ring between the two rings in allopurinol and isoallopurinol, Foster and Leonard studied the inhibitory activity of the newly synthesised pyrazolo[4,3-*g*]quinazolin-5-one (**100**) (*lin*-benzoallopurinol) and pyrazolo[3,4-*f*]quinazolin-9-one (**101**) (*prox*-benzoisoallopurinol)[122]. Both compounds were oxidised by XO to the oxypurinol analogues, which were shown to act as substrates of the enzyme.



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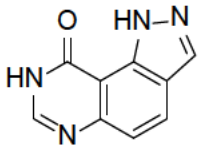
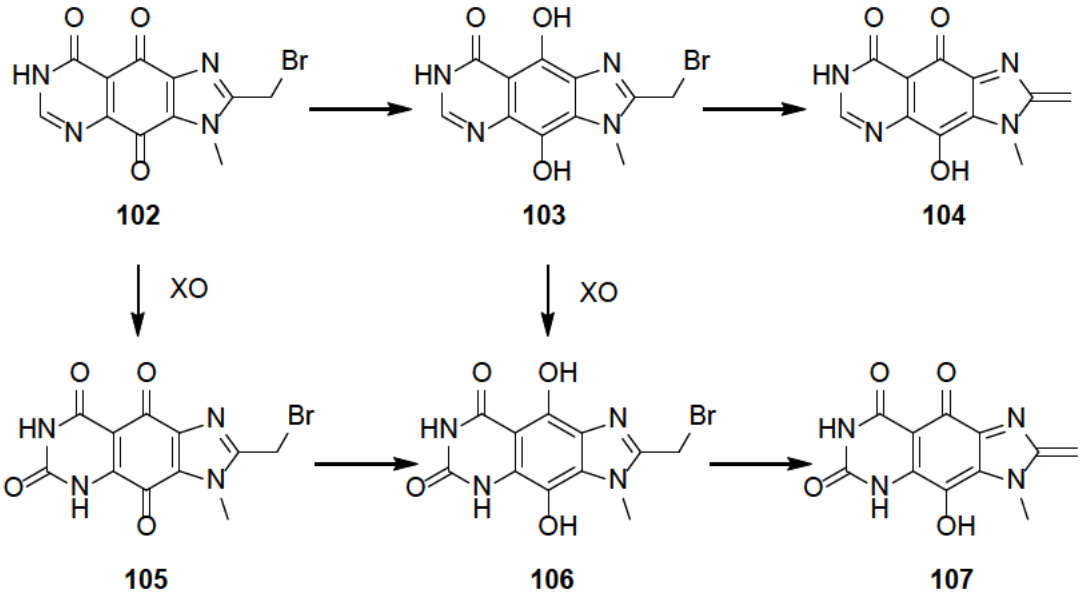


101

2.2.16 Imidazo[4,5-*g*]quinazolines

Imidazo[4,5-*g*]quinazolines were reported to act as irreversible inhibitors of XO when they were present as products of the reduction and subsequent 1,6-elimination of HBr, as shown in Fig 14[123, 124]:



102 **103** **104**

105 **106** **107**

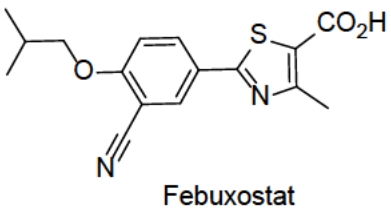
Fig 14: Oxidation of imidazo[4,5-*g*]quinazoline-4,8,9-trione (**102**) and formation of product (**107**) that could irreversibly inhibit XO

The side chain of a basic amino acid residue inside the active site of the enzyme could make a nucleophilic attack on the exocyclic double bond and place the quinazoline in a position that prevents any other molecule from interacting with the Mo centre. The 2-(bromomethyl)-3-methylimidazo[4,5-*g*] quinazoline-4,8,9(3*H*,7*H*)-trione (**102**), as well as its reduced form (**103**), behaved as

substrates for the enzyme and underwent oxidation at position 6. The oxidised forms (**105** and **106**) in the active site could undergo 1,6-elimination and form a species (**107**) susceptible of nucleophilic attack from a basic amino acid residue in the active site of XO. Enzyme studies supported this mechanism of action and the authors concluded that this alkylation/inhibition mechanism could be extended to other enzymes as well.

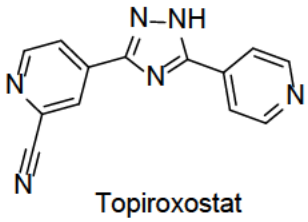
2.3 Non-purine like inhibitors of xanthine oxidase

The possible adverse effects that may arise following the administration of allopurinol to patients suffering from hyperuricemia has driven research to find alternatives to this drug. Several research groups have focused their study on novel, non-purine like XO inhibitors with the hope that such inhibitors would not interfere with other aspects of purine metabolism. A potent inhibitor of XO, febuxostat (**108**) was the first approved in Europe in 2008, then in the USA in 2009, and in other countries afterwards, for the treatment of hyperuricemic patients after almost 50 years of exclusive use of allopurinol[125]. More recently in 2013 the Pharmaceutical and Food Safety Bureau in Japan approved a new drug called topiroxostat (**109**)[126] for the treatment of hyperuricemia and gout.



Febuxostat

108



Topiroxostat

109

Febuxostat, also known as TEI-6720 during its development, binds very tightly to both forms of XOR, oxidised (Mo VI) and reduced (Mo IV), with K_i of 1.2 x 10^{-10} M and K_i' of 9 x 10^{-10} M, respectively, making it more efficient than oxypurinol, which binds only to the reduced form of the enzyme. Strong binding was demonstrated to be due to hydrogen bonds and hydrophobic interactions, but no evidence of direct bonding with the Mo centre was found[127]. Fig 15 highlights the hydrogen bonding interactions between febuxostat and the active site amino acids Asn768, Arg880 and Thr1010. Fig 16 highlights the hydrophobic interactions between febuxostat and the active site amino acids Leu648, Phe649, Leu873, Phe914, Phe1009, Val1011, Leu1014, Ala1078 and Ala1079.

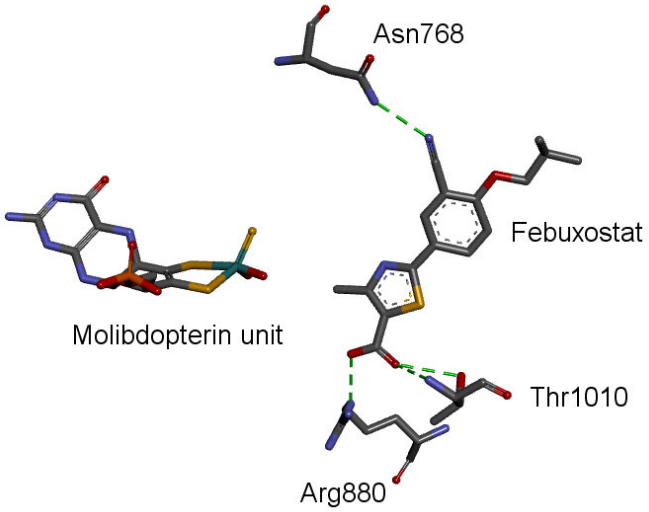


Fig 15: Hydrogen bonding interactions between febuxostat and the active site amino acids

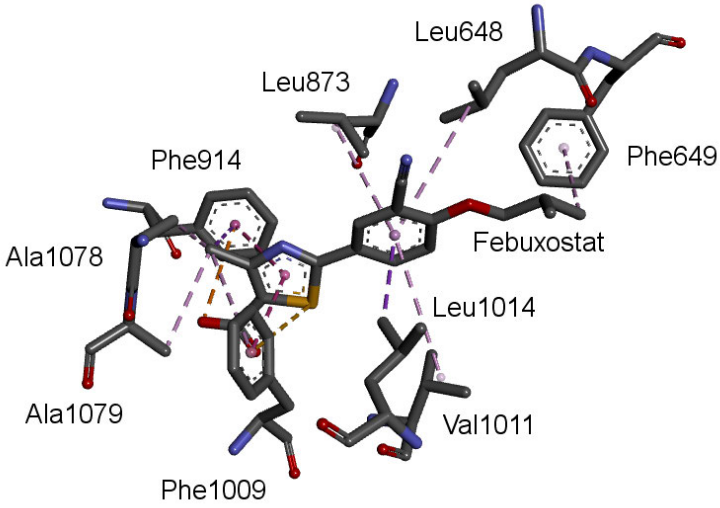
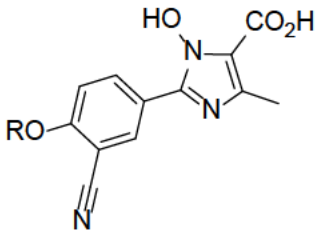


Fig 16: Hydrophobic interactions between febuxostat and the active site amino acids

Febuxostat seems to act as a “plug” in the channel leading to the Mo centre, blocking the substrate from interacting with it. The carboxylic acid group was also identified as the group most tightly bound to the active site of the enzyme. Most recently it was also found that carboxamide derivatives of febuxostat are more potent inhibitors of XO than febuxostat itself[128].

Imidazole derivatives maintaining similarities with febuxostat were synthesised by Chen et al. and reported in 2015[129]. Several compounds for the two series of analogues of 1-hydroxy and 1-methoxy substituted 2-aryl-4-methyl-1*H*-imidazole-5-carboxylic acids (**110** and **111**) were tested *in vitro* as XO inhibitors. The derivatives with a free hydroxyl group at position 1 of the imidazole ring demonstrated better activity than derivatives with a methoxy group at the same

position. Further studies on the representative compound of the most active series (**112**) (IC_{50} = 6 nM compared to febuxostat with IC_{50} = 10 nM) revealed a mixed type mechanism of inhibition.



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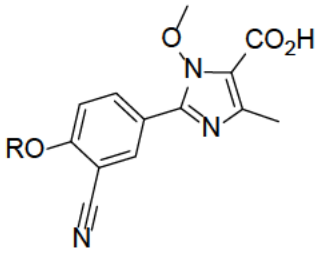

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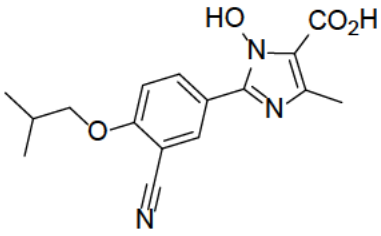


112

Molecular docking studies showed that the position of the inhibitor inside the enzyme was similar to that of febuxostat, with the carboxylate group interacting with Arg 880 in the region close to the Mo centre in the active site (Fig 17A). By contrast, docking of the 1-methoxy derivatives predicted an orientation of the molecules with the alkoxy group oriented towards the Mo centre (Fig 17B), which could explain the limited interactions with active site residues and, therefore, poor inhibitory activity. The authors suggested that the activity of febuxostat itself could be improved with the introduction of a hydroxyl group on the thiazole ring.

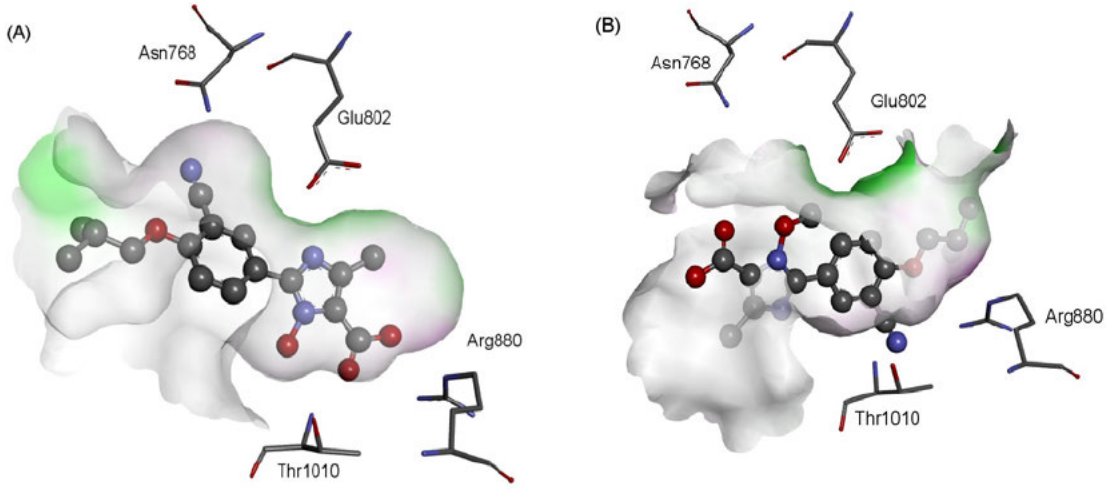
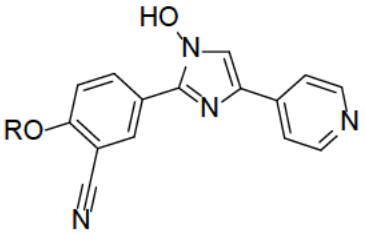


Fig 17. Docking studies showing the position of (A) 2-(3-cyano-4-*iso*butyloxyphenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxylic acid and (B) 2-(3-cyano-4-*iso*butyloxyphenyl)-1-methoxy-4-methyl-1*H*-imidazole-5-carboxylic acid inside the active site.

In 2018 the same group[130] published a work that continued the investigation on imidazole derivatives. They synthesised and tested a library of both 1-hydroxy-2-phenyl-4-pyridyl-1*H*-imidazole derivatives (**113**) and 1-hydroxy-2-phenyl-3-pyridyl-1*H*-imidazole derivatives (**114**). They aimed to retain the favourable interactions of the enzyme with the 1-hydroxy-2-(4-alkoxy-3-cyanophenyl) moiety of compound **110** and the pyridine moiety from topiroxostat **109**. Among the two series of compounds the derivatives with a 4-pyridyl moiety demonstrated better inhibitory activity than their 3-pyridyl analogues. The most active compound (IC_{50} = 0.64 μM) had also the longest ether tail (R = *iso*pentyl) among the synthesised structures. However all the compounds were less active than topiroxostat **109** (IC_{50} = 0.0048 μM).



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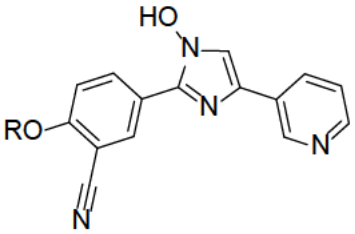
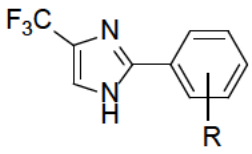


114

Some simple imidazole analogues were considered to be good inhibitors of XO by Baldwin and his co-workers back in 1975[131]. They explored a library of 4-trifluoromethyl-2-arylimidazoles (**115**). It was reported that an aryl or heteroaryl substituent at position 2 was needed in order to retain inhibitory activity. It was also found that the cyclic amidine structure of imidazole needed to remain unsubstituted to allow good activity. While most of the substituents on the phenyl ring did not have a significant effect on the activity of the parent compound, a cyano group in the *para*-position, or a chlorine group in the *ortho*-position of the phenyl ring resulted in an inactive compound. The inhibitory activity decreased when the nitrogen of the pyridine ring changed from position 4 to position 3, and completely disappeared when the nitrogen was in position 2. The authors chose 2-(4-pyridyl)-4-trifluoromethylimidazole (**116**), with an IC_{50} value of 6 μM, as the lead compound for further studies. When the trifluoromethyl group of this compound was replaced by a carboxylic acid group a 5-fold loss of activity was observed.



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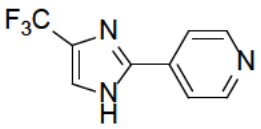
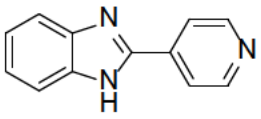


116

The same authors subsequently investigated the effect on the inhibitory activity of various substituents at positions 4 and 5 of the imidazole ring (including benzimidazoles), where position 2 was occupied by either a 3- or 4-pyridyl moiety[132]. Cyano and nitro groups at position 4 and/or position 5 gave compounds with comparable activity to the one with a trifluoromethyl group. Hydrogen, methyl or carbomethoxy substituents at the same position instead negatively affected the activity of the inhibitor. Bulky substituents at positions 4 and 5 seemed also to decrease the activity of the resulting compound, while double substitution with small substituents increased activity. More acidic amidine hydrogen was preferred as evidenced by the IC_{50} values obtained for chloro substituted 2-(4-pyridyl)benzimidazoles. Compound **117** was demonstrated to be inactive, compound **118** exhibited only 11% inhibition at 20 μM, and compound **119** had an IC_{50} value of 15 μM compared to allopurinol with an IC_{50} of 3 μM. The most potent compound obtained was 4,5-dicyano-2-(4-pyridyl)imidazole (**120**), with an IC_{50} value 150 times (IC_{50} = 20 nM) better than that of allopurinol.



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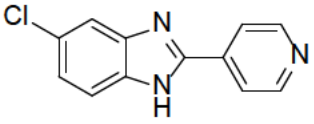

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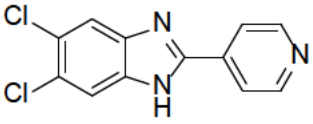

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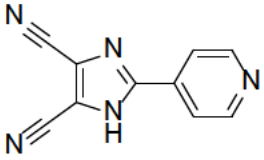
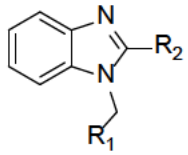


120

Nile et al. investigated 2-aryl-1-arylmethyl-1*H*-benzimidazoles (**121**)[133]. All reported analogues exhibited activity comparable to allopurinol. They possessed IC_{50} values ranging from 4.3 to 9.7 μM (with allopurinol having an IC_{50} of 5.5 μM). The preferred substitution point in the phenyl ring was the *ortho*-position for chlorine (**122**) (IC_{50} = 4.4 μM), as the IC_{50} value was almost half of the value obtained with a chlorine in the *meta*-position. On the other hand, substitution in the *meta*-position was preferred for methyl groups (**123**) (IC_{50} = 4.3 μM). Phenols

with hydroxyls in the *para*-position (**124**) (IC_{50} = 4.5 µM) were instead the most effective compared to the *ortho* and *meta*-substituted analogues, and conferred better activity than allopurinol.



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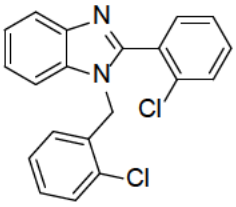

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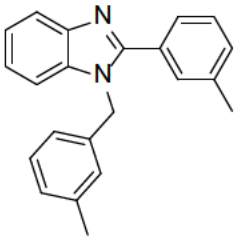

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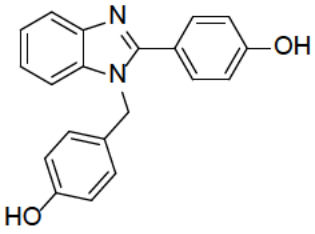
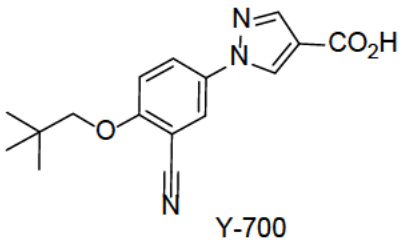


124

The structure-activity relationship study performed for a series of 1-aryl pyrazoles highlighted three essential requirements needed to maintain inhibitory activity against XO: a cyano group at position 3 on the phenyl ring, a bulky group at position 4 on the phenyl ring and a carboxylic acid group at position 4 of the pyrazole ring were essential. Y-700 (**125**) was identified as the compound with the best IC_{50} (5.8 nM compared to 260 nM for allopurinol) and good *in vivo* activity in a rat model of hyperuricemia[134].



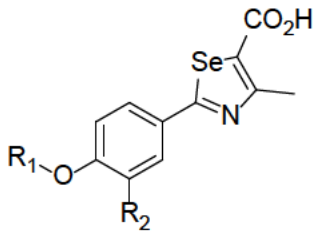
Y-700

125

The above 1-(3-cyano-4-neopentyloxyphenyl)-1*H*-pyrazole-4-carboxylic acid (**125**) exhibited inhibitory activity of a mixed type and its excretion pathway through the liver made the molecule suitable also for patients with renal disorder[135, 136]. The inhibitory activity on XO by Y-700 correlated to the ability of this molecule to prevent colon cancer in mice[137]. The mechanism of action for this anticancer activity remains unknown, but it was found that Y-700 possessed no radical scavenging effect.

The need to retain the cyano group on the phenyl ring was confirmed by Guan et al. in their evaluation of 2-aryl-4-methyl-1,3-selenazole-5-carboxylic acids (**126**) as XO inhibitors[138]. All the compounds obtained with different substituents in the R_1 position and a nitro group as R_2 consistently exhibited lower activity than the corresponding structures with a cyano group in place of the nitro group. The

derivative with an allyl group as R_1 (**127**) showed better inhibitory activity than febuxostat (IC_{50} = 5.5 nM while febuxostat had an IC_{50} value of 18.6 nM).



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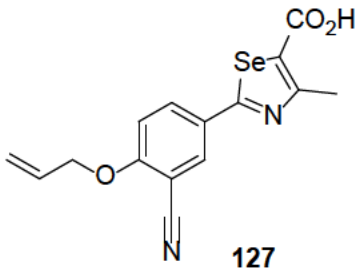
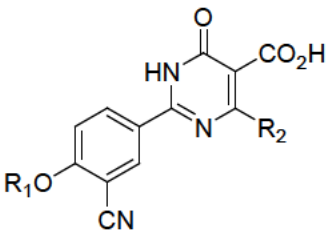


127

Enlarging the five membered heteroaromatic ring in febuxostat to a pyrimidine ring was done by Mao et al.[139] in their study of the inhibitory activity of 1,6-dihydropyrimidine derivatives (**128** and **129**). The authors found that having a methyl group in position 4 of the pyrimidinone ring (R_2) reduced in vitro potency and derivatives of type **129** were more potent than those of type **128**. The best inhibitor was found to be 419 times better than allopurinol and had a similar potency to febuxostat. The same authors subsequently [140] investigated the inhibitory activity of a series of compounds of type **130**. The new structures were designed based on two hypotheses: 1) a pyrimidinone ring could mimic the pyrimidinone ring in allopurinol without the fused pyrazole ring; 2) the cyano group in febuxostat could be substituted by a tetrazole ring. All the compounds tested were more potent in vitro than allopurinol and had comparable activity to febuxostat. The most potent compound (**131**) demonstrated to be 263 times more active than allopurinol. Further studies published the following year by the same authors[141] explored the replacement of the pyrimidinone ring with 1,2,4-oxadiazole (**132**). The inhibitory activity obtained by the new compounds was better than allopurinol, but less potent than the previously published compounds. The most potent compound (**133**) was 63 times more potent than allopurinol. In 2021 the same authors reported[142] the inhibitory activity of four more pyrimidinone derivatives (**134**, **135**, **136** and **137**). All the compounds demonstrated better activity than allopurinol. Based on the above findings it could be concluded that the beta imino acid functional group has the best interactions with the enzyme active site.



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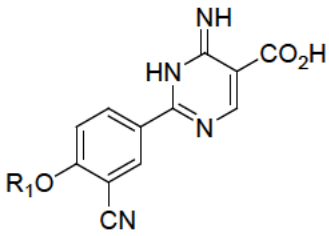

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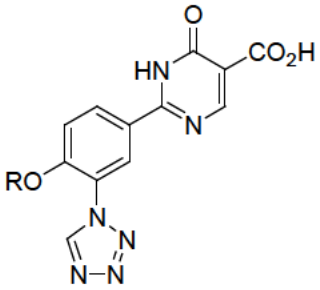

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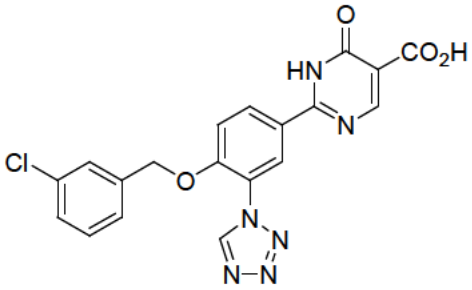

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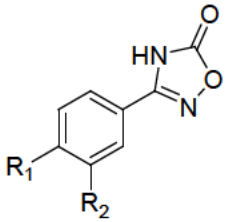

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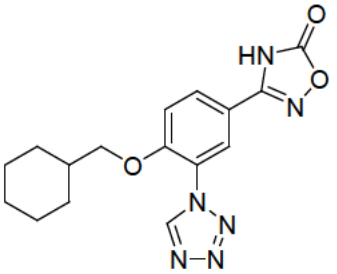

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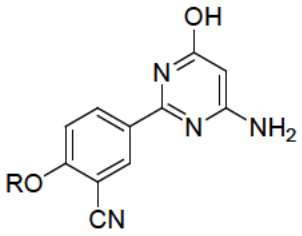

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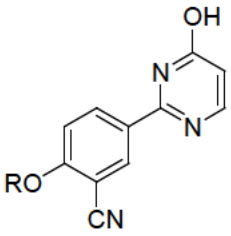

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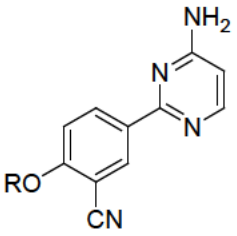

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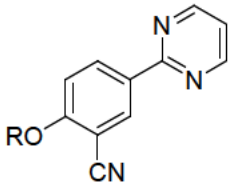
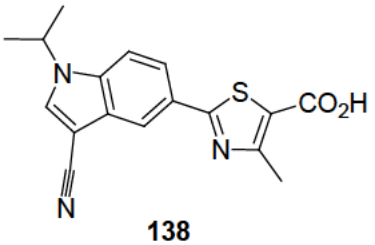


137

The thiazolo ring substituted at position 2 by an indol-5-yl group was recently explored by Song et al.[143]. The structures synthesised had the same carboxylic acid functional group at position 5 of the thiazole as in febuxostat, but had an indole ring instead of a phenyl ring at position 2 of febuxostat. All the compounds were measured to have IC_{50} values ranging from 3.0 to 12.3 nM, comparable to that of febuxostat (3.1 – 5.5 nM). Molecular docking studies supported by *in vitro* and *in vivo* studies were used to design very active 2-(3-cyano-1-isopropylindol-5-yl)-4-methylthiazole-5-carboxylic acid (**138**) (IC_{50} = 3.5 nM) and 2-(3-cyano-1-

(1-hydroxyprop-2-yl)indol-5-yl)-4-methylthiazole-5-carboxylic acid (**139**) (IC_{50} = 3.0 nM).



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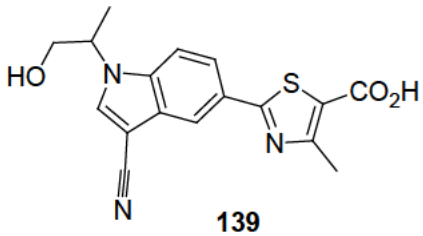

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Fig 18 illustrates the predicted fit of this type of molecules (**138**) within active site, such that the carboxylic acid group of **138** is oriented in the same manner as febuxostat (shown in yellow).

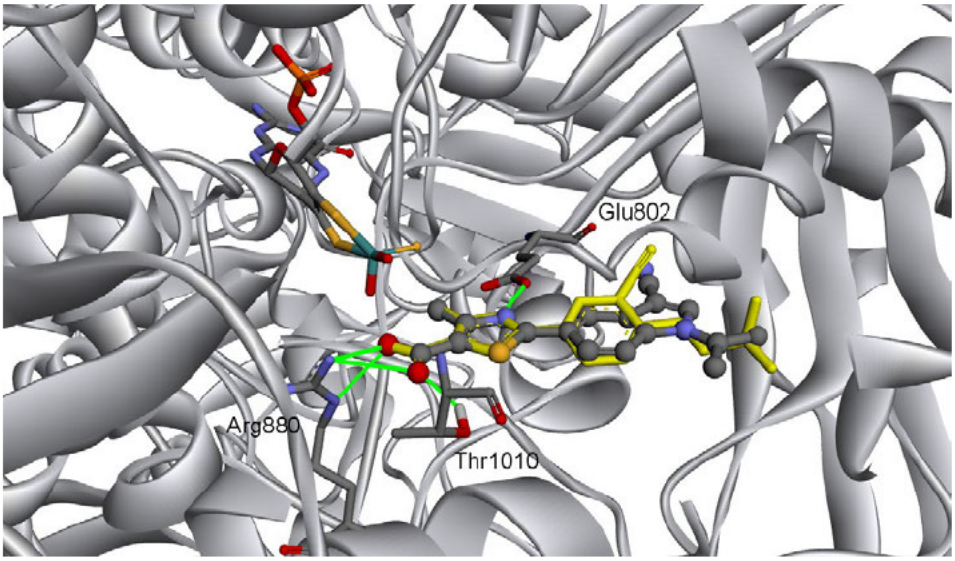
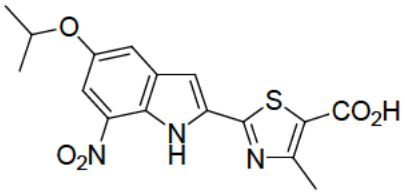


Fig 18: The orientation of 2-(3-cyano-1-isopropylindol-5-yl)-4-methylthiazole-5-carboxylic acid (**138**) inside the active site is similar to febuxostat (in yellow)

A group of four structures selected from the library of compounds on the basis of their high activity was also tested *in vivo* for the reduction of uric acid in Sprague-Dawley rats at a dose of 10 mg/kg. Compound **138** was chosen by the authors for further studies as it gave a 60% uric acid inhibition after 1 h in *in vivo* tests, although compound **139** showed better *in vitro* activity but only a 8.5% inhibition *in vivo*.

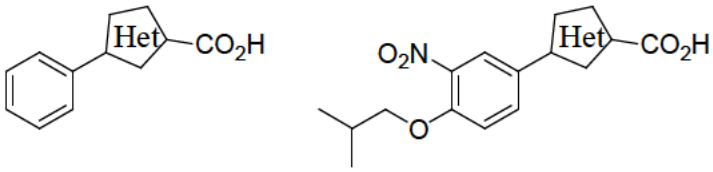
More recently, Song et al. reported the synthesis and the screening of a library 2-(indol-2-yl)thiazoles[144]. After lead optimization, the authors identified 2-(7-nitro-5-*iso*propoxy-indol-2-yl)-4-methylthiazole-5-carboxylic acid (**140**) (IC_{50} = 5.1 nM compared to febuxostat with IC_{50} = 3.1 - 5.5 nM) as a strong inhibitor of XO *in vitro* and a good inhibitor of uric acid formation in an oxonate-induced

hyperuricemic rat model. The compound was reported to inhibit uric acid production by 43%.



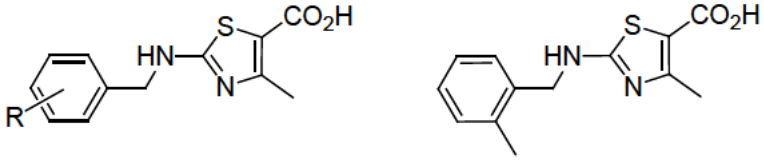
140

Xu et Al.[145] synthesised and tested in vitro a series of febuxostat analogues in an attempt to improve the inhibitory activity of febuxostat itself. The thiazole ring of the approved drug was substituted with various 5-membered heterocycle rings (**141**): 1,3-oxazole, 1,2-oxazole, pyrazole and thiazole with the nitrogen and sulpha atoms in various positions. The analogues had an unsubstitiuted phenyl ring and they all demonstrated to be less potent than febuxostat. The cyano group on the structure of the approved drug was also substituted with a nitro group (**142**), but resulted in analogues with decreased in vitro potency compared to febuxostat.



141 **142**

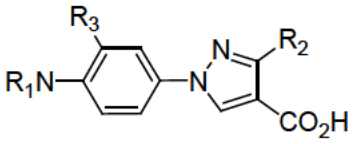
An additional linker between the phenyl ring and the thiazole ring of febuxostat capable of producing more hydrogen bonding between inhibitor and enzyme was explored by Rhamat et al.[146] in their work on 2-(benzylamino)-4-methyl-1,3-thiazole-5-carboxylic acid derivatives (**143**).



143 **144**

The most potent analogue identified in the series of 16 tested compounds was 2-(2-methylbenzylamino)-4-methyl-1,3-thiazole-5-carboxylic acid (**144**). Nevertheless, even this compound (**144**) was significantly less potent than febuxostat (IC_{50} = 0.03 μM) and possessed an IC_{50} value of 3.6 μM, which was two orders higher than that of the reference drug.

Structures mimicking both febuxostat and Y-700 were investigated by Li et Al.[147] A series of 1-phenyl-pyrazole-4-carboxylic acid derivatives were synthesised and tested in vitro. The presence of either a methyl or a trifluoromethyl group in R_2 was detrimental for the inhibitory activity. Better results were obtained when R_3 was a cyano instead of a nitro group, confirming the finding of other researcher on similar structures. Analogues of type **145** performed better than those of type **146**. Interestingly, among the cyclic amines (cyclopentylamine, cyclohexylamine and morpholine) selected as substituents in position 4' of the phenyl ring, morpholine gave the worst result. Compounds of type **145** demonstrated inhibitory activity comparable to that of febuxostat (IC_{50} = 5.4 μM). The most active compound (**147**) had an IC_{50} = 4.2 μM.



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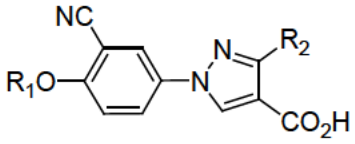

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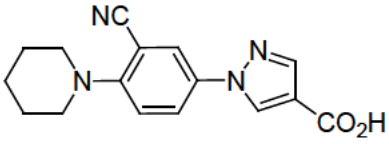
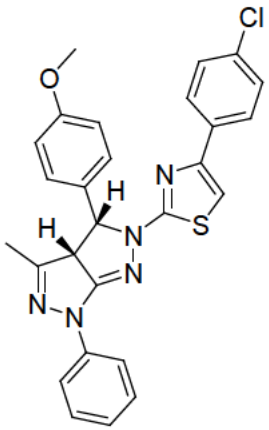


147

A thiazole ring linked to two fused pyrazoles was the central skeleton of a library of 26 derivatives synthesised and tested *in vitro* by Beedkar et al.[148]. Compounds **148** (IC_{50} = 9 μM), **149** (IC_{50} = 7.1 μM) and **150** (IC_{50} = 6.5 μM) were reported to have IC_{50} values comparable to that of allopurinol.



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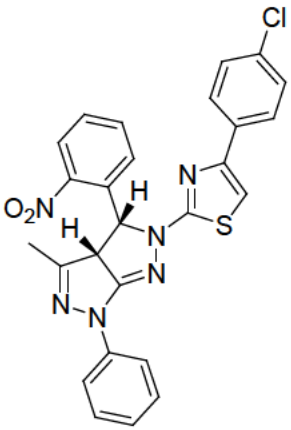

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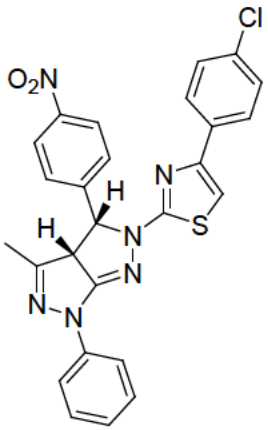
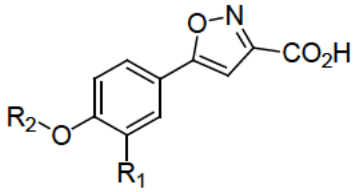


150

Enzyme kinetic studies found that these compounds act as competitive inhibitors of XO and interact with the Mo centre of the enzyme. The presence of methoxy

and nitro groups on the phenyl rings resulted in the decrease of the observed IC_{50} values. In general, it was found that substitution on the phenyl ring in either *para* or *ortho*-position with electron donating groups increased inhibitory activity, while substitution with electron withdrawing groups decreased activity. The authors suggested that the electron-rich aromatic ring favours electron transfer to Mo(VI), converting it to Mo(IV), while forming a complex with the reduced form in the same way as in the allopurinol-oxypurinol mechanism. Good interactions between the compounds and amino acid residues in the active site of the enzyme were predicted by molecular docking studies.

Isoxazole derivatives (**151**) with similar substitutions to febuxostat and Y-700 were explored by Wang et al[149]. A library of five derivatives with a nitro group at position R_1 and five derivatives with a cyano group at the same position were synthesised and tested *in vitro* against XO. The compounds with the cyano group always showed better activity than the compounds with the nitro group. Among the substituents at the R_2 position, the *iso*butyl group showed the best activity, confirming the findings of Ishibuky et al.[134]. All the compounds possessed inhibitory activity in the micromolar range and thus were less active than febuxostat. It was speculated that the position of the nitrogen in the thiazole of febuxostat and in the pyrazole in Y-700 closer to the phenyl ring contributed to their activity, as opposed to the lower activity showed by these isoxazole derivatives **151** with a nitrogen atom more distant from the phenyl ring.

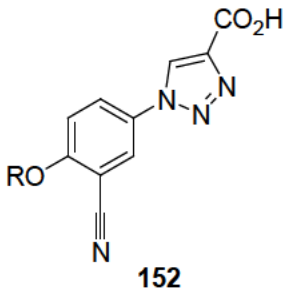


151

Exploiting the structural similarities between compound Y-700 and febuxostat, and focussing on *in vivo* activity, Sato et al. reported several studies on 3,5-disubstituted triazoles as XO inhibitors[150, 151], considering also what had earlier been done by Baldwin et al.[131, 132]. Optimization of the substituents on the pyridine rings led to the mono substituted 3-(3-cyano-4-pyridyl)-5-(4-pyridyl)-1,2,4-triazole (topiroxostat) (**109**), also known as FYX-051 during its development. Topiroxostat (**109**) is a hybrid type inhibitor that shows a behaviour typical of a competitive inhibitor at the initial interaction with the enzyme, with a K_i value of 5.3 nM[44], and turning into a tight complex with the Mo centre of the

enzyme via a covalent bond through an oxygen atom after a few minutes[152]. It is considered a slow inhibitor and its tight fit for the substrate channel avoids the presence of water in the active site of the enzyme that is responsible for the exclusion of the substrate from the Mo centre[52]. Topiroxostat (**109**) was approved for the treatment of hyperuricemia in 2013 in Japan. In January 2014 the results of a double-blind clinical trial was published, showing the effectiveness of this compound at reducing serum uric acid levels in Japanese patients with chronic kidney disease (CKD)[153].

Using febuxostat (**108**), topiroxostat (**109**) and Y700 (**125**) as lead compounds, Zhang et al. investigated series of 1,2,3-triazole derivatives, namely 1-(4-alkoxy-3-cyanophen-1-yl)-1*H*-1,2,3-triazole-4-carboxylic acids (**152**) and 2-alkoxy-5-(4(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitriles (**153**)[126, 154]. The majority of compounds showed inhibitory activities in the micromolar range and comparable to allopurinol, but never exceeded potency of the lead compounds **108**, **109** and **125**.



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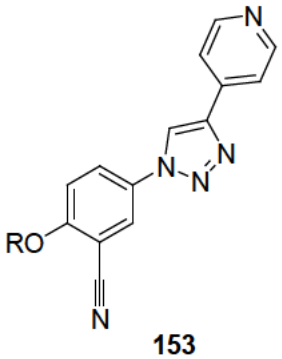
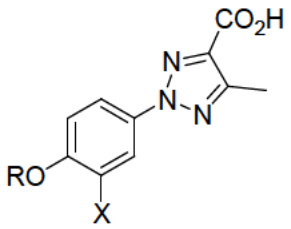


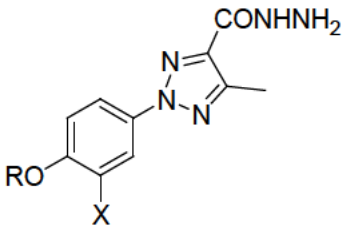
153

A variation in the position of the 3 nitrogens within the central 5-membered ring was reported by Shi et al. in their work on 2-aryl-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acids (**154**) and carbohydrazide derivatives (**155**)[155]. The carbohydrazide derivatives showed no activity at a concentration of 10 µM, while among the carboxylic acid derivatives the ones with the cyano group was in general more active than those with the nitro group. The observed IC_{50} range was from 0.084 to 0.254 µM, compared to 0.012 µM for febuxostat. The *iso*-amyloxy group showed to be the best substituent (R) for inhibitory activity.



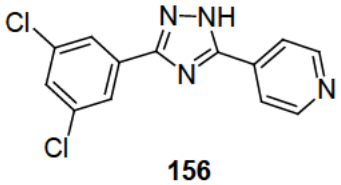
X = CN, NO_2

154



155

The use of a triazole ring instead of an imidazole ring was proven by Baldwin et Al[131]. to give better inhibitors of XO. The authors decided to maintain the 4-pyridyl substituent but use a 1,2,4-triazole ring instead of the imidazole ring. Better activities were obtained for this series, with several compounds exhibiting IC_{50} values in the nanomolar range. Both 3-(3,5-dichlorophenyl)-5-(4-pyridyl)-1,2,4-triazole (**156**) and 3-(3-pyridyl)-5-(4-pyridyl)-1,2,4-triazole (**157**) had IC_{50} values of 20 nM.



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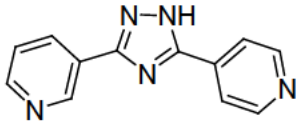
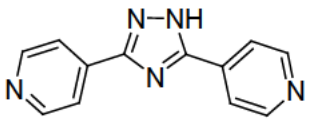


157

Three more compounds from the same 1,2,4-triazole series named 3,5-bis-(4-pyridyl)-1,2,4-triazole (**158**) (IC_{50} = 60 nM), 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole (**159**) (IC_{50} = 40 nM) and 3-(4-pyridazinyl)-5-(4-pyridyl)-1,2,4-triazole (**160**) (IC_{50} = 80 nM) demonstrated activity in the nanomolar range, and were chosen for further biological *in vivo* studies on rats, dogs and squirrel monkeys[156]. Their efficacy seemed to last for an unusually long time, a finding which was explained by the authors as arising from an uneven distribution of the inhibitor within the body, with the majority of the drug concentrated at the site where XO was more abundant (i.e. the liver and gut).



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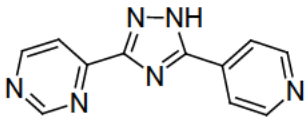

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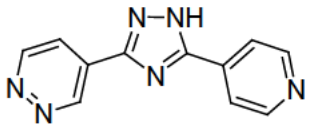
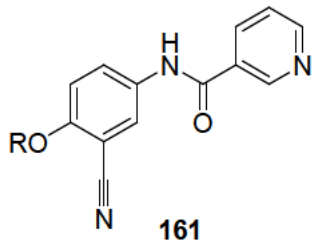


160

Zhang et al. in 2017[157] reported new derivatives containing an amide "linker" instead of a 5-membered ring between the part of the molecule interacting with amino acids close to the molibdopterin unit (either 3-pyridyl (**161**) or 4-pyridyl (**162**)) and the part of the molecule pointing away from the active site of the

enzyme. Isonicotinic acid derivatives (**162**) were more active than nicotinic acid derivatives (**161**), with IC_{50} values between 0.3 μM and 19.2 μM, compared to an IC_{50} value of 8.5 μM for allopurinol. The most active compound had an *o*-cyanobenzyl group (**164**). A few picolinic acid derivatives (**163**) were also studied but the majority of them gave less than 60% inhibition at a concentration of 50 μM and only one of them, N-(4-(benzyloxy)-3-cyanophenyl)picolinamide (**165**) showed IC_{50} value of 22.6 μM.



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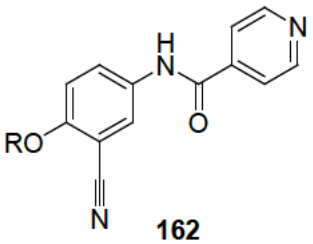

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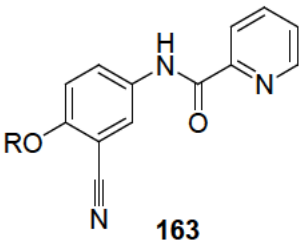

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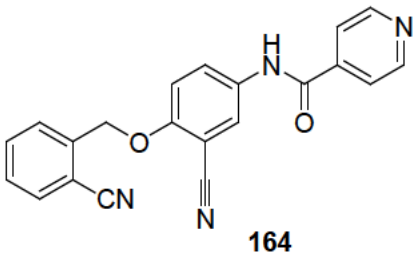

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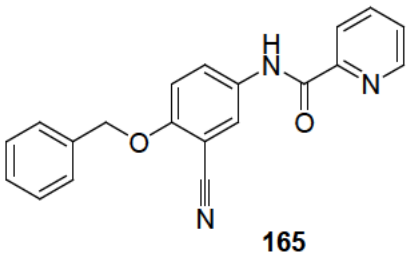
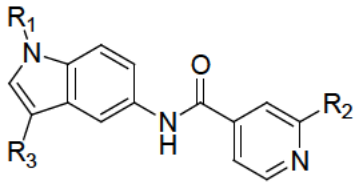


165

Molecular modelling studies predicted the presence of hydrogen bonding interactions between the nitrogen in the pyridine ring of isonicotinic acid derivatives **162** and Glu1261 that were not possible in the case of the both picolinic and nicotinic acid derivatives **161** and **163**.

In a subsequent report in 2021[158] the authors used the same amide linker between two aromatic rings larger than pyridine. Derivatives of **166** and **167** were thought to form H-bonds with Asn768 and Glu1261 in the active site, resulting in higher inhibitory activity. The in vitro potency of the analogues ranged from 0.62 to 21.79 μM (allopurinol IC50 = 8.9 μM, topiroxostat IC50 = 0.021 μM). In general compounds of type **167** performed better than compounds of type **166**. The most active in vitro compound (**168**) was shown to exhibit a hypouricemic effect in vivo in rats and its predicted properties of adsorption, distribution, metabolism, excretion and toxicity (ADMET) made the compound a good lead for future development, according to the authors.



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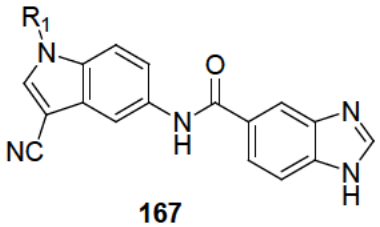

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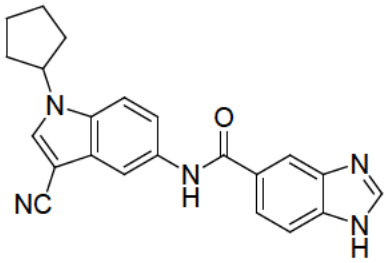
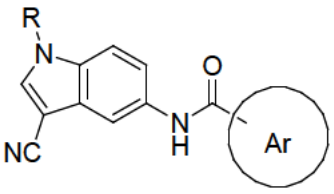


168

Molecular docking simulations suggested that the indole ring allows the correct distance between the cyano group and Asn768 in the active site for a H-bond to be formed. That bond was not possible for structures that had only a phenyl ring instead of the indole, as in compounds **161**-**163**. Based on this finding, the authors synthesised and investigated a series of molecules (**169**) that maintained the indole ring linked to the amide group and had various aromatic rings instead of the benzimidazole[159]. They also evaluated the effect of either a propyl, benzyl or cyclopentyl group on the nitrogen of the indole ring. The inhibitory activities of the new compounds were compared to the activity of topiroxostat (IC50 = 0.013 μM) and the most active compound (**170**, IC50 = 0.018 μM) demonstrated that the imidazole ring could be a potential alternative to the pyridine ring in topiroxostat.



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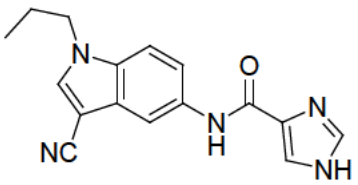
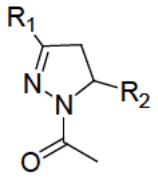


170

The synthesis of 53 analogues of 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles (**171**) was put forward by Nepali et al. using 2-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]-pyrimidines[105] (**172**) and topiroxostat **109** as models[160].



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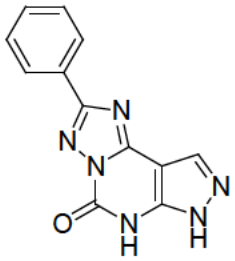
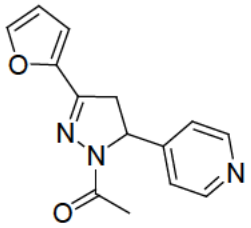


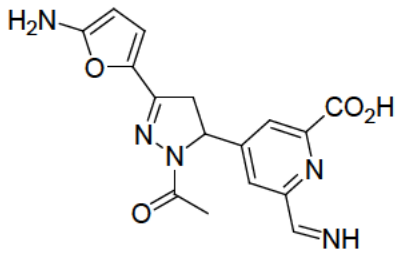
172

The key features that were thought to be necessary to retain good inhibitory activity were the two aromatic or heteroaromatic rings joined by a central five membered ring, and an acetyl group that could mimic the ureic carbonyl in compound **172**. The majority of the compounds had IC_{50} values higher than allopurinol, with the exception of 1-acetyl-3-(2-furyl)-5-(4-pyridyl)-4,5-dihydro-1*H*-pyrazole (**173**), which had an IC_{50} value of 5.3 μM, compared to an IC_{50} of 8.3 μM for allopurinol.



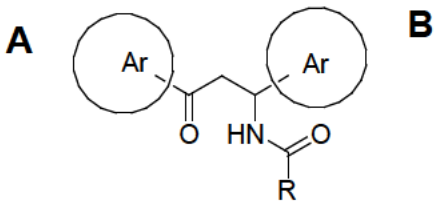
173

Li et al. studied the interactions between 1-acetyl-3,5-diaryl-4,5-dihydro-1*H*-pyrazoles and the active site of XO using 3D-QSAR analysis [161]. The authors validated two models based on comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) using data available in the literature. The models were then used to design a group of ten new analogues possessing higher predicted efficacy than the above reference compound **173**. Based on their 3D-QSAR analysis and predicted physicochemical properties, compound **174** was selected as the most potent derivative. The added groups were designed to increase the number of hydrogen bonding interactions between the molecule and the amino acid residues in the active site of the enzyme.



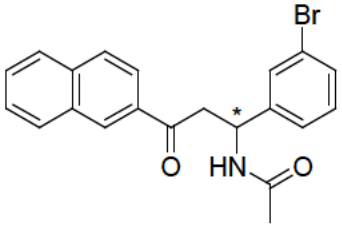
174

In subsequent work, Nepali et al. synthesised and tested *in vitro* a series of N-(1,3-diaryl-3-oxopropyl)amides (**175**)[162].



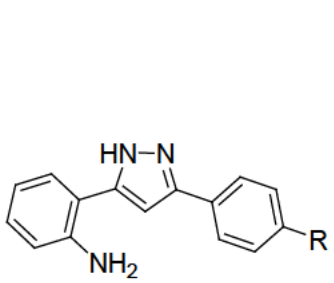
175

The SAR analyses using the *in vitro* data identified the requirements for the most active structure. Heterocycles like furan or thiophene in aromatic ring position **A** increased inhibitory activity but the best result was obtained with a 2-naphthyl group. A phenyl ring with a deactivating group in the *meta*-position in aromatic ring position **B** resulted in the lowest concentration needed to inhibit enzyme. Longer "linkers" between the two aromatic rings decreased the activity and the methyl group was preferred to a phenyl group as substituent at position R to produce compound with good activity against XO. Compound **176** was identified as the most active *in vitro*, with an IC_{50} value of 2.45 μM compared to an IC_{50} of 8.3 μM for allopurinol. *In vivo* studies on potassium oxonate-induced hyperuricemic mice of the above compound showed potency comparable to that of allopurinol. The interactions between compound **176** and the amino acid residues in the active site were predicted by molecular docking studies using the crystal structure of the enzyme. It was predicted that compound **176** fits well in the active site and that there is no significant difference between the two stereoisomers R and S.



176

As a continuation of the study of N-(1,3-diaryl-3-oxopropyl)amides and N-acetylpyrazolines, Kumar et al. reported the synthesis of 5,6-dihydropyrazolo[1,5-*c*]quinazolines[163]. The structural limitations that were considered for the synthesis of the new compounds in order to retain good interactions with the active site of the enzyme were a linker of three atoms between the two aryl or heteroaryl groups, a region susceptible of hydroxylation that can interact with the Mo centre, and replacement of the carbonyl group with an amine or imine group. Among the three different types of structures tested (**177**, **178** and **179**), only two compounds based on the structure type **179** showed better activity than allopurinol (IC_{50} = 31.62 µM) viz. **180** (IC_{50} = 10.96 µM) and **181** (IC_{50} = 20.89 µM).



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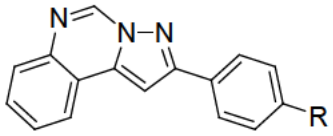

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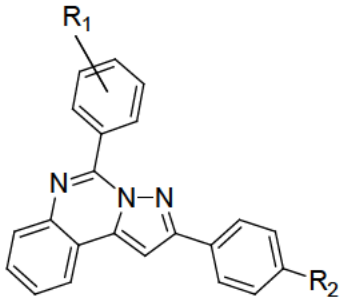

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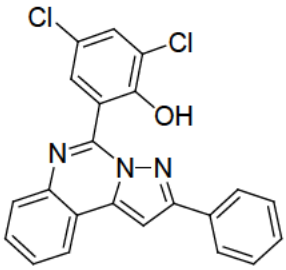

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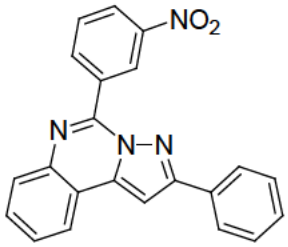
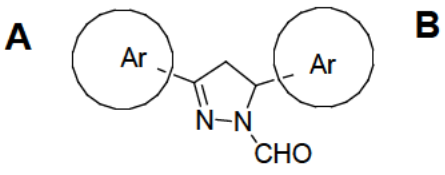


181

Further studies published in 2021[164] investigated the actvity of 3,5-diaryl-4,5 dihydro-1H-pyrazole carbaldehyde derivatives (**182**) as both XO inhibitors and anti-cancer drugs in XO overexpressing tumours. The authors identified two lead compounds, **183** and **184**, that had XO inhibitory activity comparable (**183** IC_{50} = 9.32 µM, **184** IC_{50} = 10.03 µM) to allopurinol (IC_{50} = 13.03 µM), showing promising results as anti-cancer drugs.



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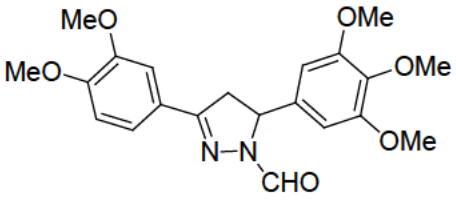

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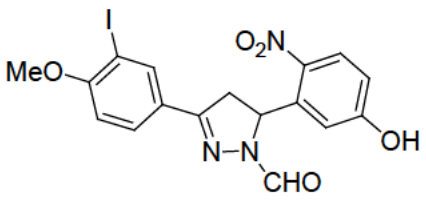
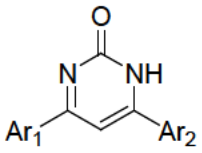


184

Maintaining the same concept of a chain linker with three atoms between two aryl groups, Shiwani et al. investigated a library of compounds with a pyrimidinone ring (**185**) variously substituted at positions 4 and 6[165]. After initial screening of the 25 compounds synthesised, only those showing more than 50% inhibition at a concentration of 50 μM were investigated further to determine their IC_{50} values. The majority of the compounds (11 analogues) showed comparable activity to allopurinol, with IC_{50} values ranging from 10.21 μM to 21.24 μM (with allopurinol having an IC_{50} of 12.24 μM). In two cases (**186** with IC_{50} = 6.45 μM and **187** with IC_{50} = 7.23 μM) the activity of the compounds exceeded that of allopurinol.



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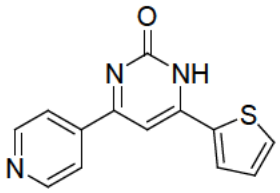

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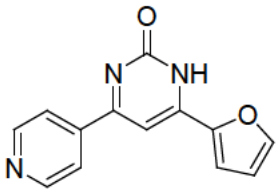
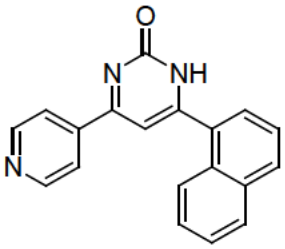


187

Two other analogues with heteroaromatic rings showed very good activity (**188** IC_{50} of 11.23 μM and **189** IC_{50} of 13.46 μM), as well as 4,6-diphenyl-1*H*-pyrimidin-2-one (**190**) with no heteroatoms on the aromatic rings (IC_{50} = 10.21 μM).



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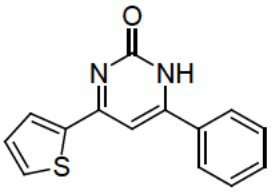

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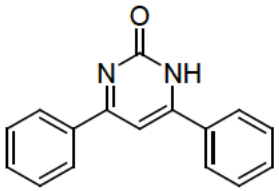
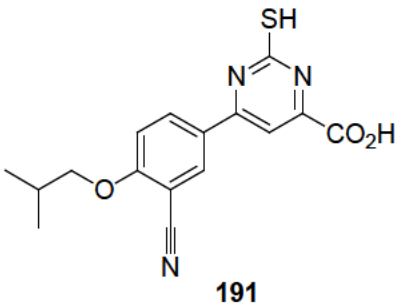


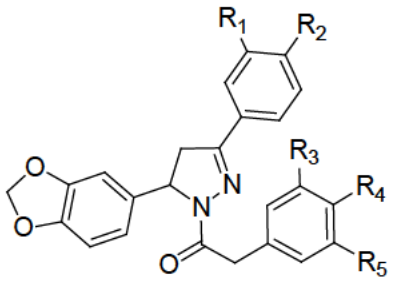
190

XO inhibitors bearing a six membered heterocycle ring instead of a 5 membered ring as a linker between the aryl substituent and the carboxylic acid group were investigated by Shi A. et Al.[166] Molecular docking simulation performed by the authors identified the 2-mercaptopyrimidine structure as a possible scaffold to improve interaction between the enzyme and the inhibitor inside the active site. Among the 18 derivatives synthesised and tested, compound (**191**) showed the best *in vitro* activity but it was still approximately 10 times weaker than febuxostat.



191

Sun et al. used a dihydro-pyrazole ring as linker between three aromatic systems (**192**)[167]. The highest inhibition was obtained with compound **193**, possessing an IC_{50} value of 9.8 µM comparable to that of allopurinol (IC_{50} of 9.5 µM). Interestingly, the activity was drastically reduced if there was only one methoxy group in the *meta*-position. The same reduction in activity was obtained when an ethoxy group was added in the *para*-position on the second phenyl ring of compound **193**.



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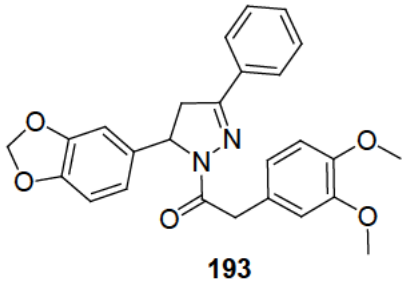
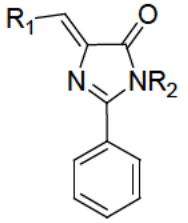


193

The concept of a five membered ring as a linker between three aromatic moieties was also exploited by Chopra et al. in their work on 1-aryl-4-arylidene-2-phenyl-1*H*-imidazol-5-ones (**194**)[168]. After a first screen for compounds with inhibition at a concentration of 50 µM, only those that showed more than 65% inhibition were investigated further. The majority of the compounds showing good activities had a 3-chloro-4-fluorophenyl group at position 1 of the imidazole ring. The most active compound, 4-(2-chlorobenzylidene)-1-(3-chloro-4-fluorophenyl)-2-phenyl-1*H*-imidazol-5(4*H*)-one (**195**), had an IC_{50} value almost half of that of allopurinol.



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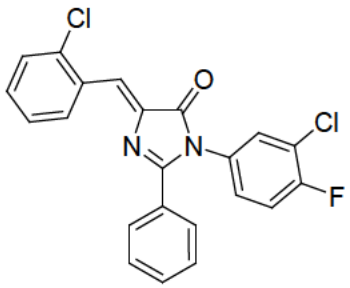
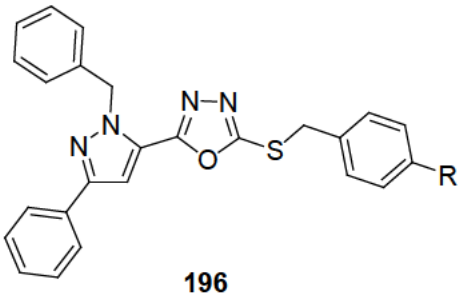


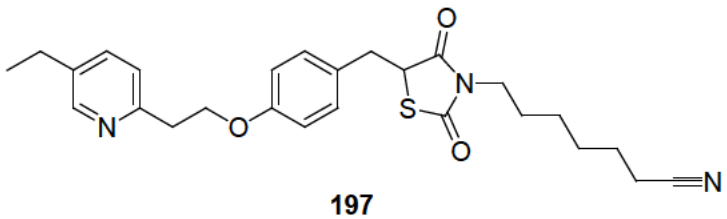
195

The XO inhibitory activity of a series of 1,3,4-oxadiazole derivatives (**196**) was tested by Qi et al.[169]. The authors concluded that further optimization needed for the two most promising compounds, although they were less active than allopurinol. A nitro group at position R generated a compound with an IC_{50} value of 72.4 μM and a cyano group in the same position generated a compound with an IC_{50} of 75.6 μM, while allopurinol was measured to have an IC_{50} of 2.3 μM.



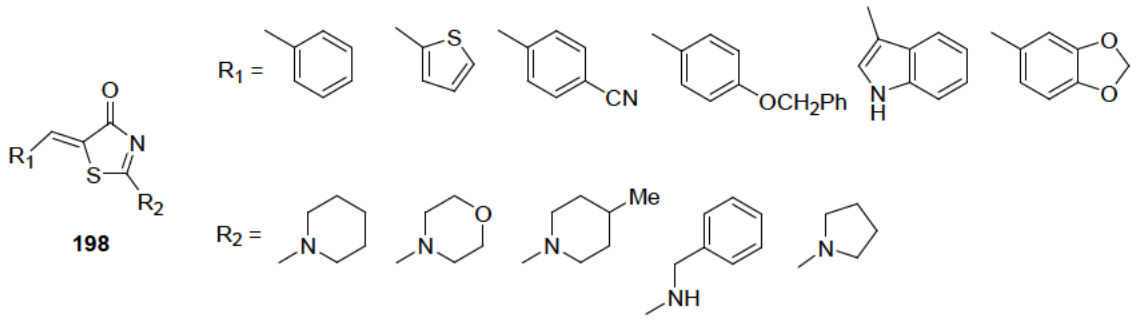
196

The thiazolidinedione motif was investigated in the structures synthesised and tested by Begum et al.[170]. They identified compound **197** possessing inhibitory activity comparable to the reference compound allopurinol.



197

A series of 30 derivatives of 2-amino-5-alkylidene-thiazol-4-ones (**198**), obtained by combining six different substituents at position 5 (R_1) with five different amines at position 2 (R_2) of the thiazole-4-one ring, were tested against both bovine milk and rat liver XO[171]. Only compounds showing more than 50% inhibition at a concentration of 50 μg/mL were selected for further studies to determine their IC_{50} values. A total of 19 analogues were successful in inhibiting bovine milk XO with an activity of more than 50%, while only 8 analogues inhibited rat liver XO with an activity of more than 50%. The IC_{50} values measured for the selected compounds demonstrated that they were less efficient in XO inhibition than allopurinol, with the most active compound of the library for both assays being 4-[(2-benzylamino-4-oxothiazol-5(4*H*)-ylidene)-methyl]benzonitrile (**199**). Its IC_{50} value measured against bovine milk XO was 53.7 μM (allopurinol IC_{50} = 1.9 μM), while its IC_{50} measured against rat liver XO was 76.7 μM (allopurinol IC_{50} = 5.8 μM).



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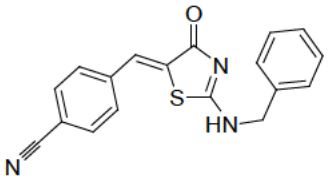
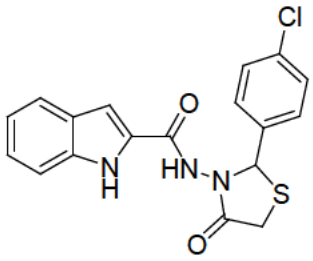


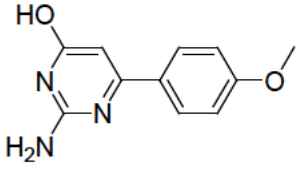
199

A series of five thiazolidinone derivatives was synthesised and investigated by Buvana et al.[172]. All the reported IC_{50} values were smaller than the one obtained for allopurinol (IC_{50} of 164.5 μM), ranging from 32.3 μM to 75.6 μM. The best inhibitory activity was obtained for compound **200**.



200

Isocytosine was reported to have good inhibitory activity towards XO[173]. Virtual screening followed by molecular docking studies and *in vitro* assays identified two isocytosine derivatives (**201** and **202**) with activities comparable to allopurinol.



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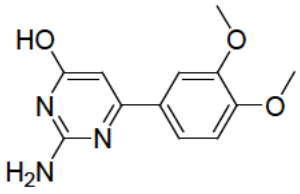

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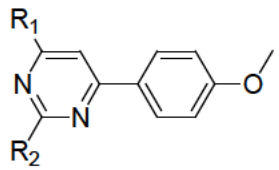
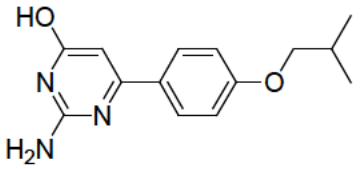


203

In vitro studies performed on the above compounds with different substituents in the R_1 and R_2 positions (**203**) showed that bulkier substituents decreased activity against the enzyme. This finding was confirmed by molecular docking studies as it was seen that there was only limited space inside the active site close to the Mo centre where the substituents R_1 and R_2 could be positioned.

The *in vitro* activity of **201** was improved when the methoxy group was substituted with either an *iso*butoxy group (**204**), with a 16-fold increase in activity, or with a phenyl ring (**205**), with a 30-fold increase in activity[174]. Compound **204** was used as a lead molecule to further explore the effect of substituents in the *ortho*-position to the *iso*butoxy group. The IC_{50} values ranged from more than 20 µM for the trifluoromethoxy group to 20 nM for the cyano group (**206**).



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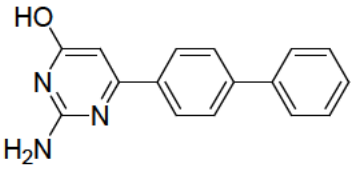

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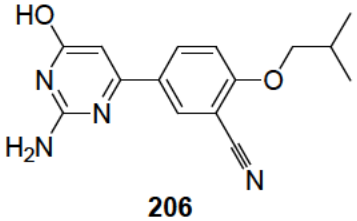

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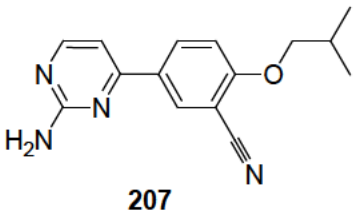
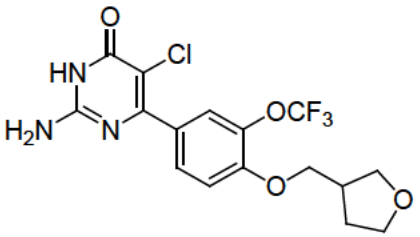


207

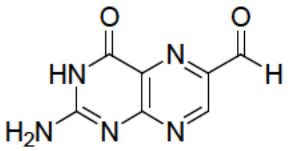
These new analogues increased the *in vitro* inhibitory activity to the nanomolar range but showed to be only comparable to the reference compounds allopurinol and febuxostat in reducing uric acid levels in a rat model if administered intraperitoneally, and very low efficacy if administered orally. A subsequent work was conducted to increase the oral bioavailability of the isocytosine derived lead compound **204**[175]. The loss of the hydroxyl group with a concomitant reduction of *in vitro* activity was necessary to increase the pharmacokinetic properties of the lead compound, which resulted in a more orally bioavailable molecule (**207**) that demonstrated to be as effective as allopurinol in reducing serum uric acid levels at the 3 h time point.

Further studies on isocytosine published by Evenas et al. were focused on the functionalities of the pyrimidone ring and on different aryl groups attached to the pyrimidone ring itself [176]. Their optimization was driven by lipophilic ligand efficiency and pharmacokinetic properties of the new synthetised compounds. Their most promising compound **208** exhibited activity in the nanomolar concentration range and was further tested in a rat model of hyperuricemia demonstrating a reduction of uric acid levels by 54% after 2 h of oral administration at a concentration of 10 mg/kg.



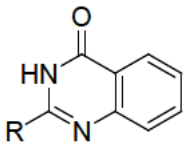
208

Among the 27 pteridines tested by Oettl and Reibnegger as potential inhibitors of XO, the most active compound was 6-formylpterin (**209**), with an IC_{50} value of 0.1 μM and a mixed type mechanism of inhibition[177]. This study outlined the essential requirements for a pteridine derivative to be an inhibitor of XO: an unsubstituted aromatic carbon atom at position 7 and an electron-deficient carbon atom adjacent to the carbon at position 6.



209

The inhibitory efficacy dropped by at least one order of magnitude when the pyrimidinone ring was fused with a benzene ring instead of the pyrazine ring, as above. Quinazolin-4(3*H*)-ones (**210**) with a variously substituted phenyl ring in the R position were tested by Zafar et al.[178]. It is interesting to note that the seven analogues tested by kinetic studies were shown to have either competitive, mixed type or non-competitive modes of action, depending on the substituents on the phenyl ring. Three different mechanisms were in fact exhibited by the three most potent derivatives. Compound **211** (IC_{50} = 2.80 μM) exhibited non-competitive inhibition, compound **212** (IC_{50} = 3.43 μM) exhibited mixed type inhibition, and compound **213** (IC_{50} = 4.80 μM) exhibited competitive inhibition. The IC_{50} values of the library of compounds were higher than that of allopurinol, ranging from 2.80 to 105.20 μM, with allopurinol having an IC_{50} value of 2.01 μM.



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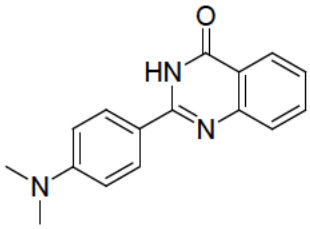

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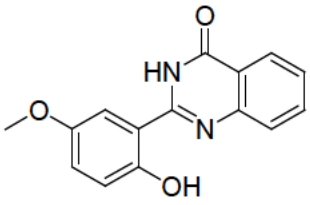

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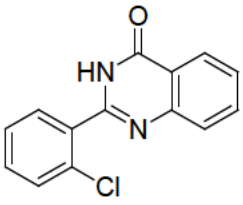
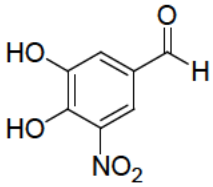


213

The same group[179] in 2018 reported that the 24 synthesised 5-aryl-1*H*-tetrazoles showed either comparable or weaker inhibitory activity towards XO than allopurinol.

A simple aldehyde, 3,4-dihydroxy-5-nitrobenzaldehyde (DHNB) (**214**) was shown to be as active as allopurinol in inhibiting XO[180]. Kinetic studies showed that it has a mixed type mechanism of inhibition, with an IC_{50} value of 3 μM, comparable to that obtained for allopurinol (1.8 μM). MS/MS spectra of the metabolites resulting from the action of XO on DHNB showed that the inhibitor was slowly oxidised to its carboxylic acid (**215**).



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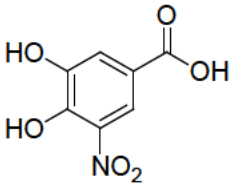
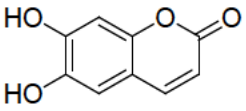


215

A similar mechanism for the oxidation of hpoxanthine to xanthine oxidation was proposed by the authors for the oxidation of DHNB[180]. The nitro group at position 5 was shown to be essential for the inhibitory activity and thought to enhance the electrophilicity of the aldehyde carbon. The hydroxyl oxygen bonded to the Mo centre in the active site of the enzyme could attack the aldehyde carbonyl group to give an intermediate, with the inhibitor covalently bonded to the Mo centre. A subsequent protolysis would generate the carboxylic acid product. DHNB proved to be slightly less effective *in vivo* than allopurinol but less toxic, and this made it a good candidate for further studies in the search for alternatives to allopurinol.

Coumarin derivatives were reported to be competitive inhibitors of XO[181]. Although esculetin (**216**) was less effective than allopurinol in inhibiting the

enzyme (its IC_{50} value of 10.84 μM was approximately ten times larger than that of allopurinol, IC_{50} = 1.07 μM), it demonstrated good activity as a scavenger of ROS. The SAR studies conducted by the authors demonstrated the need for the hydroxyl groups to maintain both characteristics. When the hydroxyl groups were either removed (**217**) (IC_{50} > 100 μM) or replaced by a methoxy (**218**) group (IC_{50} > 100 μM) the inhibitory activity and the scavenging potency were affected negatively.



Esculetin

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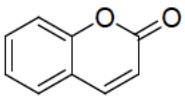

Coumarin

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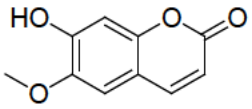
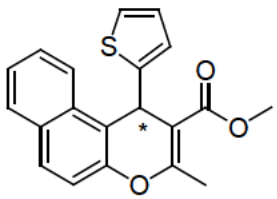


Scopoletin

218

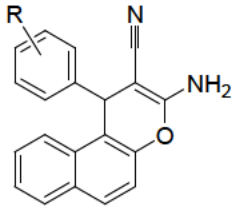
The inhibitory activity of 4*H*-naphtho[2,1-*b*]pyran derivatives was evaluated by Sharma et al.[182]. Compound **219** was shown to have the lowest IC_{50} value (4 μM) among the naphthopyran series. It was almost 3-fold lower than the IC_{50} value obtained for allopurinol (IC_{50} = 11.10 μM), but much higher than that of febuxostat (IC_{50} = 0.025 μM). The *R*-enantiomer of compound **219** was also found by molecular docking studies to fit better in the active site of the enzyme than its *S*-enantiomer.



219

The pyran motif was also investigated by Nepali et al.[183] with eight different libraries of compounds (**220**-**227**). More than 100 compounds were synthesised and tested for inhibitory activity against XO at a concentration of 50 μM. Only those compounds with more than 80% inhibition were studied further. A total of 41 compounds met this condition, and were measured to have IC_{50} values ranging from 34.3 μM down to 0.59 μM for compound **228** (compared to allopurinol with IC_{50} = 8.29 μM). SAR studies suggested that a halogen or nitro group was preferred over methoxy or hydroxy group as a substituent on the phenyl ring. Substitution in the *para*-position of the phenyl ring was also essential to have the required minimum level of enzyme inhibition. The best activity was obtained when the phenyl ring was replaced by a thiophene ring. Among the eight different libraries, the substituted coumarins (**224**) were the strongest XO

inhibitors. The most active compound (**228**) was shown to exhibit the mixed type of inhibition, and molecular docking studies predicted the *S*-enantiomer to have the best fit in the active site of the enzyme.



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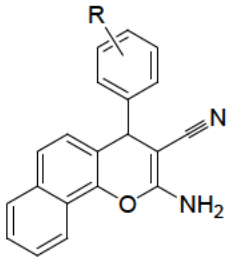

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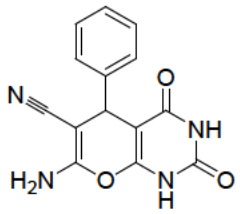

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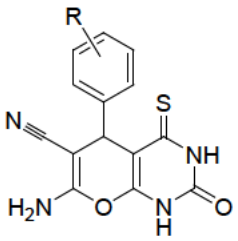

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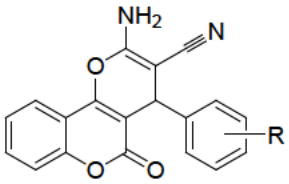

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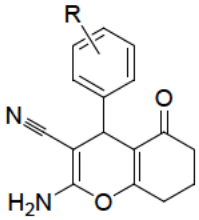

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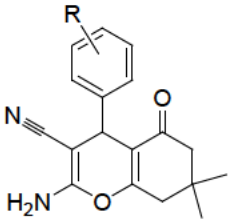

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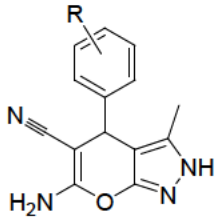

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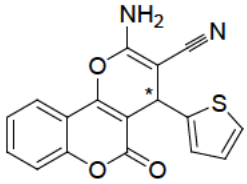
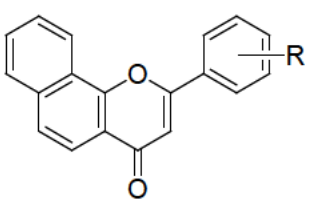


228

Sharma and co-workers combined the characteristics of naphthopyran and flavone scaffolds to synthesise naphthoflavone compounds (**229**)[184]. *In vitro* testing revealed that their IC_{50} values ranged from 0.62 to 41.2 μM (with allopurinol having IC_{50} = 8.69 μM). A single substituent on the phenyl ring resulted in better inhibitory activity than the unsubstituted compound. The order of decreasing activity for a single substituent was halogen and nitro > methoxy > methyl > hydrogen. Substitution at the *para*-position also resulted in the lowest IC_{50} values, while *meta*-substitution resulted in the highest. By contrast, a double substitution was not tolerated, resulting in compounds with significantly reduced inhibitory activity. The highest inhibitory activity was observed for compound **230** (IC_{50} = 0.62 μM), which exhibited a mixed type mechanism of inhibition. Molecular docking studies suggested that a fluorine atom could be involved in hydrogen bonding with side chain NH group of Asn768.



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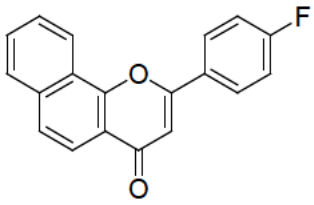
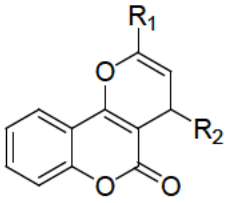


230

Virdi et al. instead combined the characteristics of coumarins and chalcones and synthesised a library of 2,4-diarylpyrano[3,2-*c*]chromen-5(4*H*)-ones (**231**)[185]. SAR studies revealed that any electron withdrawing (EWG) or electron donating (EDG) substituent on the phenyl at R_1 affected negatively the activity of resulting compounds. When the substituent at R_2 was a phenyl ring with an EDG, the activity of the derivate increased vs. analogues with a EWG, which instead had a negative impact on activity. Thiophene was the only heteroaryl ring present among the compounds in the library and gave rise to the best inhibitor (**232**) (IC_{50} = 2.21 μM compared to allopurinol IC_{50} = 8.79 μM). Two other compounds exhibited better or comparable activity to allopurinol, and they both had an unsubstituted phenyl ring at position R_1 and a naphthyl group at position R_2, with 1-naphthyl giving better activity than 2-naphthyl (IC_{50} values of 4.31 vs. 8.21 μM, respectively).



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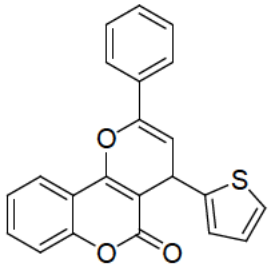
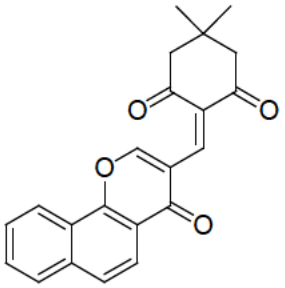


232

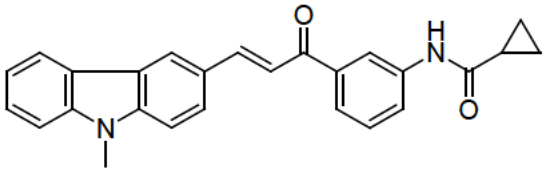
As a continuation of their work on benzopyran derivatives, Charanjeet et al. synthesised a library of 14 compounds with a benzochromone scaffold[186]. A few compounds showed comparable inhibitory activity to allopurinol, with one (**233**) (IC_{50} = 0.65 μM) of them in particular being approximately 13-fold more active than allopurinol (IC_{50} = 8.69 μM).



233

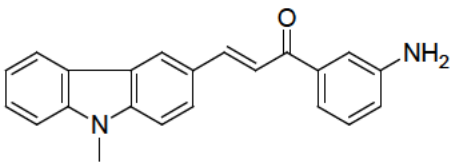
Fused pyrano[3,2-*d*]pyrimidine derivatives were synthesised and tested by Manroopraj et al.[187]. Most of the 100 analogues exhibited lower activity than allopurinol and only three of them had a slightly better activity than the reference compound.

The inhibitory activity of carbazole derivatives was investigated by Bandgar et al.[188]. A library of 19 compounds was synthesised and tested against XO, and 7 of these compounds showed higher activity than allopurinol (IC_{50} = 8.5 μM), with the most active compound (**234**) (IC_{50} = 4.3 μM) being approximately twice as active as allopurinol.



234

When the cyclopropyl group was replaced with a phenyl ring, the activity decreased, but it was found that, in general, some specific substitutions on the phenyl ring helped to bring the activity back to IC_{50} values in the range of 4.4-5.6 μM. The amide functionality was shown to be essential for the activity, as the synthetic precursor (**235**) was also tested and demonstrated an IC_{50} value of 48.5 μM. Good activity was also observed when the amide "linker" was extended with a cinnamoyl moiety (**236**) (IC_{50} = 5.1 μM).



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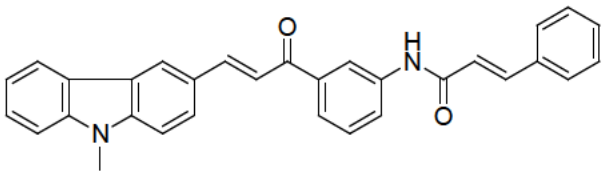
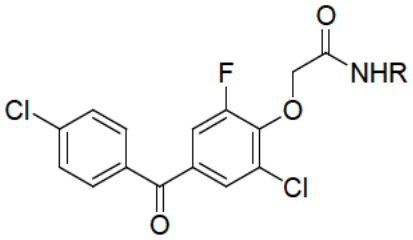


236

A group of 11 heterocyclic amides linked to a benzophenone carrying a chlorine atom in the *para*-position on the distant phenyl ring, and fluorine and chlorine atoms on the derivatised phenyl ring (**237**) were synthesised and tested by Girish et al.[189]. Two of these compounds (**238** with IC_{50} = 0.23 μM and **239** with IC_{50} =

0.26 μM) were shown to have better activity than allopurinol (IC_{50} = 0.71 μM) inhibiting bovine milk XO. The authors also tested the same compounds using rat liver XO and it was found that compound **238** (IC_{50} = 0.88 μM) had only comparable activity to allopurinol while compound **239** was inactive, although allopurinol itself (IC_{50} = 0.70 μM) demonstrated the same activity against both XO types. Interestingly, the most active compound against rat liver XO was compound **240** (IC_{50} = 0.24 μM), with an IC_{50} value almost 3 times better than this parameter for allopurinol. Compound **240** also showed activity against bovine milk XO (IC_{50} = 0.87 μM) comparable to that of allopurinol.



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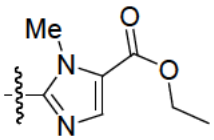

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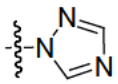

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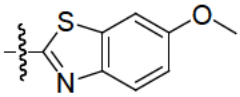
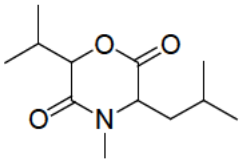


240

Two morpholine derivatives, namely 3-isobutyl-6-isopropyl-4-methylmorpholine-2,5-dione (**241**) and 3,6-diisopropyl-4-methylmorpholine-2,5-dione (**242**), were investigated for their inhibitory activity against XO and for their anti-inflammatory activity[190]. Both compounds were found to have *in vitro* inhibitory activity comparable to that of allopurinol. Allopurinol was shown to be a stronger inhibitor when the compounds were tested using rat liver homogenate. Molecular docking studies of the two morpholine derivatives showed that their binding modes resembled those for salicylic acid and febuxostat.



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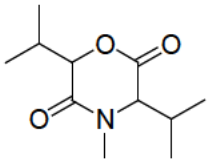
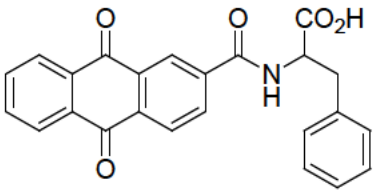


242

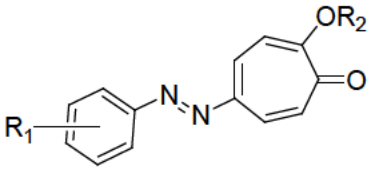
The anthraquinone moiety was exploited by Zhang et al.[191, 192] to increase enzyme-inhibitor interactions outside the active site while the portion of the molecule interacting inside the active site was made of various acylaminoacids. Of the eight amino acids derivatives synthesised, only the one with phenylalanine

(**243**) (IC_{50} = 3.0 µM) demonstrated better activity than allopurinol (IC_{50} = 8.1 µM). When the 9,10-anthraquinone moiety was replaced by a phenyl group activity decreased drastically, showing that the anthraquinone group was necessary for retaining inhibitory activity.



243

Tropolone is considered a bioisostere of carboxylic acid and is also a good ligand for metal complexes. Sato et al.[193] synthesised a library of 22 derivatives (**244**) build on the 5-arylazotropolone skeleton and tested their *in vitro* inhibitory activity against XO.



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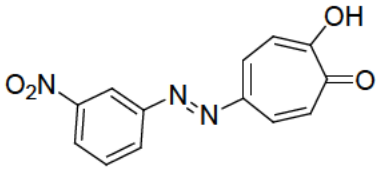
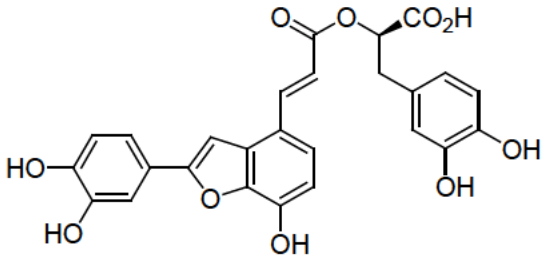


245

They found that electron withdrawing groups in *meta*-position of the phenyl ring gave more potent inhibitors when compared with derivatives with the same substituents in *para*-position. (*E*)-5-(3-Nitrophenyl)-2-hydroxycyclohepta-2,4,6-trienone (**245**) demonstrated to be a mixed type inhibitor (IC_{50} = 0.46 µM), more than 5 times more potent than allopurinol (IC_{50} = 2.6 µM). Docking studies revealed that the free hydroxyl group was essential for hydrogen bonding with Arg880. When the hydroxyl group of (*E*)-5-((3,5-bis (trifluoromethyl)phenyl) diazenyl) -2-hydroxycyclohepta-2,4,6-trienone was substituted with a methoxy group, the obtained compound completely lost the inhibitory activity. The carbonyl moiety of the tropolone ring in the docking model showed hydrogen bonding with both Ala1079 and the hydroxyl group bound to the molybdenum atom.

Salvianolic acid C (**246**), extracted from the dried root and rhizome of *Salvia miltiorrhiza* Bge., demonstrated good inhibitory activity towards XO. Tang et al.[194] synthesised benzofuran derivatives based on the salvianolic acid C scaffold and obtained compounds comparable to allopurinol in their activity. In a subsequent work[195] the authors reported the synthesis and results of *in vitro*

experiments for 3 different libraries prepared by the derivatisation of a precursor of salvianolic acid C (**246**) called tournefolic acid A (TAA) (**247**).



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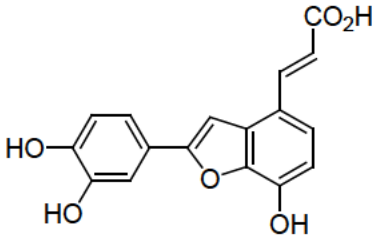
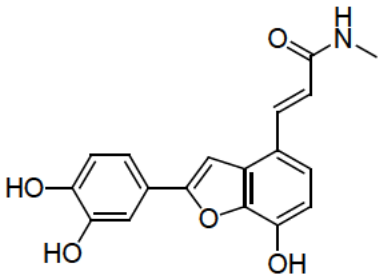


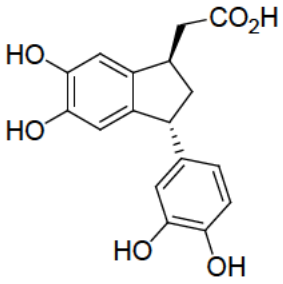
247

The 3 libraries were obtained by converting the carboxylic acid group into either esters or amides and by reducing the acyclic double bond of the amide derivatives. The obtained IC_{50} values indicated that the acyclic double bond was essential to retain inhibitory activity and the smaller the substituent on the carboxy group the better was the potency. The most active compound (**248**) showed to be more than twice stronger XO inhibitor than allopurinol.



248

A phenylindane derivative (**249**) was identified by Fukuyama et al.[196] during their work on the isolation of constituents from the roasted coffee beans and testing their effect on the XO activity. Among six isolated phenylindanes, compound (**249**) was found to be active but at a rather high concentration. Only 62% of the enzyme inhibition was demonstrated by **249** at a concentration of 200 µM.



249

2.4 Conclusion

For more than 50 years the only drug inhibitor of XO available on the market was allopurinol. However, there has been an increase in the search for new inhibitors of XO in the last decade as the activity of the enzyme has been associated not only with hyperuricemia and gout, but also with a variety of other conditions like diabetes, hypertension and cardiovascular disease. Non purine-like inhibitors febuxostat and topiroxostat have recently been approved in various countries for the treatment of hyperuricemia.

A multitude of various chemical structures have been synthesised and tested for their activities as XO inhibitors since the discovery of allopurinol. The availability of X-ray diffraction crystal structures of XO is allowing the rationalization of the activities of new inhibitors, allowing the creation of SAR models that can predict the potency and selectivity of new inhibitors. A group of five amino acids Glu802, Arg880, Phe914, Phe1009 and Glu1261 has been identified as the core enzyme active site that gives the strongest interactions with the inhibitor. The potency of the majority of the newly synthesised inhibitors is rationalized by molecular modelling via hydrogen bonds and aromatic-aromatic interaction with the five amino acids within the enzyme active site.

The characteristics identified in various chemical structures as essential for a good XO inhibitor are a chemical group capable of forming hydrogen bonds with Glu802, Glu1261 and Arg880 and a non-polar region capable of forming non-polar interactions with Phe914 and Phe1009 not too distant from the polar group. Unfortunately the above characteristics do not necessarily translate into a successful candidate as not always good in vitro inhibitors translate into good in vivo inhibitors as in the case of isocytosine derivatives.

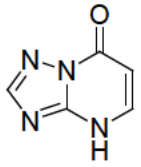
Various molecules have demonstrated to interact with not only the five core amino acids but also other residues within the narrow channel of the enzyme active site. There is a need to find alternatives to the current available drugs with better inhibitory activity and less side effects. The focus of this project is to find novel purine analogue inhibitors of XO more active than allopurinol in vitro that could potentially be developed into drugs.

Chapter 3

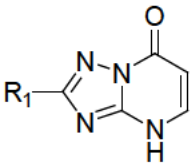
3 Synthesis, in-vitro testing and molecular modelling of 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a] pyrimidine derivatives.

3.1 Introduction

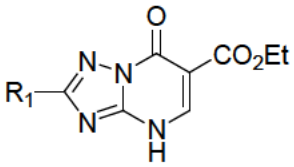
Purine analogues have been reported to possess various biological activities[197]. More specifically, 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives have demonstrated activity as CB_2 cannabinoid receptor inverse agonists[198]. Compounds with 1,2,4-triazolo[1,5-*a*]pyrimidine moiety have also been reported as agents potentially useful in the treatment of tumour[199] and neurodegenerative disorders[200-202]. The antiviral[203], antifungal[204] and antiparasitic activities[203, 205] have been identified for some compounds of this class. In particular, analogues of 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one have been recently studied for their activity as agonists of benzodiazepine receptors[206]. Our study focuses on expanding the library of analogues reported by Robinson et al.[8] and, more specifically, on the synthesis of 2-substituted-7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidinones (**1, 2** and **3**) and the evaluation of their inhibitory activity against xanthine oxidase (XO).



1,2,4-triazolo[1,5-*a*]pyrimidin-7-one



1



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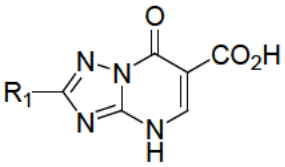


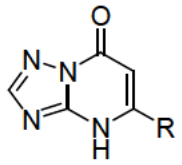
3

R_1 = a)H, b)MeS, c)Ph, d)2-FC_6H_4, e)3-FC_6H_4, f)4-FC_6H_4, g)2-ClC_6H_4, h)3-ClC_6H_4, i)4-ClC_6H_4, j)3-MeC_6H_4, k)4-MeC_6H_4, l)3-$MeOC_6H_4$, m)4-$MeOC_6H_4$, n)3-$EtOC_6H_4$, o)4-$EtOC_6H_4$, p)3-*i*$PrOC_6H_4$, q)4-*i*$PrOC_6H_4$, r)3-*i*$BuOC_6H_4$, s)4-*i*$BuOC_6H_4$, t)3-$CF_3C_6H_4$, u)4-$CF_3C_6H_4$, v)3-$CF_3OC_6H_4$, w)Pyridin-3-yl, x)Pyridin-4-yl, y)Thien-2-yl.

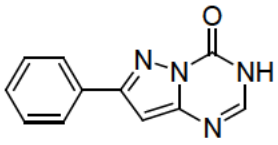
Robinson et al.[8] published the synthesis and *in vitro* XO inhibitory activity of a series of 5-substituted-7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidinones (**4a-e**) and in the

same work the authors identified 7-phenyl-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (**5**) as one of the most active inhibitor of XO (Table 1).

Table 1: Derivatives of 5-substituted-7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidinones (**4**) and their IC_{50} values, reported by Robinson et al.[8], together with one of the most active compound (**5**) and allopurinol.



4



5

Compound	R	IC_{50} (μM)
4a	H	45
4b	HO	>150
4c	Ph	21
4d	H_2N	73
4e	Cl	78
5		0.047
Allopurinol		5.9

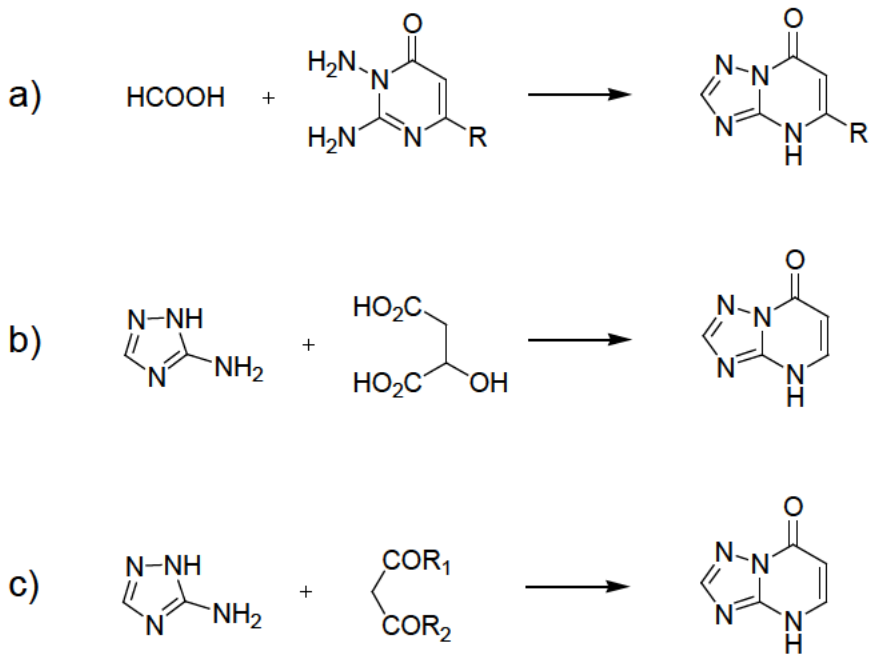
None of the structures reported with the scaffold of type **4** had a substituent in position 2 that could have mimicked the substituent in position 2 of the more potent compound **5**. Therefore,

Three different libraries **1**, **2** and **3** with a total of 75 analogues of the 2-substituted-7-oxo-1,2,4-triazolo pyrimidine scaffold were synthesised. Their IC_{50} values were determined using xanthine as substrate and compared with the enzyme inhibition measured using Allopurinol. The most active structures were docked into the active site of XO using docking simulations.

3.2 Chemistry

The synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one derivatives can be performed by either the cyclization of 2,3-diamino-4(3*H*)-pyrimidone at reflux in formic acid for 8 hours[207] (Scheme 1a), or the condensation of 5-amino-1,2,4-triazole and malic acid in fuming sulphuric acid at low temperature[208] (Scheme

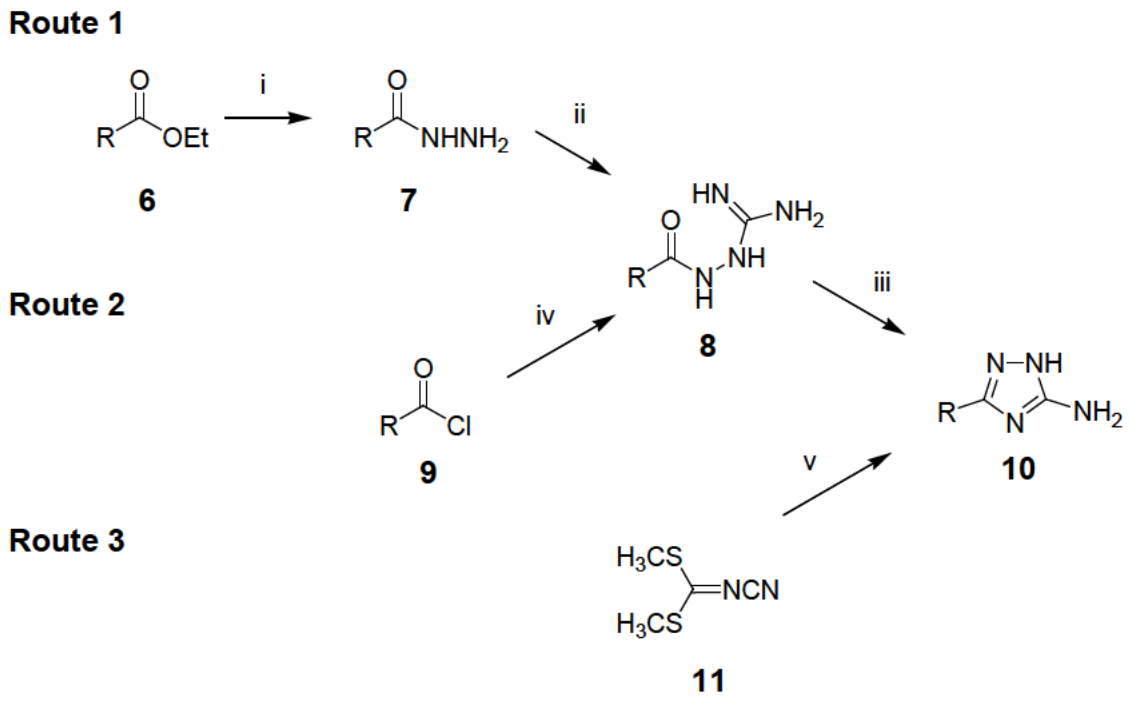
1b). More commonly nowadays they are prepared by the condensation between 5-amino-1,2,4-triazole derivatives and β-dicarbonyl compounds[209] (Scheme 1c).



Scheme 1: Approaches for the synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one derivatives.

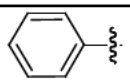
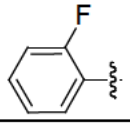
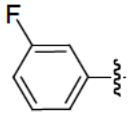
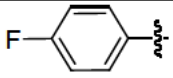
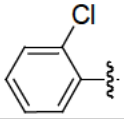
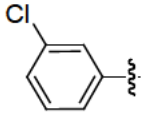
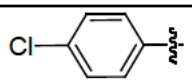
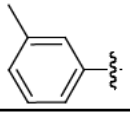
These procedures require refluxing temperatures, long reaction times and presence of corrosive acids. In this study, application of microwave irradiation as a tool for improving the reaction efficiency is attempted.

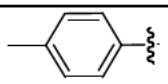
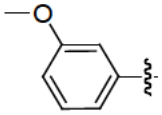
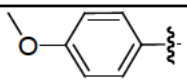
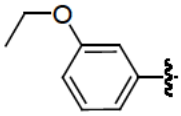
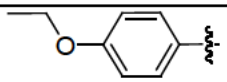
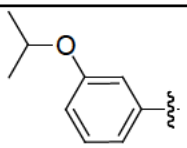
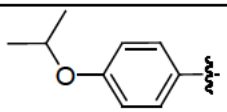
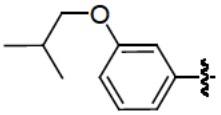
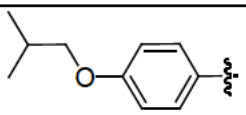
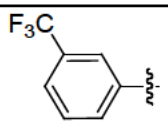
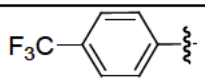
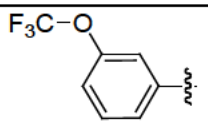
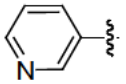
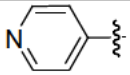
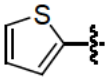
Here 3-substituted-5-amino-1,2,4-triazoles (**10**) were used as starting material to obtain 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one derivatives. The library of triazole derivatives was prepared by the cyclisation of the corresponding amidoguanidines (**8**) following the Route outlined in Scheme 2 and Table 2, depending on the nature of the group R.



Scheme 2: i) NH_2NH_2, overnight, room temp.; ii) H_2O, $HN=C(NH_2)SCH_3$ $1/2H_2SO_4$, NaOH, 2 – 11 days, room temp.; iii) H_2O, μW, 10 min, 170°C; iv) MeOH, $NH_2NHC(=NH)NH_2 \cdot HCl$, overnight, reflux; v) NH_2NH_2, MeOH, reflux.

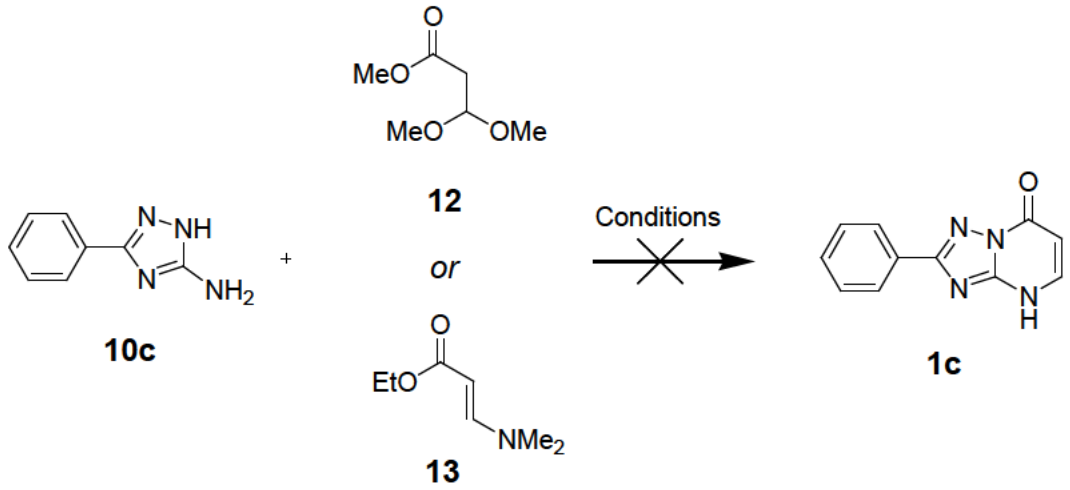
Table 2: Routes applied for the synthesis of 5-amino-1,2,4-triazoles (**10**).

Compound 10	R	Route
a	H	/
b	MeS	3
c		1
d		2
e		2
f		2
g		2
h		2
i		2
j		1

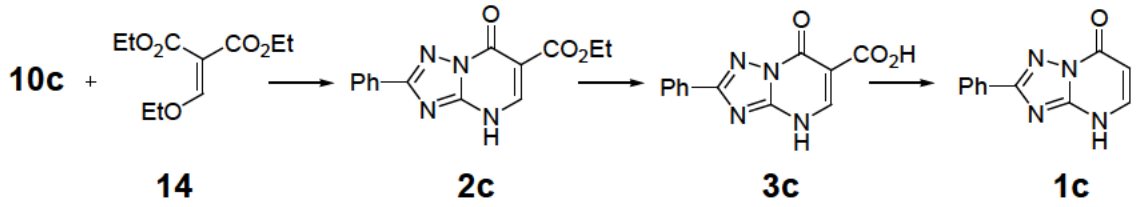
k		1
l		1
m		1
n		1
o		1
p		1
q		1
r		1
s		1
t		2
u		2
v		2
w		1
x		1
y		2

The pyrimidinone ring annulation to aminotriazoles was attempted using a model reaction of **10c** with methyl 3,3-dimethoxypropionate (**12**) under microwave irradiation. Aminotriazole **10c** remained unreacted when this reaction was carried out in water in the presence of hydrochloric acid (for the *in situ* formation of reactive aldehyde *via* acetal hydrolysis) at 80 ºC. Further attempts to find suitable

conditions using other solvent systems and higher temperatures (up to 220 ºC) were also unsuccessful. The typical outcome was a complex mixture with small quantities of the desired product, which was not possible to be conveniently isolated. To improve the reaction selectivity, we attempted to apply ethyl 3-(*N,N*-dimethylamino)acrylate (**13**) as a dielectrophile. The reaction of **10c** with **13** in acetic acid under microwave irradiation (220 ºC, 20 min) resulted in a complex mixture of products. In the further screening of reaction conditions, the desired product **1c** was detected (^{1}H NMR) in the mixture of reaction products obtained in toluene (180 ºC, 25 min). Higher reaction temperatures did not help to increase the formation of 1c but instead increased the complexity of the reaction product mixture. These results inclined our further method development toward a different approach with the initial formation of **2c** with subsequent hydrolysis and decarboxylation.



A different β-dicarbonyl compound, diethyl ethoxymethylene malonate (**14**), was then considered as starting reagent for the synthesis of compounds of type **1**, as illustrated in Scheme 3.



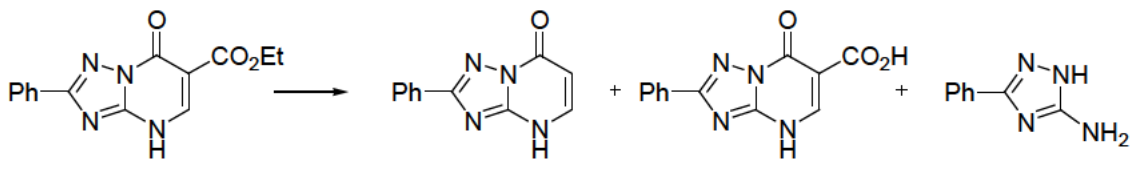
Scheme 3: Reaction steps required to produce compound **1c**

Reaction conditions were optimised using compound **10c** as starting material. Acetic acid at 180 °C under microwave irradiation was selected as the condition for the reaction to obtain product **2c**. Amongst the two tested reaction times, 10 min and 15 min, the longer time gave better results.

As shown in Scheme 3, the use of reagent **14** permitted the production of two more series of 2-substituted-1,2,4-triazolo[1,5-*a*]pyrimidinone analogues (**2** and **3**) that, upon testing *in vitro* for their inhibition of XO, added valuable information about the interaction between inhibitor and the active site of the enzyme.

During the optimisation of the reaction conditions to form compound **2c**, it was noted that compound **1c** was also formed. Identification of the reaction conditions required to form product **1c** directly from **2c** was then attempted. Several experiments, all starting from 100 mg of **2c**, were performed under controlled conditions in the microwave reactor and they all produced compound **1c** but not as the main product. Table 3 reports the isolated material amount and the product composition obtained at the end of the reaction. The ratios of constituents of the crude material recovered at the end of the reaction have been estimated based on ^{1}H NMR analysis of the final mixture. The aim of the experiments was to optimise the reaction conditions in order to obtain total consumption of the starting material **2c** and a product **3c** that would have required very minimal purification step. Changing the base from potassium hydroxide to 10% potassium carbonate increased the quantity of the collected material, but it also contributed to the degradation of the starting material to the aminotriazole **10c**. Milder conditions, using 5% potassium carbonate, and shorter reaction time, allowed for the formation of the desired product in higher amount. A series of experiments were also conducted in slightly acidic environment resulting in mixtures containing, in most of the cases, **1c** as main product.

Table 3. Estimated product composition, estimated on the basis of ^{1}HNMR analysis, of either **2c** or **3c** into various products obtained during the optimisation of the reaction conditions. All the experiments were performed starting from 100 mg of **2c**, unless stated differently.



2c **1c** **3c** **10c**

Entry	Reagent	Temperature / time	Amount and composition of product recovered
1[a]	5% KOH	203°C / 30 min	15 mg of **3c** and **10c** (main products), **1c** (traces)
2[a]	5% KOH	180°C / 1 h	15 mg of **3c** (main product), **1c** (traces)
3[a]	10% K_2CO_3	180°C / 1 h	45 mg of **1c**/**3c**/**10c** = 1.0/1.6/0.9
4[a]	10% K_2CO_3	210°C / 30 min	20 mg of **1c**/**3c**/**10c** = 1.0/0.3/0.3
5[a]	10% K_2CO_3	190°C / 1.5 h	33 mg of **1c**/**3c**/**10c** = 1.0/0.6/0.6
6[a]	5% K_2CO_3	150°C / 30 min	75 mg of **1c** (traces), **3c** (main product)
7[a]	5% K_2CO_3	160°C / 2 h	55 mg of **1c**/**3c**/**10c** = 1.0/8.9/0.8
8[a]	5% NaH_2PO_4 x H_2O	180°C / 1 h	75 mg of **1c**/**2c**/**10c** = 1.0/6.1/0.7
9[a]	5% NaH_2PO_4 x H_2O	200°C / 1 h	62 mg of **1c**/**2c**/**10c** = 1.0/0.5/0.1
10[a]	5% NaH_2PO_4 x H_2O	200°C / 2 h	55 mg of **1c**/**2c**/**10c** = 1.0/0.1/0.2
11[a]	5% NaH_2PO_4 x H_2O	210°C / 1 h	54 mg of **1c**/**2c**/**10c** = 1.0/0.1/0.2
12[a]	5% NaH_2PO_4 x H_2O	205°C / 1.5 h	62 mg of **1c**/**2c**/**10c** = 1.0/0.1/0.4
13[a]	5% NaH_2PO_4 x H_2O	200°C / 3 h	49 mg of **1c**/**2c**/**10c** = 1.0/traces/0.4
14[b]	5% NaH_2PO_4 x H_2O	200°C / 1 h	54 mg of **1c**
15[b]	5% NaH_2PO_4 x H_2O	190°C / 30 min	71 mg of **1c**
16[b]	5% NaH_2PO_4 x H_2O	190°C / 15 min	61 mg of **1c**

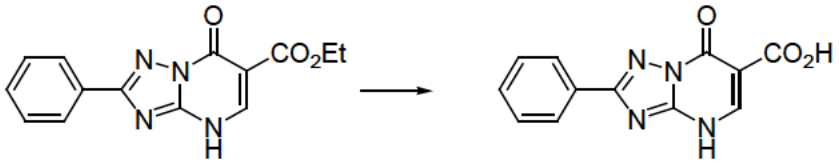
[a] 100 mg of **2c** in 2 mL of reagent
[b] 100 mg of **3c** in 2 mL of reagent.

The internal pressure of the reaction vessel during the trials listed in Table 3, entries 1–7, always fluctuated and was sometimes high enough to cause the microwave instrument to shut down for the safety reason when the maximum allowed pressure was reached. Carbon dioxide, formed during the decarboxylation step, caused these pressure fluctuations. To avoid the formation of carbon dioxide and instead drive the equilibrium of carbonic acid towards bicarbonate, the reaction was buffered with sodium dihydrogen phosphate, which has a pK_a higher than carbonic acid. All attempts to optimise the reaction

conditions to obtain product **1c** from the decarboxylation of **3c** focused on the use of this phosphate buffer.

All experiments conducted using compound **2c** to produce **1c** resulted in a mixture of products. The best outcome was obtained instead when **3c** was used as starting material for the formation of **1c**.

Finally, a series of experiments were performed to optimise the reaction condition for the hydrolysis of **2c** to **3c** as summarised in Table 4. All experiments were conducted using a microwave reactor. Changing the counter ion of the base from sodium to potassium (entry 1 and 3) slightly inproved the product yield. This trend was confirmed also when the base was 10% carbonate (entry 2 and 8). The use of carbonate as base gave also better result compared to the use of potassium hydroxide. The investigation then focused on the use of potassium carbonate and optimization of temperature and time.



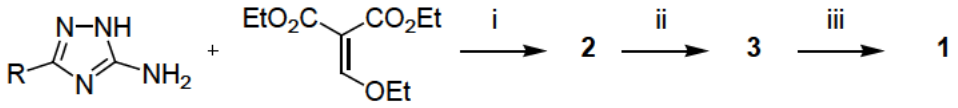
2c **3c**

Table 4. Product recovery of the hydrolysis reaction of **2c** to **3c**. All reactions were performed in a microwave reactor and starting from 100 mg of **2c** in 2 mL of reagent, unless otherwise stated.

Entry	Reagent	Temperature / time	Product recovery (mg)	Yield (%)
1	5% KOH	150°C / 30 min	55.5	62
2	10% Na_2CO_3	150°C / 30 min	59.9	66
3	5% NaOH	150°C / 30 min	49.9	55
4	H_2O	150°C / 30 min	No product	/
5	1% K_2CO_3	150°C / 30 min	No product	/
6	5% K_2CO_3	150°C / 30 min	70.2	78
7	5% K_2CO_3	140°C / 15 min	66.4	74
8	10% K_2CO_3	150°C / 30 min	65.2	72
9	10% K_2CO_3	180°C / 15 min	42.0	47
10	Glacial AcOH	180°C / 30 min	No product	/

11	Glacial AcOH (6 mL)	Reflux / overnight	No product	/
12	40% H_2SO_4	Reflux / 30 min	32.3	34

Three different libraries **1**, **2** and **3** with a total of 75 analogues of the 2-substituted-1,2,4-triazolo[1,5-*a*]pyrimidinone scaffold were then synthesised using the optimised reaction conditions summarised in Scheme 4.

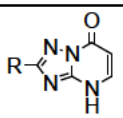
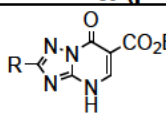
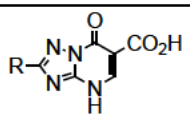
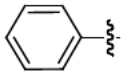
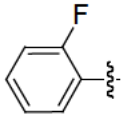


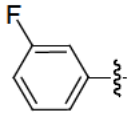
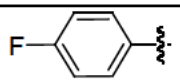
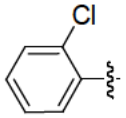
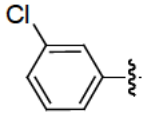
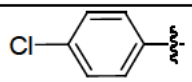
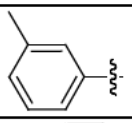
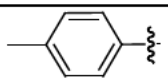
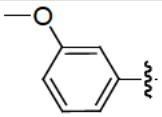
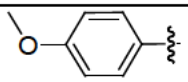
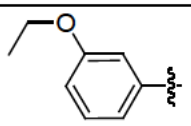
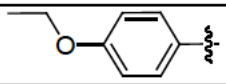
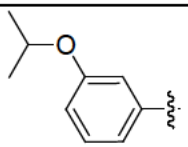
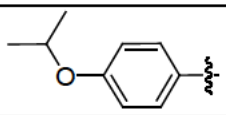
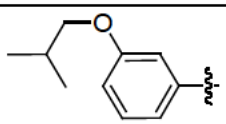
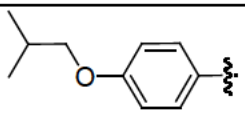
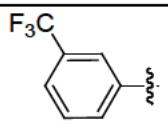
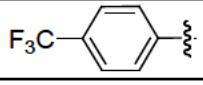
Scheme 4: i) AcOH, μW, 20 min, 150°C; ii) K_2CO_3 5%, μW, 15 min, 140°C; iii) NaH_2PO_4 5%, μW, 30 min, 190°C.

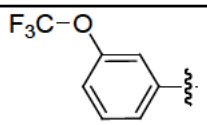
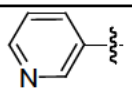
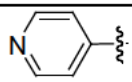
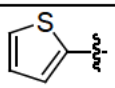
3.3 *In vitro* inhibition of XO

The XO inhibitory activity of compounds was assessed monitoring production of uric acid by oxidation of xanthine under the bovine milk XO catalysis. The concentration of uric acid was measured spectrophotometrically at 295 nm and rate of reaction was calculated in the absence of an inhibitor and at five different concentrations of each tested compound. Every sample was tested in triplicate and its average IC_{50} (i.e. the concentration of the inhibitor required to inhibit 50% of the enzyme activity) is reported in Table 5. Allopurinol was used as a reference and its IC_{50} value was estimated to be 18.81 μM.

Table 5. *In vitro* inhibition data of XO for all synthesised inhibitors.[a]

Compound	R	IC_{50} (μM)		
		 **1**	 **2**	 **3**
a	H	26.27 ± 1.61	65.34 ± 12.28	32.32 ± 1.15
b	MeS	28.96 ± 2.49	>100	22.79 ± 5.85
c		78.94 ± 7.62	31.10 ± 1.94	67.76 ± 9.04
d		33.43 ± 6.35	>100	39.17 ± 0.36

e		44.01 ± 3.49	76.50 ± 0.76	34.45 ± 5.49
f		28.80 ± 0.75	>100	37.28 ± 6.31
g		>100	>100	>100
h		16.76 ± 0.21	60.37 ± 12.18	14.90 ± 2.58
i		23.16 ± 0.22	>100	33.53 ± 4.07
j		1.92 ± 0.32	41.79 ± 7.61	33.53 ± 3.14
k		28.42 ± 2.63	48.97 ± 6.18	22.55 ± 3.52
l		13.49 ± 1.07	87.07 ± 9.14	12.12 ± 1.82
m		21.63 ± 1.85	54.77 ± 10.62	10.22 ± 1.68
n		2.27 ± 0.52	82.42 ± 11.13	5.76 ± 1.87
o		10.22 ± 0.78	42.97 ± 1.50	3.24 ± 0.15
p		1.73 ± 0.22	75.46 ± 3.43	12.95 ± 1.78
q		1.39 ± 0.07	53.22 ± 3.84	0.80 ± 0.08
r		5.26 ± 1.28	63.14 ± 6.24	18.21 ± 1.14
s		20.01 ± 1.34	>100	1.93 ± 0.30
t		1.01 ± 0.18	60.75 ± 16.04	2.90 ± 0.50
u		19.47 ± 6.88	55.58 ± 8.94	12.52 ± 1.94

v		6.94 ± 0.30	96.79 ± 2.19	10.39 ± 0.56
w		65.09 ± 5.73	>100	39.89 ± 4.76
x		12.09 ± 0.89	22.61 ± 2.04	46.20 ± 6.37
Y		28.60 ± 0.41	57.04 ± 9.62	38.83 ± 5.85
Allopurinol[b]		18.81 ± 1.78		

[a] Experiments were conducted at 37°C in 55 mM phosphate buffer at pH 7.5, 40 µM xanthine, 0.01 U/mL of XO and < 1% of DMSO.
[b] Positive control.

All ethyl 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate analogues (**2**) were weaker inhibitors than Allopurinol. The most active compound had a pyridin-4-yl group in position 2 (**2x**). A benzene ring could be tolerated in the same position (**2c**), but any substitution that was added to that ring was detrimental to the inhibitory activity. By contrast, most of the 2-substituted-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one analogues (**1**) and 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acids (**3**) exhibited either comparable or better activity than Allopurinol. When the R-substituent was just either a phenyl ring or a phenyl ring carrying a halogen atom, the inhibitory activity was either weaker or comparable to that of Allopurinol (**1c-g** and **1i**, **3c-g** and **3i**). The only exception was when a chlorine atom was placed in position 3 of the phenyl ring (**1h** and **3h**). The activity of the analogues of series **1** with the halogen substituent on the phenyl ring in either position 2 ("ortho") (**1d**) or 4 ("para") (**1f** and **1i**) was always improved compared to the corresponding analogues of series **3** (**3d**, **3f**, and **3i**). The opposite was observed when the halogen substituent was in position 3 ("meta") of the phenyl ring (**1e** and **1h** *vs.* **3e** and **3h**). When the R group of series **1** and **3** was different from a substituted phenyl ring, the resulting inhibitory activity was weaker than that of Allopurinol (**1b**, **1w, 1y, 3b**, **3w-y**,), with the exception of compound **1x,** which exhibited comparable activity to Allopurinol. When the R group was an alkyloxyphenyl substituent, the inhibitory activity was either comparable or better than that of Allopurinol (**1l-s**, **3l-s**). Analogues of series **3** with a phenyl ring carrying either Me (**3k**) or an alkyloxy group (**3m**, **3o**, **3q**, and **3s**) in position 4 of the phenyl ring always exhibited better activity than the corresponding analogue (**3j**, **3l**, **3n**, **3p**,

and **3r**) with the same substituent in position 3. The opposite was observed when the substituent on the phenyl ring was CF_3 (**3t** and **3u**). Interestingly, almost the opposite behaviour was observed within the compounds in series **1**. Having a phenyl ring carrying either Me (**1j**), alkyloxy (**1l**, **1n**, and **1r**) or CF_3 (**1t**) in position 3 of the phenyl ring resulted always in better activity than the corresponding analogues with the same substituent in position 4 (**1k**, **1m**, **1o**, **1s**, and **1u**). Having the isopropyloxy group either in position 3 or 4 of the phenyl ring in series **1** did not make any difference in inhibitory activity. Unsubstituted 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (**1a**) and the acid analogue (**3a**) demonstrated comparable activity to the same molecule carrying either a thiophene ring (**1y** and **3y**) or a methylthio group (**1b** and **3b**) in position 2 of the 1,2,4-triazolo[1,5-*a*]pyrimidine scaffold. Amongst all compounds in series **1**, the best inhibitory activity was observed for **1t,** and replacing the CF_3 group with a CF_3O group (**1v**) resulted in a 7-fold decrease in inhibitory activity. Amongst all compounds in series **3**, the best inhibitory activity was obtained by **3q**.

A clear picture of which substituent resulted in stronger inhibition can be seen when the top five active compounds are compared in each of series **1** and **3**:

Series **1**: 3-$CF_3C_6H_4$>4-*i*$PrOC_6H_4$>3-*i*$PrOC_6H_4$>3-MeC_6H_4>3-$EtOC_6H_4$
Series **3**: 4-*i*$PrOC_6H_4$>4-*i*$BuOC_6H_4$>3-$CF_3C_6H_4$>4-$EtOC_6H_4$>3-$EtOC_6H_4$

Bulky alkyloxy groups on the phenyl ring were preferred to smaller alkyloxy groups. The trifluoromethyl group provided good inhibitory activity in both series when in position 3 of the phenyl ring. Also, the methyl group provided better activity when in position 3 of the phenyl ring.

Enzyme kinetic studies were performed on the most active compounds (**1t** and **3q**). Each compound was tested at different concentrations of the substrate xanthine. The resulting Lineweaver-Burk plots (Figure 1) show a mixed type of inhibition for both compounds.

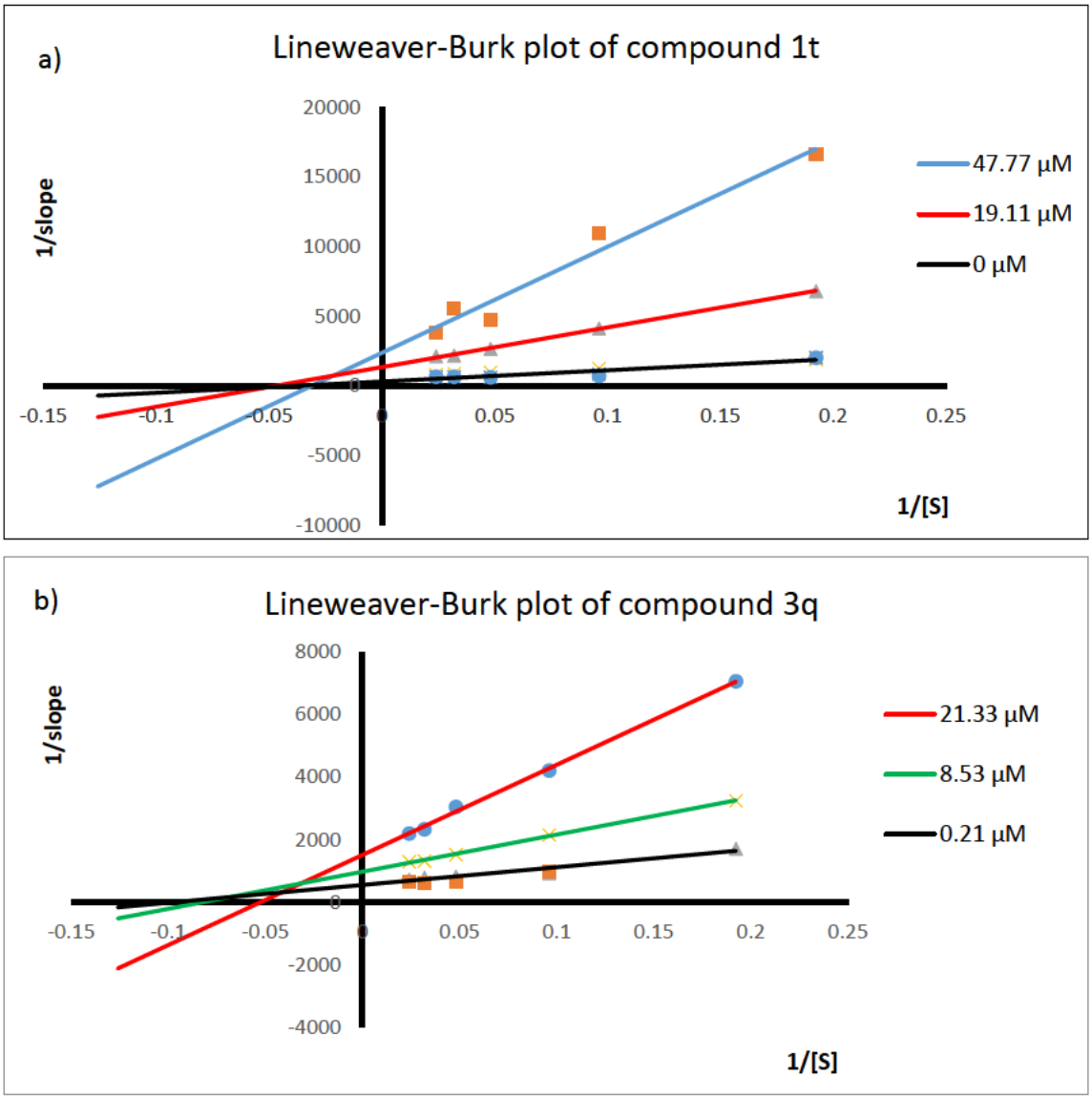
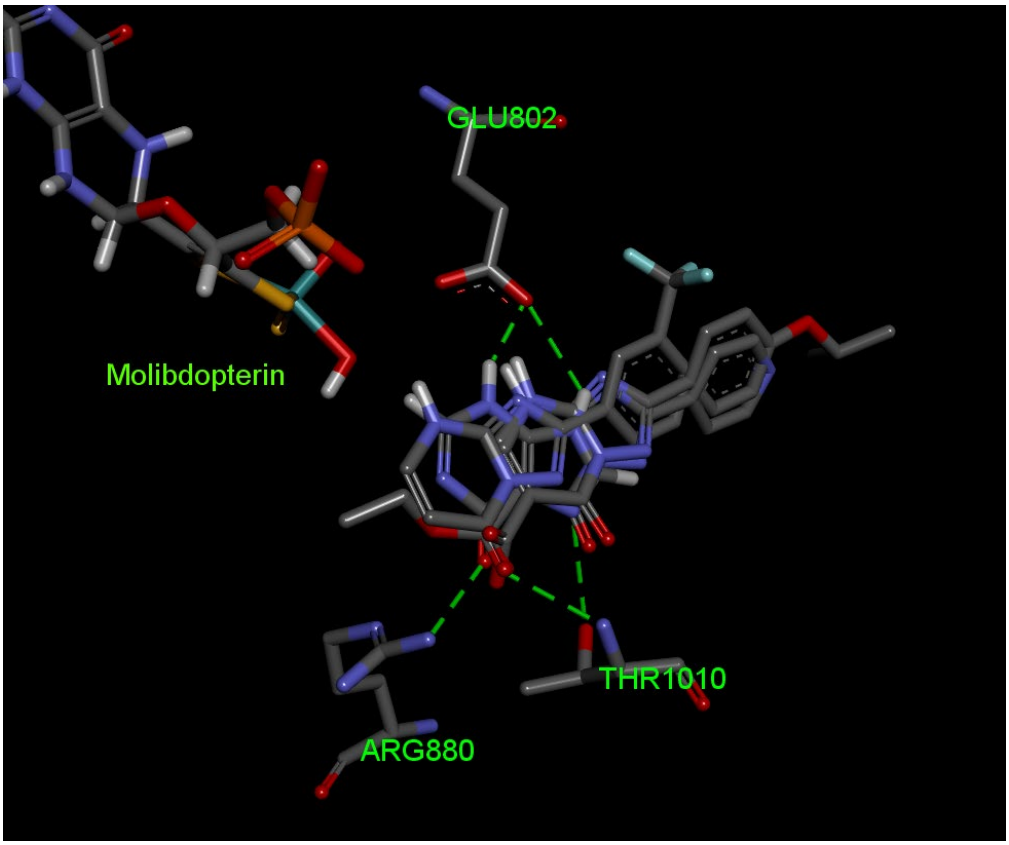


Fig 1: Lineweaver-Burk plot of compounds **1t** (a) and **3q** (b).

3.4 Molecular docking

The crystal structure of bovine milk XO in complex with hypoxanthine (PDB entry 3nrz) was used to study the interactions between the inhibitor and the enzyme. The internal energy of each molecule was minimized using Biovia Discovery Studio 2021 (Dassault Systèmes). The structure of the enzyme was prepared by removing the ligand hypoxanthine and all of the water molecules. CHARMM charges were assigned to both the enzyme and the inhibitors. Molecular docking simulations were then performed using Autodock 4.0[(210)] with an interaction grid of 0.375 Å spacing. The final predicted docked poses were visualised using Biovia Discovery Studio 2021 (Dassault Systèmes). Most of the poses of all the compounds belonging to series **1** and **3** were predicted to fit inside the enzyme

active site with the triazolopyrimidinone ring in the inner, deeper region, and the substituent in position 2 located towards the entrance of the active site. The compounds belonging to series **2** were generally predicted to have two main possible binding orientations: one with the substituent in position 2 towards the entrance of the active site, like in compounds **1** and **3**, and the other one with the substituent in position 2 oriented towards the Mo atom. The presence of these alternative binding modes could explain the lower inhibitory activity demonstrated by this type of analogues. Figure 2 shows the overlap of the most active inhibitors of each series (**1t**, **2x**, and **3q**) as an example along with their predicted binding poses. Hypoxanthine and its hydrogen bonds with the side chains of Glu802, Arg880 and Thr1010 have also been included in the figure to show the similarities between the inhibitors and the natural substrate. Compounds belonging to series **1** were predicted to fit deeper inside the active site than compounds belonging to series **2** and **3**.



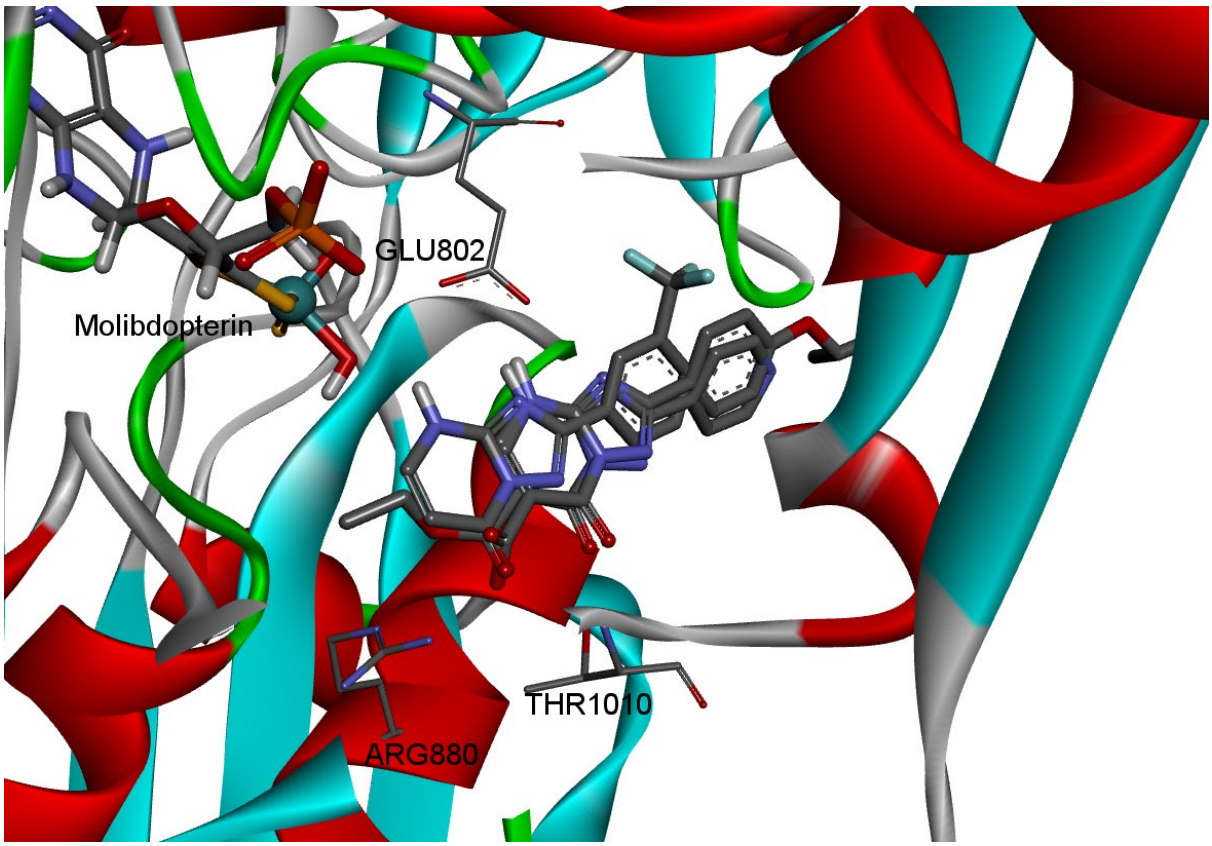


Fig 2: Visualisation of molecular docking predictions. (a) Predicted poses of compounds **1t**, **2x**, **3q**, overlapped with hypoxanthine. Predicted hydrogen bonds between hypoxanthine and Glu802, Arg880 and Thr1010 of the enzyme active site are shown with green dashed lines. (b) Predicted orientation of compounds **1t**, **2x**, and **3q**, with the heterocycle rings in the inner site of the enzyme and the substituents pointing towards the exit of the active site.

The deeper positioning of compounds belonging to series **1** inside the active site compared to the positioning of compounds belonging to series **2** and **3** is predicted to result in the loss of a hydrogen bond (H-bond) between the side chain of Glu802 and the NH in position 4, and a H-bond between the main chain of Val1011 and the C=O in position 7. The C=O group of compounds belonging to series **1** instead is predicted to form H-bonds with both side chains of Arg880 and Thr1010, and between the NH group in position 4 and the oxygen of the hydroxyl group in the molybdopterin co-factor bonded to the Mo atom.

Two-dimensional representation of the predicted drug-target interactions of compounds **1t**, **2x**, and **3q** in the active site of the enzyme are shown in Figure 3.

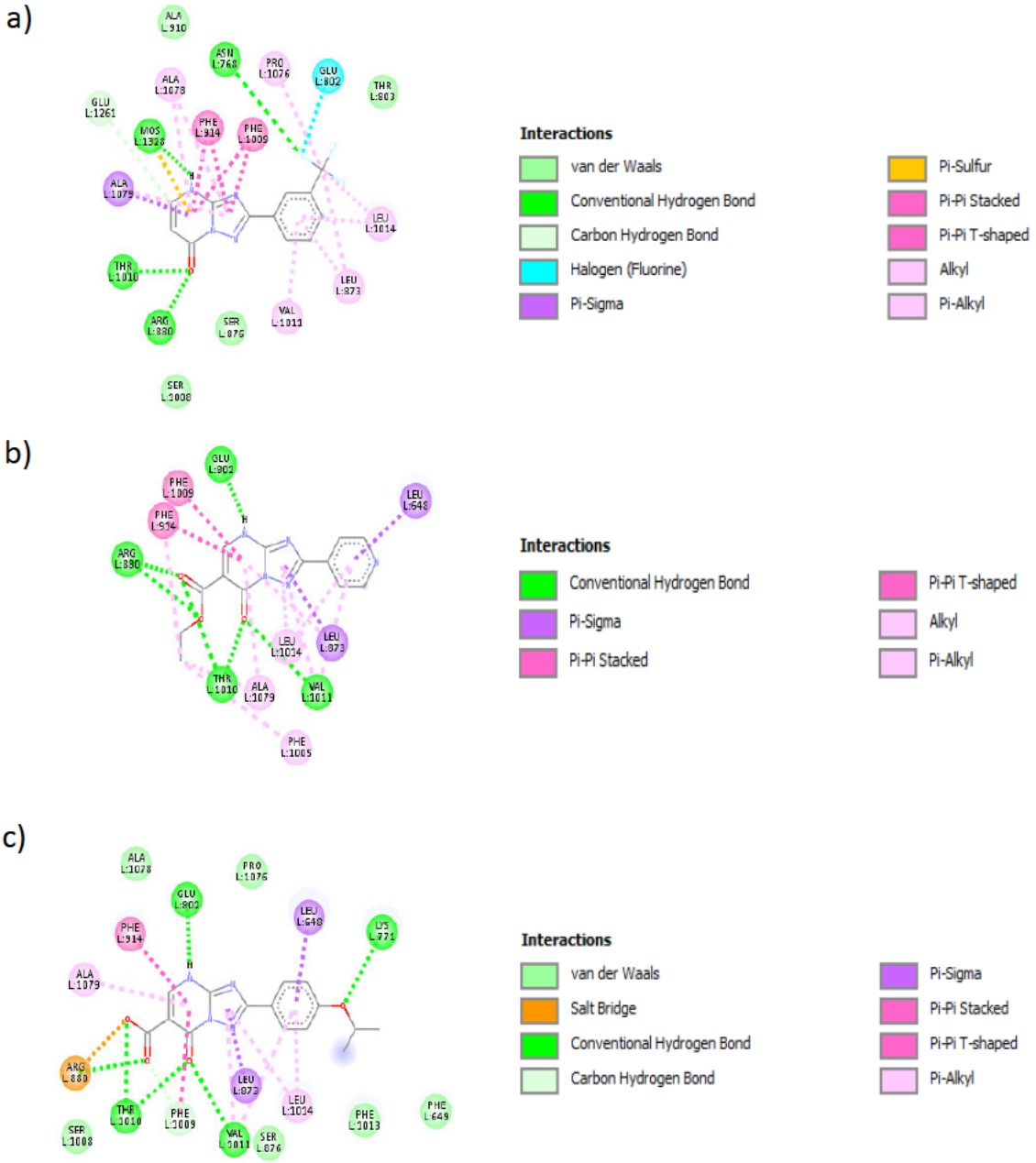


Fig 3: 2D representation of the predicted interactions between **1t** (a), **2x** (b), **3q** (c) and the active site of xanthine oxidase.

Analogues in series **1** have both triazole and pyrimidine rings predicted to interact with Phe914 and Phe1009, whilst compounds in series **2** and **3**, due to their more retrograde positions in the active site, are predicted to only have the pyrimidinone ring interact with them.

3.5 Conclusions

Facile methods for the synthesis of 2-substituted-7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidinone derivatives were developed using a microwave reactor. The optimised reaction conditions resulted in short reaction times. Three libraries of purine analogues, 75 compounds in total, were prepared and *in vitro* testing was performed on each compound to determine the inhibitory activity against XO. None of the prepared ethyl 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate analogues (**2**) demonstrate strong inhibitory activity, while most of the 2-substituted-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one analogues (**1**) and 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (**3**) exhibited good inhibitory activity. The strongest inhibitors of series **1** and **3** were, respectively, compound **1t** (19 times more active than Allopurinol) and compound **3q** (23 times more active than Allopurinol). Enzyme kinetics studies using the most active compounds (**1t** and **3q**) demonstrated a mixed type inhibition mechanism. Molecular docking simulations predicted that the two series of analogues **1** and **3** adopt the same orientation inside the enzyme active site, but the compounds belonging to series **1** are positioned deeper in the active site due to the absence of the carboxylic acid group. Compounds belonging to series **1** demonstrated comparable inhibitory activity to the compounds belonging to series **3,** except for a few cases. Bulky alkyloxy groups were among the best substituents on the phenyl ring. The presence of a carboxylic group on the analogues of type **3** allows for straightforward conversion of these compounds into the corresponding salts suitable for required drug formulations.

3.6 Experimental

3.6.1 Chemistry

Reagents were purchased either from Alfa Aesar or Sigma-Aldrich. Microwave assisted reactions were conducted using a CEM Discover SP instrument. Melting point were measured using an Electrothermal Digital melting point apparatus (IA9100). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance III 400 MHz spectrometer using DMSO as a solvent. Purity was measured using Agilent HPLC 1260 Infinity II with a PDA detector. Enzyme inhibition was evaluated using a Shimadzu UV-1280 spectrophotometer and 1 mL quartz

cuvette. Bovine milk XO was purchased from Sigma-Aldrich. Statistical analysis was performed using GraphPad Prism 9.2.0.

3.6.2 General method for the synthesis of ethyl 3(4)-alkyloxybenzoates (**6**)

The proper ethyl 3(4)-hydroxybenzoate (1 mmol) and potassium carbonate (1.1 mmol) were stirred in acetonitrile (11 mL) for five min at room temperature before adding the proper alkyl bromide (1.5 mmol). The mixture was heated until there was no sign of starting ethyl 3(4)-hydroxybenzoate by TLC. The solvent was evaporated under vacuum and the resulting oil was re-dissolved in dichloromethane (15 mL) and washed with 5% potassium carbonate solution (3 x 15 mL). The combined organic fractions were dried over sodium sulphate, filtered and evaporated under vacuum to afford the desired ethyl 3(4)-alkyloxybenzoate.

Ethyl 3-*iso*propyloxyphenylbenzoate (6p)

Ethyl 3-hydroxybenzoate was reacted with 2-bromopropane as per general method 3.6.2 to yield **6p** (84%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.51 (ddd, 1H, ^{3}J=7.7 Hz, ^{4}J=1.3 Hz, ^{4}J=1.3 Hz, H-6); 7.42 (t, 1H, ^{3}J=7.9 Hz, H-5); 7.41 (dd, 1H, ^{4}J=2.6 Hz, ^{4}J=1.6 Hz, H-2); 7.20 (ddd, 1H, ^{3}J=8.2 Hz, ^{4}J=2.7 Hz, ^{4}J=1.0 Hz, H-4); 4.67 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 4.30 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.31 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H_3}$); 1.28 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 157.5 (C-3); 131.3 (C-1); 130.0 (C-5); 121.1 (C-6); 120.6 (C-4); 115.8 (C-2); 69.6 (CH); 60.8 (CH_2); 21.7 (CH(<u>C</u>$H_3)_2$); 14.1 (CH_2<u>C</u>H_3).

Ethyl 4-*iso*propyloxyphenylbenzoate (6q)

Ethyl 4-hydroxybenzoate was reacted with 2-bromopropane as per general method 3.6.2 to yield **6q** (64%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.88 (d, 2H, ^{3}J=9.0 Hz, H-2 and H-6); 7.01 (d, 2H, ^{3}J=9.0 Hz, H-3 and H-5); 4.72 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 4.27 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H_3}$); 1.29 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.4 (C=O); 161.4 (C-4); 131.2 (C-2 and C-6); 121.7 (C-1); 115.2 (C-3 and C-5); 69.6 (CH); 60.2 (CH_2); 21.6 (CH(<u>C</u>$H_3)_2$); 14.2 (CH_2<u>C</u>H_3).

Ethyl 3-*iso*butyloxyphenylbenzoate (6r)

Ethyl 3-hydroxybenzoate was reacted with 1-bromo-2-methylpropane as per general method 3.6.2 to yield **6r** (61%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.53 (ddd, 1H, ^{3}J=7.6 Hz, ^{4}J=1.3 Hz, ^{4}J=1.3 Hz, H-6); 7.43 (dd, 1H, ^{4}J=1.6 Hz, H-2); 7.42 (t, 1H, ^{3}J=7.9 Hz, H-5); 7.22 (ddd, 1H, ^{3}J=8.2 Hz, ^{4}J=2.6 Hz, ^{4}J=1.0 Hz, H-4); 4.31 (q, 2H, ^{3}J=7.1 Hz, O$\underline{CH_2}$CH$_3$); 3.80 (d, 2H, ^{3}J=6.5 Hz, $\underline{CH_2}$CH(CH$_3$)$_2$); 2.02 (m, 1H, ^{3}J=6.6 Hz, CH$_2$C$\underline{H}$(CH$_3$)$_2$); 1.32 (t, 3H, ^{3}J=7.1 Hz, OCH$_2$C$\underline{H_3}$); 0.99 (d, 6H, ^{3}J=6.7 Hz, CH$_2$CH(C$\underline{H_3}$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 158.9 (C-3); 131.3 (C-1); 130.0 (C-5); 121.3 (C-6); 119.6 (C-4); 114.5 (C-2); 74.0 ($\underline{C}$H$_2$CH(CH$_3$)$_2$); 60.8 (O$\underline{C}$H$_2$CH$_3$); 27.7 (CH$_2\underline{C}$H(CH$_3$)$_2$); 19.0 (2C, CH$_2$CH($\underline{C}$H$_3$)$_2$); 14.2 (OCH$_2\underline{C}$H$_3$).

Ethyl 4-*iso*butyloxyphenylbenzoate (6s)

Ethyl 4-hydroxybenzoate was reacted with 1-bromo-2-methylpropane as per general method 3.6.2 to yield **6s** (66%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.89 (d, 2H, ^{3}J=9.0 Hz, H-2 and H-6); 7.03 (d, 2H, ^{3}J=9.0 Hz, H-3 and H-5); 4.27 (q, 2H, ^{3}J=7.1 Hz, O$\underline{CH_2}$CH$_3$); 3.82 (d, 2H, ^{3}J=6.5 Hz, $\underline{CH_2}$CH(CH$_3$)$_2$); 2.03 (m, 1H, ^{3}J=6.7 Hz, CH$_2$C$\underline{H}$(CH$_3$)$_2$); 1.30 (t, 3H, ^{3}J=7.1 Hz, OCH$_2$C$\underline{H_3}$); 0.98 (d, 6H, ^{3}J=6.7 Hz, CH$_2$CH(C$\underline{H_3}$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.5 (C=O); 162.7 (C-4); 131.2 (C-2 and C-6); 122.0 (C-1); 114.5 (C-3 and C-5); 74.0 ($\underline{C}$H$_2$CH(CH$_3$)$_2$); 60.3 (O$\underline{C}$H$_2$CH$_3$); 27.7 (CH$_2\underline{C}$H(CH$_3$)$_2$); 19.0 (2C, CH$_2$CH($\underline{C}$H$_3$)$_2$); 14.3 (OCH$_2\underline{C}$H$_3$).

3.6.3 General method for the synthesis of benzhydrazide derivatives (**7**)

Hydrazine (1.2 mmol) was added to a stirring solution of the substituted ethyl benzoate (1 mmol) in methanol (20 mL). The mixture was stirred at reflux temperature for 4 h. After this time the reaction was left to cool down to room temperature and the solid was filtered and washed with water before drying it in vacuum oven at 40ºC overnight.

3-Methylbenzhydrazide (7j)

Ethyl 3-methylbenzoate was reacted as per general method 3.6.3 to yield **7j** (82%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.69 (br, 1H, NH); 7.64 (br, 1H, H-2); 7.63 – 7.57 (m, 1H, H-6); 7.35 – 7.28 (m, 2H, H-4 and H-5); 4.46 (br, 2H, NH$_2$);

2.34 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.0 (C=O); 137.5 (C-3); 133.3 (C-1); 131.6 (C-4); 128.2 (C-5); 127.6 (C-2); 124.0 (C-6); 20.9 (CH_3).

3-Ethoxybenzhydrazide (7n)

Compound **6n** was reacted as per the general method 3.6.3 to yield **7n** (89%). *Anal* ^1H NMR (400 MHz, DMSO-d_6) δ: 9.73 (br, 1H, NH); 7.42 – 7.29 (m, 3H, H-2, H-5 and H-6); 7.08 – 7.01 (m, 1H, H-4); 4.47 (br, 2H, NH_2); 4.06 (q, 2H, ^3J=7.0 Hz, CH_2); 1.33 (t, 3H, ^3J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 158.4 (C-3); 134.7 (C-1); 129.4 (C-5); 119.1 (C-6); 117.4 (C-4); 112.5 (C-2); 63.1 (CH_2); 14.6 (CH_3).

4-Ethoxybenzhydrazide (7o)

Compound **6o** was reacted as per the general method 3.6.3 to yield **7o** (96%). *Anal* ^1H NMR (400 MHz, DMSO-d_6) δ: 9.58 (br, 1H, NH); 7.78 (d, 2H, ^3J=8.9 Hz, H-2 and H-6); 6.95 (d, 2H, ^3J=8.9 Hz, H-3 and H-5); 4.40 (br, 2H, NH_2); 4.07 (q, 2H, ^3J=7.0 Hz, CH_2); 1.33 (t, 3H, ^3J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (CO); 160.7 (C-4'); 128.4 (C-2 and C-6); 125.3 (C-1); 113.9 (C-3 and C-5); 63.2 (CH_2); 14.5 (CH_3).

3-*iso*Propyloxybenzhydrazide (7p)

Compound **6p** was reacted as per the general method 3.6.3 to yield **7p** (81%). *Anal* ^1H NMR (400 MHz, DMSO-d_6) δ: 9.72 (br, 1H, NH); 7.40 – 7.28 (m, 3H, H-2, H-5, and H-6); 7.03 (ddd, 1H, ^3J=7.8 Hz, ^4J=2.5 Hz, ^4J=1.3 Hz, H-4); 4.65 (m, 1H, ^3J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 4.46 (br, 2H, NH_2); 1.27 (d, 6H, ^3J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 157.3 (C-3); 134.7 (C-1); 129.5 (C-5); 119.0 (C-6); 118.5 (C-4); 113.7 (C-2); 69.3 (<u>C</u>H$(CH_3)_2$); 21.8 (2C, CH(<u>C</u>H$_3)_2$).

4-*iso*Propyloxybenzhydrazide (7q)

Compound **6q** was reacted as per the general method 3.6.3 to yield **7q** (79%). The reaction was refluxed for 4 h. *Anal* ^1H NMR (400 MHz, DMSO-d_6) δ: 9.57 (br, 1H, NH); 7.77 (d, 2H, ^3J=8.9 Hz, H-2 and H-6); 6.94 (d, 2H, ^3J=8.9 Hz, H-3 and H-5); 4.68 (m, 1H, ^3J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 4.39 (br, 2H, NH_2); 1.27 (d, 6H, ^3J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 159.7 (C-4);

128.7 (C-2 and C-6); 125.1 (C-1); 114.8 (C-3 and C-5); 69.3 ($\underline{C}H(CH_3)_2$); 21.7 (2C, $CH(\underline{C}H_3)_2$).

3-*iso*Butyloxybenzhydrazide (7r)

Compound **6r** was reacted as per the general method 3.6.3 to yield **7r** (96%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (br, 1H, NH); 7.41 – 7.35 (m, 2H, H-2 and H-6); 7.33 (t, 1H, ^3J=7.8 Hz, H-5); 7.05 (ddd, 1H, ^3J=8.0 Hz, ^4J=2.6 Hz, ^4J=1.2 Hz, H-4); 4.47 (br, 2H, NH_2); 3.78 (d, 2H, ^3J=6.6 Hz, CH_2); 2.02 (m, 1H, ^3J=6.7 Hz, $C\underline{H}(CH_3)_2$); 0.98 (d, 6H, ^3J=6.7 Hz, $CH(C\underline{H_3})_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 158.7 (C-3); 134.6 (C-1); 129.4 (C-5); 119.1 (C-6); 117.5 (C-4); 112.6 (C-2); 73.9 (CH_2); 27.7 ($\underline{C}H(CH_3)_2$); 19.0 (2C, $CH(\underline{C}H_3)_2$).

4-*iso*Butyloxybenzhydrazide (7s)

Compound **6s** was reacted as per the general method 3.6.3 to yield **7s** (89%). The reaction was refluxed for 4 h. *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.58 (br, 1H, NH); 7.78 (d, 2H, ^3J=8.9 Hz, H-2 and H-6); 6.96 (d, 2H, ^3J=8.9 Hz, H-3 and H-5); 4.40 (br, 2H, NH_2); 3.79 (d, 2H, ^3J=6.5 Hz, CH_2); 2.02 (m, 1H, ^3J=6.6 Hz, $C\underline{H}(CH_3)_2$); 0.98 (d, 6H, ^3J=6.7 Hz, $CH(C\underline{H_3})_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (C=O); 161.0 (C-4); 128.7 (C-2 and C-6); 125.3 (C-1); 114.0 (C-3 and C-5); 74.3 (CH_2); 27.6 ($\underline{C}H(CH_3)_2$); 19.0 (2C, $CH(\underline{C}H_3)_2$)

3.6.4 General method for the synthesis of 3-substituted-5-amino triazoles (**10**)

a) Isothiourea hemisulphate (60 mmol) was added portionwise to a solution of sodium hydroxide (60 mmol) in water (100 mL) maintaining the reaction flask in an ice/water bath. The proper benzhydrazide (43 mmol) was then added portionwise to the above slurry whilst still maintaining the reaction flask in an ice/water bath. The mixture was left to stir at room temperature and the progress of the reaction was controlled by thin layer chromatography (TLC) until all the starting material was consumed. When no more starting benzhydrazide was observed by TLC, the reaction mixture was filtered and the solid was washed with water (5 x 100 mL). The wet solid was transferred into a microwave reaction tube and suspended in water (13 mL). The mixture was reacted in the microwave instrument at 170°C for 10 minutes. After this time, the solid was filtered

and washed with water before drying it overnight in a vacuum oven at 60°C.

b) The mixture of the proper substituted acid chloride (40 mmol) and aminoguanidine hydrochloride (80 mmol) was warmed up to 170°C in an oil bath while stirring with a glass rod. After five min, the mixture consistency was changed. The mixture was kept at 170°C for a further five min whilst stirring before removing it from the oil bath. Hot water (40 mL) and sodium hydroxide (120 mmol) were added and the mixture was left to stir for five minutes at room temperature. The mixture was then filtered and the solid was washed with water before transferring it into a microwave reaction tube. Fresh water was added (30 mL) and the mixture was reacted in the microwave instrument at 170°C for 10 min. After cooling, the precipitated solid was filtered and washed with water before drying it overnight in a vacuum oven at 60°C.

5-Amino-3-(2-chlorophenyl)-1,2,4-triazole (10g)

Compound **9g** was reacted as per the general method 3.6.4b to yield **10g** (80%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.98* and 12.17 (br, 1H, NH); 7.85 – 7.75 (m, 1H, H-6'); 7.53 – 7.43 (m, 1H, H-3'); 7.41 – 7.31 (m, 2H, H-4' and H-5'); 6.07 and 5.31* (br, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.1 (C-3); 156.8 (C-5); 131.2 (C-1' and C-2'); 131.0 (C-4'); 130.4 (C-6'); 129.6 (C-3'); 126.9 (C-5'). * - signals of the minor tautomer

5-Amino-3-(3-methylphenyl)-1,2,4-triazole (10j)

Compound **7j** was reacted as per the general method 3.6.4a to yield **7j** (41%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.13* and 12.02 (br, 1H, NH); 7.73 – 7.69 (m, 1H, H-6'); 7.69 – 7.63 (m, 1H, H-2'); 7.40 – 7.20 (m, 1H, H-5'); 7.20 – 7.06 (m, 1H, H-4'); 6.01 and 5.24* (br, 2H, NH_2); 2.33 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.5 (C-5); 157.2 (C-3); 137.3 (C-3'), 132.3 (C-1'); 128.7 (C-2'); 128.3 (C-4'); 126.0 (C-5'); 122.5 (C-6'); 21.1 (CH_3). * - signals of the minor tautomer.

5-Amino-3-(3-ethoxyphenyl)-1,2,4-triazole (10n)

Compound **7n** was reacted as per the general method 3.6.4a to yield **10n** (65%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.21* and 12.08 (br, 1H, NH); 7.53 – 7.45

(m, 1H, H-6'); 7.45 – 7.39 (m, 1H, H-2'); 7.36 – 7.21 (m, 1H, H-5'); 7.02 – 6.76 (m, 1H, H-4'); 6.11 and 5.35* (br, 2H, NH_2); 4.03 (q, 2H, ^3J=6.9 Hz, CH_2); 1.32 (t, 3H, ^3J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 158.6 (C-3'); 158.4 (C-5); 157.4 (C-3); 133.8 (C-1'); 129.5 (C-5'); 117.7 (C-6'); 114.6 (C-4'); 110.9 (C-2'); 63.0 (CH_2); 14.7 (CH_3). * - signals of the minor tautomer.

5-Amino-3-(4-ethoxyphenyl)-1,2,4-triazole (10o)

Compound **7o** was reacted as per the general method 3.6.4a to yield **10o** (59%). *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 12.96* and 11.89 (br, 1H, NH); 7.80 (d, 2H, ^3J=8.8 Hz, H-2' and H-6'); 7.07 - 6.87 (m, 2H, H-3' and H-5'); 5.97 and 5.20* (br, 2H, NH_2); 4.04 (q, 2H, ^3J=6.9 Hz, CH_2); 1.33 (t, 3H, ^3J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 158.6 (C-4'); 158.3 (C-5); 157.2 (C-3); 126.7 (C-2' and C-6'); 125.0 (C-1'); 114.2 (C-3' and C-5'); 63.0 (CH_2); 14.6 (CH_3). * - signals of the minor tautomer.

5-Amino-3-(3-*iso*propyloxyphenyl)-1,2,4-triazole (10p)

Compound **7p** was reacted as per the general method 3.6.4a to yield **10p** (56%). *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 13.14* and 12.03 (br, 1H, NH); 7.48 – 7.41 (m, 1H, H-6'); 7.41 – 7.36 (m, 1H, H-2'); 7.27 (t, 1H, ^3J=7.8 Hz, H-5'); 7.00 – 6.78 (m, 1H, H-4'); 6.04 and 5.27* (br, 2H, NH_2); 4.60 (m, 1H, ^3J=5.9 Hz, C<u>H</u>$(CH_3)_2$); 1.27 (d, 6H, ^3J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 158.3 (C-5); 157.5 (C-3'); 157.3 (C-3); 133.8 (C-1'); 129.5 (C-5'); 117.5 (C-6'); 115.8 (C-4'); 112.0 (C-2'); 69.2 (<u>C</u>H$(CH_3)_2$); 21.8 (2C, CH(<u>C</u>H$_3)_2$). * - signals of the minor tautomer.

5-Amino-3-(4-*iso*propyloxyphenyl)-1,2,4-triazole (10q)

Compound **7q** was reacted as per the general method 3.6.4a to yield **10q** (72%). *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 12.95* and 11.89 (br, 1H, NH); 7.77 (d, 2H, ^3J=8.8 Hz, H-2' and H-6'); 6.91 (d, 2H, ^3J=8.3 Hz, H-3' and H-5'); 5.97 and 5.19* (br, 2H, NH_2); 4.62 (m, 1H, ^3J=5.9 Hz, C<u>H</u>$(CH_3)_2$); 1.27 (d, 6H, ^3J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 158.3 (C-5); 157.5 (C-4'); 157.1 (C-3); 126.7 (C-2' and C-6'); 124.8 (C-1'); 115.3 (C-3' and C-5'); 69.1 (<u>C</u>H$(CH_3)_2$); 21.8 (2C, CH(<u>C</u>H$_3)_2$). * - signals of the minor tautomer.

5-Amino-3-(3-*iso*butyloxyphenyl)-1,2,4-triazole (10r)

Compound **7r** was reacted as per the general method 3.6.4a to yield **10r** (20%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.16* and 12.06 (br, 1H, NH); 7.45 (dt, 1H, ^{3}J=7.6 Hz, ^{4}J=1.0 Hz, H-6'); 7.43 – 7.39 (m, 1H, H-2'); 7.28 (t, 1H, ^{3}J=7.8 Hz, H-5'); 6.93 – 6.84 (m, 1H, H-4'); 6.04 and 5.27* (br, 2H, NH_2); 3.76 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.03 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 0.99 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.8 (C-3'); 158.3 (C-5); 157.2 (C-3); 133.7 (C-1'); 129.4 (C-5'); 117.6 (C-6'); 114.6 (C-4'); 110.9 (C-2'); 73.7 (CH_2); 27.7 (<u>C</u>H$(CH_3)_2$); 19.0 (2C, CH(<u>C</u>H$_3)_2$). * - signals of the minor tautomer.

5-Amino-3-(4-*iso*butyloxyphenyl)-1,2,4-triazole (10s)

Compound **7s** was reacted as per the general method 3.6.4a to yield **10s** (66%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.95* and 11.88 (br, 1H, NH); 7.78 (d, 2H, ^{3}J=8.8 Hz, H-2' and H-6'); 6.93 (d, 2H, ^{3}J=8.7 Hz, H-3' and H-5'); 5.96 and 5.18* (br, 2H, NH_2); 3.76 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.02 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 0.98 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.9 (C-4'); 158.3 (C-5); 157.2 (C-3); 126.7 (C-2' and C-6'); 125.0 (C-1'); 114.3 (C-3' and C-5'); 73.8 (CH_2); 27.7 (<u>C</u>H$(CH_3)_2$); 19.1 (2C, CH(<u>C</u>H$_3)_2$). * - signals of the minor tautomer.

5-Amino-3-(3-trifluoromethylphenyl)-1,2,4-triazole (10t)

Compound **9t** was reacted as per the general method 3.6.4b to yield **10t** (79%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.45* and 12.24 (br, 1H, NH); 8.24 – 8.09 (m, 2H, H-2' and H-4'); 7.80 – 7.60 (m, 2H, H-5' and H-6'); 6.17 and 5.39* (br, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.6 (C-5); 157.1 (C-3); 133.3 (C-6'); 129.7 (C-5'); 129.3 (q, $^{2}J_{CF}$=30.5 Hz, C-3'); 128.9 (C-1'); 124.6 (q, $^{3}J_{CF}$=3.5 Hz, C-2'); 124.2 (q, $^{1}J_{CF}$=272.2 Hz, CF_3); 121.4 (q, $^{3}J_{CF}$=4.0 Hz, C-4'). * - signals of the minor tautomer.

5-Amino-3-(3-trifluoromethoxylphenyl)-1,2,4-triazole (10v)

Compound **9v** was reacted as per the general method 3.6.4b to yield **10v** (82%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.40* and 12.20 (br, 1H, NH); 7.90 (dt, 1H, ^{3}J=7.6 Hz, ^{4}J=0.8 Hz, H-6'); 7.80 – 7.73 (m, 1H, H 2'); 7.54 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.39 – 7.27 (m, 1H, H-4'); 6.15 and 5.38* (br, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.5 (C-5); 157.1 (C-3); 149.1 (q, $^{3}J_{CF}$=1.7 Hz, C-3'); 134.6 (C-1');

130.6 (C-5'); 124.1 (C-6'); 120.5 (C-4'); 120.6 (q, $^{1}J_{CF}$=256.3 Hz, CF_3); 117.1 (C-2'). * - signals of the minor tautomer.

5-Amino-3-(pyridin-4-yl)-1,2,4-triazole (10x)

Compound **7x** was reacted as per the general method 3.6.4a to yield **10x** (72%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.35 (br, 1H, NH); 8.59 (dd, 2H, ^{3}J=4.6 Hz, ^{4}J=1.4 Hz, H-2' and H-6'); 7.77 (dd, 2H, ^{3}J=4.5 Hz, ^{4}J=1.6 Hz, H-3' and H-5'); 6.20 (br, 2H, NH_2).

5-Amino-3-(thien-2-yl)-1,2,4-triazole (10y)

Compound **11** was reacted as per the general method 3.6.4b to yield **10y** (76%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.17* and 12.01 (br, 1H, NH); 7.50 – 7.43 (m, 1H, H-5'); 7.42 – 7.35 (m, 1H, H-3'); 7.07 (dd, 1H, ^{3}J=4.8 Hz, ^{3}J=3.7 Hz, H-4'); 6.08 and 5.29* (br, 2H, NH_2). * - signals of the minor tautomer.

3.6.5 General method for the synthesis of ethyl 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylates (**2a-y**)

The appropriate 5-aminotriazole (1 mmol) was dissolved in 2 mL of glacial acetic acid in a microwave glass tube. Diethyl ethoxymethylene malonate (1.2 mmol) was added to the above solution before heating it at 150°C for 20 minutes in a microwave reactor. The precipitate formed after cooling to room temperature, was filtered and the product was washed on the filter with water before drying it in a vacuum oven at 60°C overnight. Copies of NMR spectra are reported in Appendix 2.

Ethyl 7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2a).

Compound **10a** was reacted as per the general method 3.6.5 to yield **2a** (46%); Purity 97.8%; mp 328 – 330°C [Lit.[201] >300°C]; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (s, 1H, H-5); 7.95 (s, 1H, H-2); 4.16 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.25 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.9 ($\underline{C}O_2CH_2CH_3$); 159.1 (C-2); 157.2 (C-3a); 155.6 (C-5); 152.2 (C-7); 98.2 (C-6); 58.6 (CH_2); 14.5 (CH_3).

Ethyl 2-methylthio-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2b).

Compound **10b** was reacted as per the general method 3.6.5 to yield **2b** (46%); Purity 99.7%; mp 306 – 308°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.56 (s, 1H, H-5); 4.24 (q, 2H, ^{3}J=7.1 Hz, CH_2); 2.60 (s, 3H, SCH_3); 1.28 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H_3}$). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.7 ($\underline{C}O_2CH_2CH_3$); 163.0 (C-2); 152.0 (C-3a); 151.1 (C-5); 146.2 (C-7); 102.8 (C-6); 60.4 (CH_2); 14.2 ($CH_2\underline{C}H_3$); 13.5 (SCH_3).

Ethyl 2-phenyl-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2c).

Compound **10c** was reacted as per the general method 3.6.5 to yield **2c** (59%); Purity 96.8%; mp 307 – 308°C [Lit.[201] >300°C]; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.64 (s, 1H, H-5); 8.20 – 8.05 (m, 2H, H-2' and H-6'); 7.45 – 7.63 (m, 3H, H-3', H-4' and H-5'); 4.26 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.0 ($\underline{C}O_2CH_2CH_3$); 161.1 (C-2); 152.8 (C-3a); 151.1 (C-5); 146.8 (C-7); 130.6 (C-4'); 129.7 (C-1'); 129.0 (C-3' and C-5'); 126.7 (C-2' and C-6'); 102.8 (C-6); 60.4 (CH_2); 14.2 (CH_3).

Ethyl 2-(2-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2d).

Compound **10d** was reacted as per the general method 3.6.5 to yield **2d** (52%); Purity 99.2%; mp 288 – 289°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.67 (s, 1H, H-5); 8.09 (ddd, 1H, ^{3}J=7.9 Hz, $^{4}J_{HF}$=7.5 Hz, ^{4}J=1.8 Hz, H-6'); 7.59 (tdd, ^{3}J=8.3 Hz, $^{4}J_{HF}$=5.0 Hz, ^{4}J=1.8 Hz, 1H, H-4'); 7.45 – 7.34 (m, 2H, H-3' and H-5'); 4.26 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, *J*=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 162.9 ($\underline{C}O_2CH_2CH_3$); 159.9 (C-2', $^{1}J_{CF}$=255.2 Hz); 157.9 (C-2, $^{3}J_{CF}$=4.3 Hz); 152.7 (C-3a); 150.5 (C-5); 146.6 (C-7); 132.4 (C-4', $^{3}J_{CF}$=8.4 Hz); 130.4 (C-5', $^{4}J_{CF}$=2.1 Hz); 124.8 (C-6', $^{3}J_{CF}$=3.5 Hz); 117.7 (C-1', $^{2}J_{CF}$=11.2 Hz); 116.8 (C-3', $^{2}J_{CF}$=21.2 Hz); 102.8 (C-6); 60.4 (CH_2); 14.2 (CH_3).

Ethyl 2-(3-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2e).

Compound **10e** was reacted as per the general method 3.6.5 to yield **2e** (63%); Purity 98.1%; mp 304 – 305°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.66 (s, 1H, H-5); 7.98 (ddd, 1H, ^{3}J=8.1 Hz, ^{4}J=1.0 Hz, ^{4}J=1.0 Hz, H-6'); 7.82 (ddd, 1H, $^{3}J_{HF}$=9.9 Hz, ^{4}J=2.6 Hz, ^{4}J=1.4 Hz, H-2'); 7.61 (td, 1H, ^{3}J=8.0 Hz, $^{4}J_{HF}$=5.9 Hz, H-

5'); 7.39 (dddd, 1H, $^{3}J_{HF}$=8.6 Hz, ^{3}J=8.6 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 4.26 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.5 ($\underline{C}O_2CH_2CH_3$); 162.3 (C-3', $^{1}J_{CF}$=243.8 Hz); 160.0 (C-2); 152.7 (C-3a); 151.3 (C-5); 147.0 (C-7); 132.1 (C-1', $^{3}J_{CF}$=8.4 Hz); 131.2 (C-5', $^{3}J_{CF}$=8.4 Hz); 122.7 (C-6', $^{4}J_{CF}$=2.7 Hz); 117.3 (C-4', $^{2}J_{CF}$=21.2 Hz); 113.0 (C-2', $^{2}J_{CF}$=23.3 Hz); 102.6 (C-6); 60.3 (CH_2); 14.1 (CH_3).

Ethyl 2-(4-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2f).

Compound **10f** was reacted as per the general method 3.6.5 to yield **2f** (78%); Purity 97.8%; mp 332 – 333°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.64 (s, 1H, H-5); 8.16 (dd, 2H, ^{3}J=8.9 Hz, $^{4}J_{HF}$=5.5 Hz, H-2' and H-6'); 7.38 (dd, 2H, ^{3}J=8.9 Hz, $^{3}J_{HF}$=8.9 Hz, H-3' and H-5'); 4.26 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.5 (C-4', $^{1}J_{CF}$=247.9 Hz); 163.0 ($\underline{C}O_2CH_2CH_3$); 160.3 (C-2); 152.8 (C-3a); 151.1 (C-5); 146.8 (C-7); 129.1 (C-2' and C-6', $^{3}J_{CF}$=8.8 Hz); 126.3 (C-1', $^{4}J_{CF}$=3.0 Hz); 116.1 (C-3' and C-5', $^{2}J_{CF}$=22.0 Hz); 102.8 (C-6); 60.4 (CH_2); 14.2 (CH_3).

Ethyl 2-(2-chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2g).

Compound **10g** was reacted as per the general method 3.6.5 to yield **2g** (58%); Purity 99.2%; mp 281 – 282°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.67 (s, 1H, H-5); 7.94 (dd, 1H, ^{3}J=7.5 Hz, ^{4}J=1.9 Hz, H-3'); 7.64 (dd, 1H, ^{3}J=7.6 Hz, ^{4}J=1.6 Hz, H-6'); 7.59 – 7.48 (m, 2H, H-4' and H-5'); 4.27 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.9 ($\underline{C}O_2CH_2CH_3$); 160.1 (C-2); 152.7 (C-3a); 150.3 (C-5); 146.8 (C-7); 132.0 (C-1'); 131.8 (C-2'); 131.6 (C-4'); 130.8 (C-6'); 128.9 (C-3'); 127.4 (C-5'); 102.8 (C-6); 60.4 (CH_2); 14.2 (CH_3).

Ethyl 2-(3-chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2h).

Compound **10h** was reacted as per the general method 3.6.5 to yield **2h** (75%); Purity 97.2%; mp 319 – 321°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.66 (s, 1H, H-5); 8.12 – 8.05 (m, 2H, H-2' and H-4'); 7.64 – 7.56 (m, 2H, H-5' and H-6'); 4.27 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz,

DMSO-*d*$_6$) δ: 163.0 (<u>C</u>O$_2$CH$_2$CH$_3$); 159.9 (C-2); 152.8 (C-3a); 151.3 (C-5); 147.0 (C-7); 133.7 (C-1'); 131.9 (C-3'); 131.1 (C-4'); 130.3 (C-5'); 126.1 (C-2'); 125.3 (C-6'); 102.8 (C-6); 60.4 (CH$_2$); 14.2 (CH$_3$).

Ethyl 2-(4-chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2i).

Compound **10i** was reacted as per the general method 3.6.5 to yield **2i** (81%); Purity 96.2%; mp 326 – 327°C [Lit.[201] >300°C]; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.65 (s, 1H, H-5); 8.13 (d, 2H, ^3J=8.7 Hz, H-2' and H-6'); 7.61 (d, 2H, ^3J=8.7 Hz, H-3' and H-5'); 4.26 (q, 2H, ^3J=7.1 Hz, CH$_2$); 1.30 (t, 3H, ^3J=7.1 Hz, CH$_3$). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.1 (<u>C</u>O$_2$CH$_2$CH$_3$); 160.2 (C-2); 152.8 (C-3a); 151.4 (C-5); 147.0 (C-7); 135.2 (C-4'); 129.1 (C-2' and C-6'); 128.7 (C-1'); 128.4 (C-3' and C-5'); 102.7 (C-6); 60.3 (CH$_2$); 14.2 (CH$_3$).

Ethyl 2-(3-methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2j).

Compound **10j** was reacted as per the general method 3.6.5 to yield **2j** (40%); Purity 98.2%; mp 292 – 295°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.62 (s, 1H, H-5); 7.94 (s, 1H, H-2'); 7.90 (d, 1H, ^3J=7.7 Hz, H-6'); 7.42 (t, 1H, ^3J=7.6 Hz, H-5'); 7.34 (d, 1H, ^3J=7.5 Hz, H-4'); 4.25 (q, 2H, ^3J=7.1 Hz, CH$_2$); 2.40 (s, 3H, CH$_3$); 1.29 (t, 3H, ^3J=7.1 Hz, CH$_2$C<u>H$_3$</u>). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.4 (<u>C</u>O$_2$CH$_2$CH$_3$); 161.4 (C-2); 153.2 (C-3a); 151.4 (C-5); 147.4 (C-7); 138.5 (C-3'); 131.5 (C-1'); 129.8 (C-4'); 129.1 (C-2'); 127.3 (C-5'); 124.1 (C-6'); 102.8 (C-6); 60.6 (CH$_2$); 21.2 (CH$_3$); 14.4 (CH$_2$<u>C</u>H$_3$).

Ethyl 2-(4-methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2k).

Compound **10k** was reacted as per the general method 3.6.5 to yield **2k** (15%); Purity 96.7%; mp > 400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.55 (s, 1H, H-5); 8.00 (d, 2H, ^3J=8.1 Hz, H-2' and H-6'); 7.28 (d, 2H, ^3J=7.9 Hz, H-3' and H-5'); 4.17 (q, 2H, ^3J=7.1 Hz, CH$_2$); 2.36 (s, 3H, CH$_3$); 1.26 (t, 3H, ^3J=7.1 Hz, CH$_2$C<u>H$_3$</u>). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 165.9 (<u>C</u>O$_2$CH$_2$CH$_3$); 160.9 (C-2); 159.9 (C-3a); 157.3 (C-5); 155.4 (C-7); 138.7 (C-4'); 129.2 (C-1'); 129.1 (C-3' and C-5'); 126.4 (C-2' and C-6'); 98.5 (C-6); 58.6 (CH$_2$); 21.0 (CH$_3$); 14.5 (CH$_2$<u>C</u>H$_3$).

Ethyl 2-(3-methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2l).

Compound **10l** was reacted as per the general method 3.6.5 to yield **2l** (57%); Purity 95.7%; mp 298 – 301°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.65 (s, 1H, H-5); 7.72 (dt, 1H, ^{3}J=7.7 Hz, ^{4}J=1.2 Hz, H-6'); 7.62 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.5 Hz, H-2'); 7.46 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.11 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.7 Hz, ^{4}J=0.9 Hz, H-4'); 4.26 (q, 2H, ^{3}J=7.1 Hz, CH_2); 3.85 (s, 3H, OCH_3); 1.30 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 ($\underline{C}O_2CH_2CH_3$); 160.9 (C-2); 159.6 (C-3'); 152.9 (C-3a); 151.1 (C-5); 146.9 (C-7); 131.1 (C-1'); 130.3 (C-5'); 119.1 (C-6'); 116.5 (C-2'); 111.5 (C-4'); 102.8 (C-6); 60.4 (CH_2); 55.3 (OCH_3); 14.2 ($CH_2\underline{C}H_3$).

Ethyl 2-(4-methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2m).

Compound **10m** was reacted as per the general method 3.6.5 to yield **2m** (42%); Purity 98.2%; mp 320 – 323°C [Lit.[201] >300°C]; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.62 (s, 1H, H-5); 8.06 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.09 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.25 (q, 2H, ^{3}J=7.1 Hz, CH_2); 3.85 (s, 3H, OCH_3); 1.29 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.2 ($\underline{C}O_2CH_2CH_3$); 161.1 (C-4'), 160.9 (C-2); 152.9 (C-3a); 151.2 (C-5); 147.1 (C-7); 128.3 (C-2' and C-6'); 122.1 (C-1'); 114.4 (C-3' and C-5'); 102.6 (C-6); 60.3 (CH_2); 55.3 (OCH_3); 14.2 ($CH_2\underline{C}H_3$).

Ethyl 2-(3-ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2n).

Compound **10n** was reacted as per the general method 3.6.5 to yield **2n** (25%); Purity 97.0%%; mp 290 – 292°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.63 (s, 1H, H-5); 7.69 (dt, 1H, ^{3}J=7.6 Hz, ^{4}J=1.1 Hz, H-6'); 7.60 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.5 Hz, H-2'); 7.44 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.07 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 4.24 (q, 2H, ^{3}J=7.1 Hz, $CO_2C\underline{H_2}CH_3$); 4.11 (q, 2H, ^{3}J=6.9 Hz, $OC\underline{H_2}CH_3$); 1.36 (t, 3H, ^{3}J=6.9 Hz, $OCH_2C\underline{H_3}$); 1.29 (t, 3H, ^{3}J=7.1 Hz, $CO_2CH_2C\underline{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.2 ($\underline{C}O_2CH_2CH_3$); 161.0 (C-2); 158.9 (C-3'); 153.1 (C-3a); 151.4 (C-5); 147.1 (C-7); 131.1 (C-1'); 130.3 (C-5'); 119.1 (C-6'); 117.1 (C-2'); 112.0 (C-4'); 102.7 (C-6); 63.3 ($O\underline{C}H_2CH_3$); 60.5 ($CO_2\underline{C}H_2CH_3$); 14.7 ($OCH_2\underline{C}H_3$); 14.3 ($CO_2CH_2\underline{C}H_3$).

Ethyl 2-(4-ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2o).

Compound **10o** was reacted as per the general method 3.6.5 to yield **2o** (33%); Purity 95.0%; mp 312 – 314°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.61 (s, 1H, H-5); 8.04 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.07 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.25 (q, 2H, ^{3}J=7.1 Hz, $CO_2C\underline{H}_2CH_3$); 4.11 (q, 2H, ^{3}J=6.9 Hz, $OC\underline{H}_2CH_3$); 1.36 (t, 3H, ^{3}J=7.0 Hz, $OCH_2C\underline{H}_3$); 1.29 (t, 3H, ^{3}J=7.1 Hz, $CO_2CH_2C\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.2 ($\underline{C}O_2CH_2CH_3$); 160.9 (C-4'); 160.4 (C-2), 152.8 (C-3a); 151.1 (C-5); 147.1 (C-7); 128.3 (C-2' and C-6'); 121.9 (C-1'); 114.8 (C3' and C-5'); 102.7 (C-6); 63.3 ($O\underline{C}H_2CH_3$); 60.3 ($CO_2\underline{C}H_2CH_3$); 14.6 ($OCH_2\underline{C}H_3$); 14.2 ($CO_2CH_2\underline{C}H_3$).

Ethyl 2-(3-*iso*propyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2p).

Compound **10p** was reacted as per the general method 3.6.5 to yield **2p** (7%); Purity 98.7%; mp 322 – 324°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.64 (s, 1H, H-5); 7.67 (dt, 1H, ^{3}J=7.7 Hz, ^{4}J=1.2 Hz, H-6'); 7.59 (dd, 1H, ^{4}J=2.4 Hz, ^{4}J=1.5 Hz, H-2'); 7.43 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.07 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.8 Hz, H-4'); 4.69 (m, 1H, ^{3}J=6.0 Hz, $C\underline{H}(CH_3)_2$); 4.25 (q, 2H, ^{3}J=7.1 Hz, $C\underline{H}_2CH_3$); 1.31 (d, 6H, ^{3}J=6.0 Hz, $CH(C\underline{H}_3)_2$); 1.29 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.2 ($\underline{C}O_2CH_2CH_3$); 161.0 (C-2); 157.8 (C-3'); 153.0 (C-3a); 151.3 (C-5); 147.2 (C-7); 131.1 (C-1'); 130.4 (C-5'); 118.9 (C-6'); 118.2 (C-2'); 113.2 (C-4'); 102.7 (C-6); 69.6 ($O\underline{C}H(CH_3)_2$); 60.4 ($\underline{C}H_2CH_3$); 21.9 (2C, $OCH(\underline{C}H_3)_2$); 14.3 ($CH_2\underline{C}H_3$).

Ethyl 2-(4-*iso*propyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2q).

Compound **10q** was reacted as per the general method 3.6.5 to yield **2q** (31%); Purity 96.8%; mp 340 – 343°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60 (s, 1H, H-5); 8.02 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.05 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.70 (m, 1H, ^{3}J=6.0 Hz, $C\underline{H}(CH_3)_2$); 4.24 (q, 2H, ^{3}J=7.1 Hz, $C\underline{H}_2CH_3$); 1.29 (d, 6H, ^{3}J=6.2 Hz, $CH(C\underline{H}_3)_2$); 1.28 (t, 3H, ^{3}J=7.1 Hz, $CH_2C\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.4 ($\underline{C}O_2CH_2CH_3$); 161.1 (C-2); 159.6 (C-4'), 153.2 (C-3a); 151.4 (C-5); 147.4 (C-7); 128.6 (C-2' and C-6'); 121.8 (C-1'); 115.9 (C3' and C-

5'); 102.8 (C-6); 69.7 (O<u>C</u>H(CH_3)$_2$); 60.6 (<u>C</u>$H_2$$CH_3$); 22.0 (2C, OCH(<u>C</u>H_3)$_2$); 14.4 (CH_2<u>C</u>H_3).

Ethyl 2-(3-*iso*butyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2r).

Compound **10r** was reacted as per the general method 3.6.5 to yield **2r** (35%); Purity 97.6%; mp 292 – 294°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.64 (s, 1H, H-5); 7.69 (dt, 1H, ^3J=7.6 Hz, ^4J=1.2 Hz, H-6'); 7.61 (dd, 1H, ^4J=2.5 Hz, ^4J=1.5 Hz, H-2'); 7.43 (t, 1H, ^3J=8.0 Hz, H-5'); 7.08 (ddd, 1H, ^3J=8.3 Hz, ^4J=2.6 Hz, ^4J=0.9 Hz, H-4'); 4.26 (q, 2H, ^3J=7.1 Hz, OC<u>H</u>$_2$$CH_3$); 3.84 (d, 2H, ^3J=6.6 Hz, OC<u>H</u>$_2$CH(CH_3)$_2$); 2.06 (m, 1H, ^3J=6.6 Hz, OCH$_2$C<u>H</u>(CH_3)$_2$); 1.30 (t, 2H, ^3J=7.1 Hz, OCH$_2$C<u>H</u>$_3$); 1.01 (d, 6H, ^3J=6.7 Hz, OCH$_2$CH(C<u>H</u>$_3$)$_2$).

Ethyl 2-(4-*iso*butyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2s).

Compound **10s** was reacted as per the general method 3.6.5 to yield **2s** (49%); Purity 97.5%; mp 315 – 316°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.61 (s, 1H, H-5); 8.04 (d, 2H, ^3J=8.9 Hz, H-2' and H-6'); 7.08 (d, 2H, ^3J=8.9 Hz, H-3' and H-5'); 4.25 (q, 2H, ^3J=7.1 Hz, OC<u>H</u>$_2$$CH_3$); 3.83 (d, 2H, ^3J=6.6 Hz, OC<u>H</u>$_2$CH(CH_3)$_2$); 2.05 (m, 1H, ^3J=6.7 Hz, OCH$_2$C<u>H</u>(CH_3)$_2$); 1.29 (t, 3H, ^3J=7.1 Hz, OCH$_2$C<u>H</u>$_3$); 1.00 (d, 6H, ^3J=6.7 Hz, OCH$_2$CH(C<u>H</u>$_3$)$_2$). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.2 (<u>C</u>O$_2$CH$_2$CH$_3$); 160.9 (C-2); 160.7 (C-4'), 152.9 (C-3a); 151.4 (C-5); 147.3 (C-7); 128.3 (C-2' and C-6'); 122.0 (C-1'); 114.9 (C-3' and C-5'); 102.6 (C-6); 73.9 (O<u>C</u>H$_2$CH(CH_3)$_2$); 60.3 (CO$_2$<u>C</u>H$_2$CH$_3$); 27.7 (OCH$_2$<u>C</u>H(CH_3)$_2$); 19.1 (2C, OCH$_2$CH(<u>C</u>H$_3$)$_2$); 14.3 (CO$_2$CH$_2$<u>C</u>H$_3$).

Ethyl 2-(3-trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2t).

Compound **10t** was reacted as per the general method 3.6.5 to yield **2t** (28%); Purity 99.9%; mp 343 – 346°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.60 (s, 1H, H-5); 8.41 (d, 1H, ^3J=7.8 Hz, H-6'); 8.35 (br, 1H, H-2'); 7.91 (d, 1H, ^3J=7.9 Hz, H-4'); 7.81 (t, 1H, ^3J=7.8 Hz, H-5'); 4.26 (q, 2H, ^3J=7.1 Hz, CH_2); 1.30 (t, 3H, ^3J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.0 (<u>C</u>O$_2$CH$_2$CH$_3$); 159.9 (C-2); 152.9 (C-3a); 151.4 (C-5); 147.1 (C-7); 130.9 (C-6'); 130.6 (C-1' and C-5');

129.8 (C-3', $^{2}J_{CF}$=32.2 Hz); 127.1 (C-4', $^{3}J_{CF}$=1.2 Hz); 124.0 (CF_3, $^{1}J_{CF}$=272.6 Hz); 122.8 (C-2', $^{3}J_{CF}$=1.3 Hz); 102.9 (C6); 60.5 (CH_2); 14.3 (CH_3).

Ethyl 2-(4-trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2u).

Compound **10u** was reacted as per the general method 3.6.5 to yield **2u** (36%); Purity 97.2%; mp 341 - 343°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H, H-5); 8.33 (d, 2H, ^{3}J=8.1 Hz, H-3' and H-5'); 7.92 (d, 2H, ^{3}J=8.2 Hz, H-2' and H-6'); 4.27 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 ($\underline{C}O_2CH_2CH_3$); 159.9 (C-2); 152.8 (C-3a); 151.3 (C-5); 147.0 (C-7); 133.7 (C-1'); 130.4 (C-4', $^{2}J_{CF}$=32.0 Hz); 127.4 (C-2' and C-6'); 126.0 (C-3' and C-5', $^{3}J_{CF}$=3.7 Hz); 124.1 (CF_3, $^{1}J_{CF}$=272.1 Hz); 102.8 (C-6); 60.4 (CH_2); 14.2 (CH_3).

Ethyl 2-(3-trifluoromethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylate (2v).

Compound **10v** was reacted as per the general method 3.6.5 to yield **2v** (26%); Purity 99.4%; mp 307 – 309°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.67 (s, 1H, H-5); 8.15 (ddd, 1H, ^{3}J=5.6 Hz, ^{4}J=3.3 Hz, ^{4}J=2.1 Hz, H-6'); 8.01 – 7.95 (m, 1H, H-2'); 7.70 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.59 – 7.51 (m, 1H, H-4'); 4.26 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.30 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 ($\underline{C}O_2CH_2CH_3$); 159.8 (C-2); 152.8 (C-3a); 151.3 (C-5); 148.8 (q, $^{3}J_{CF}$=1.8 Hz, C-3'); 146.9 (C-7); 132.0 (C-1'); 131.4 (C-5'); 125.6 (C-6'); 123.0 (C-2'); 120.1 (CF_3, $^{1}J_{CF}$=256.8 Hz); 118.5 (C-4'); 102.8 (C-6); 60.4 (CH_2); 14.2 (CH_3).

Ethyl 2-(pyridin-3-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2w).

Compound **10w** was reacted as per the general method 3.6.5 to yield **2w** (28%); Purity 99.2%; mp > 400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.30 (dd, 1H, ^{4}J=2.0 Hz, ^{4}J=0.6 Hz, H-2'); 8.64 (dd, 1H, ^{3}J=4.8 Hz, ^{3}J=1.7 Hz, H-4'); 8.63 (s, 1H, H-5); 8.45 (dt, 1H, ^{3}J=7.9 Hz, ^{4}J=1.9 Hz, H-6'); 7.52 (ddd, 1H, ^{3}J=7.9 Hz, ^{3}J=4.8 Hz, ^{3}J=0.8 Hz, H-5'); 4.20 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.28 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.8 ($\underline{C}O_2CH_2CH_3$); 159.8 (C-2); 159.0 (C-3a); 157.6 (C-5); 155.7 (C-7); 150.3 (C-4'); 147.5 (C-2'); 133.8 (C-5'); 127.5 (C-1'); 123.8 (C-6'); 98.7 (C-6); 58.9 (CH_2); 14.5 (CH_3).

Ethyl 2-(pyridin-4-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2x).

Compound **10x** was reacted as per the general method 3.6.5 to yield **2x** (18%); Purity 99.8%; mp 375 – 377°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.69 (dd, 2H, ^{3}J=4.4 Hz, ^{4}J=1.6 Hz, H-3' and H-5'); 8.61 (s, 1H, H-5); 8.03 (dd, 2H, ^{3}J=4.5 Hz, ^{4}J=1.5 Hz, H-2' and H-6'); 4.18 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.27 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.8 ($\underline{C}O_2CH_2CH_3$); 159.9 (C-2); 159.0 (C-3a); 157.8 (C-5); 155.6 (C-7); 150.3 (C-3' and C-5'); 139.0 (C-1'); 120.6 (C-2' and C-6'); 98.8 (C-6); 58.9 (CH_2); 14.5 (CH_3).

Ethyl 2-(thien-2-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (2y).

Compound **10y** was reacted as per the general method 3.6.5 to yield **2y** (39%); Purity 98.4%; mp 320 – 322°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.63 (s, 1H, H-5); 7.78 (dd, 1H, ^{3}J=3.6 Hz, ^{4}J=1.2 Hz, H-5'); 7.76 (dd, 1H, ^{3}J=5.0 Hz, ^{4}J=1.2 Hz, H-3'); 7.22 (dd, 1H, ^{3}J=5.0 Hz, ^{4}J=3.7 Hz, H-4'); 4.25 (q, 2H, ^{3}J=7.1 Hz, CH_2); 1.29 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 ($\underline{C}O_2CH_2CH_3$); 157.6 (C-2); 152.7 (C-3a); 151.0 (C-5); 146.8 (C-7); 132.5 (C-2'); 129.4 (C-3'); 128.5 (C-5'); 128.3 (C-4'); 102.9 (C-6); 60.5 (CH_2); 14.3 (CH_3).

3.6.6 General method for the synthesis of 7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acids (**3a-y**)

The appropriate ethyl 7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (**2**, 1 mmol) in 2 mL of 5% potassium carbonate was heated at 140°C for 15 min in a microwave reactor. The pH of the resulting solution, cooled to room temperature, was adjusted to approximately 5 by adding glacial acetic acid dropwise. The precipitated solid was filtered and the filtrate was washed with water before drying it in a vacuum oven at 60°C overnight. Copies of NMR spectra are presented in Appendix 3.

7-Oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3a).

Compound **2a** was reacted as per the general method 3.6.6 to yield **3a** (13%); Purity 98.7%; mp 360 – 362°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.54 (s,

1H, NH); 8.63 (s, 1H, H-5); 8.20 (s, 1H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.8 (CO_2H); 160.7 (C-2); 159.0 (C-3a); 156.0 (C-5); 153.8 (C-7); 97.3 (C-6).

2-Methylthio-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3b).

Compound **2b** was reacted as per the general method 3.6.6 to yield **3b** (97%); Purity 98.2%; mp 289 – 291°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.59 (s, 1H, H-5); 2.59 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.4 (CO_2H); 164.3 (C-2); 156.6 (C-3a); 155.2 (C-5); 150.9 (C-7); 100.2 (C-6); 13.4 (CH_3).

2-Phenyl-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3c).

Compound **2c** was reacted as per the general method 3.6.6 to yield **3c** (73%); Purity 95.5%; mp 348 – 350°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.23 (br, 1H, NH); 8.66 (s, 1H, H-5); 8.19 – 8.12 (m, 2H, H-2' and H-6'); 7.58 – 7.47 (m, 3H, H-3', H-4' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.7 (CO_2H); 162.0 (C-2); 158.1 (C-3a); 156.0 (C-5); 152.2 (C-7); 130.5 (C-4'); 130.2 (C-1'); 128.9 (C-3' and C-5'); 126.7 (C-2' and C-6'); 99.8 (C-6).

2-(2-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3d).

Compound **2d** was reacted as per the general method 3.6.6 to yield **3d** (83%); Purity 98.3%; mp 348 – 350°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.70 (s, 1H, H-5); 8.11 (ddd, 1H, ^3J=7.9 Hz, $^4J_{HF}$=7.6 Hz, ^4J=1.7 Hz, H-6'); 7.59 (dddd, 1H, ^3J=8.3 Hz, ^3J=7.3 Hz, $^4J_{HF}$=5.0 Hz, ^4J=1.8 Hz, H-4'); 7.47 – 7.33 (m, 2H, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.0 (CO_2H); 160.1 (C-2', $^1J_{CF}$=254.9 Hz); 158.7 (C-2, $^3J_{CF}$=4.8 Hz); 156.1 (C-3a); 152.8 (C-5); 149.3 (C-7); 132.5 (C-4', $^3J_{CF}$=8.4 Hz); 130.7 (C-5', $^4J_{CF}$=2.3 Hz); 125.0 (C-6', $^3J_{CF}$=3.6 Hz); 118.1 (C-1', $^2J_{CF}$=11.1 Hz); 117.0 (C-3', $^2J_{CF}$=21.2 Hz); 101.7 (C-6).

2-(3-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3e).

Compound **2e** was reacted as per the general method 3.6.6 to yield **3e** (48%); Purity 97.9%; mp 309 – 311°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.67 (s, 1H, H-5); 7.99 (ddd, 1H, ^3J=7.8 Hz, ^4J=1.1 Hz, ^4J=1.1 Hz, H-6'); 7.84 (ddd, 1H,

$^{3}J_{HF}$=10.0 Hz, ^{4}J=2.6 Hz, ^{4}J=1.4 Hz, H-2'); 7.59 (td, 1H, ^{3}J=8.0 Hz, ^{3}J=6.0 Hz, H-5'); 7.37 (dddd, 1H, $^{3}J_{HF}$=9.0 Hz, ^{3}J=8.2 Hz, ^{4}J=2.7 Hz, ^{4}J=0.8 Hz, H-4'). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 165.3 (CO_2H); 162.4 (C-3', $^{1}J_{CF}$=243.7 Hz); 160.8 (C-2); 156.9 (C-3a); 154.7 (C-5); 150.8 (C-7); 132.7 (C-1', $^{3}J_{CF}$=8.4 Hz); 131.3 (C-5', $^{3}J_{CF}$=8.3 Hz); 122.9 (C-6', $^{4}J_{CF}$=2.7 Hz); 117.3 (C-4', $^{2}J_{CF}$=21.1 Hz); 113.2 (C-2', $^{2}J_{CF}$=23.2 Hz); 100.9 (C-6).

2-(4-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3f).

Compound **2f** was reacted as per the general method 3.6.6 to yield **3f** (49%); Purity 96.3%; mp 348 – 350°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.64 (s, 1H, H-5); 8.19 (dd, 2H, ^{3}J=9.0 Hz, $^{4}J_{HF}$=5.6 Hz, H-2' and H-6'); 7.34 (dd, 2H, ^{3}J=9.0 Hz, $^{3}J_{HF}$=9.0 Hz, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 166.8 (CO_2H); 163.2 (C-4', $^{1}J_{CF}$=246.6 Hz); 161.7 (C-2); 160.7 (C-3a); 159.9 (C-5); 156.3 (C-7); 128.9 (C-2' and C-6', $^{3}J_{CF}$=8.6 Hz); 127.8 (C-1', $^{4}J_{CF}$=3.6 Hz); 115.7 (C-3' and C-5', $^{2}J_{CF}$=21.8 Hz); 97.6 (C-6).

2-(2-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3g).

Compound **2g** was reacted as per the general method 3.6.6 to yield **3g** (71%); Purity 97.7%; mp 211 – 213°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 13.23 (br, 1H, NH); 8.69 (s, 1H, H-5); 7.93 (dd, 1H, ^{3}J=7.0 Hz, ^{4}J=2.4 Hz, H-6'); 7.62 (dd, 1H, ^{3}J=7.7 Hz, ^{4}J=1.4 Hz, H-3'); 7.53 (td, 1H, ^{3}J=7.1 Hz, ^{4}J=1.7 Hz, H-4'); 7.49 (td, 1H, ^{3}J=7.1 Hz, ^{4}J=2.0 Hz, H-5'). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 165.9 (CO_2H); 161.2 (C2); 158.6 (C-3a); 156.2 (C-5); 153.1 (C-7); 132.0 (C-1'); 131.9 (C-4'); 131.1 (C-2'); 130.6 (C-6'); 130.0 (C-3'); 127.2 (C-5'); 99.4 (C-6).

2-(3-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3h).

Compound **2h** was reacted as per the general method 3.6.6 to yield **3h** (63%); Purity 97.1%; mp 344 – 346°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.66 (s, 1H, H-5); 8.14 – 8.05 (m, 2H, H-2' and H-4'); 7.67 – 7.53 (m, 2H, H-5' and H-6'). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 164.7 (CO_2H); 160.4 (C-2); 155.7 (C-3a); 153.0 (C-5); 148.9 (C-7); 133.8 (C-1'); 132.1 (C-3'); 131.1 (C-4'); 130.4 (C-5'); 126.2 (C-2'); 125.4 (C-6'); 101.9 (C-6).

2-(4-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3i).

Compound **2i** was reacted as per the general method 3.6.6 to yield **3i** (82%); Purity 95.1%; mp 385 – 387°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H, H-5); 8.14 (d, 2H, ^{3}J=8.7 Hz, H-2' and H-6'); 7.61 (d, 2H, ^{3}J=8.7 Hz, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.7 (CO_2H); 160.7 (C-2); 155.8 (C-3a); 153.0 (C-5); 148.9 (C-7); 135.2 (C-4'); 129.1 (C-2' and C-6'); 128.9 (C-1'); 128.5 (C-3' and C-5'); 101.7 (C-6).

2-(3-Methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3j).

Compound **2j** was reacted as per the general method 3.6.6 to yield **3j** (93%); Purity 96.3%; mp 352 - 354°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.66 (s, 1H, H-5); 7.96 (s, 1H, H-2'); 7.93 (d, 1H, ^{3}J=7.7 Hz, H-6'); 7.41 (t, 1H, ^{3}J=7.6 Hz, H-5'); 7.32 (d, 1H, ^{3}J=7.5 Hz, H-4'); 2.40 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (CO_2H); 162.1 (C-2); 157.3 (C-3a); 154.7 (C-5); 151.0 (C-7); 138.4 (C-3'); 131.3 (C-1'); 130.2 (C-4'); 129.1 (C-2'); 127.4 (C-5'); 124.1 (C-6'); 100.8 (C-6); 21.2 (CH_3).

2-(4-Methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3k).

Compound **2k** was reacted as per the general method 3.6.6 to yield **3k** (66%); Purity 95.6%; mp 362 - 364°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.65 (s, 1H, H-5); 8.04 (d, 2H, ^{3}J=8.2 Hz, H-2' and H-6'); 7.34 (d, 2H, ^{3}J=8.0 Hz, H-3' and H-5'); 2.38 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.8 (CO_2H); 162.0 (C-2); 158.3 (C-3a); 156.3 (C-5); 152.5 (C-7); 139.9 (C-4'); 129.4 (C-3' and C-5'); 127.8 (C-1'); 126.6 (C-2' and C-6'); 99.6 (C-6); 21.0 (CH_3).

2-(3-Methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3l).

Compound **2l** was reacted as per the general method 3.6.6 to yield **3l** (62%); Purity 95.7%; mp 311 - 312°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.67 (s, 1H, H-5); 7.73 (ddd, 1H, ^{3}J=7.7 Hz, ^{4}J=1.1 Hz, ^{4}J=1.1 Hz, H-6'); 7.65 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.4 Hz, H-2'); 7.45 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.08 (ddd, 1H, ^{3}J=8.3 Hz,

^{4}J=2.7 Hz, ^{4}J=0.8 Hz, H-4'); 3.85 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.4 (CO_2H); 161.7 (C-2); 159.6 (C-3'); 157.4 (C-3a); 155.0 (C-5); 151.2 (C-7); 131.7 (C-1'); 130.2 (C-5'); 119.1 (C-6'); 116.4 (C-4'); 111.5 (C-2'); 100.5 (C-6); 55.3 (CH_3).

2-(4-Methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3m).

Compound **2m** was reacted as per the general method 3.6.6 to yield **3m** (98%); Purity 95.8%; mp 332 - 333°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.66 (s, 1H, H-5); 8.07 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.10 (d, 2H, ^{3}J=9.0 Hz, H-3' and H-5'); 3.84 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.5 (CO_2H); 161.20 (C-2); 161.16 (C-4'), 155.4 (C-3a); 151.8 (C-5); 148.0 (C-7); 128.4 (C-2' and C-6'); 121.9 (C-1'); 114.4 (C-3' and C-5'); 102.2 (C-6); 55.4 (CH_3).

2-(3-Ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3n).

Compound **2n** was reacted as per the general method 3.6.6 to yield **3n** (69%); Purity 96.8%; mp 292 - 294°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H, H-5); 7.71 (ddd, 1H, ^{3}J=7.6 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.62 (dd, 1H, ^{4}J=2.4 Hz, ^{4}J=1.5 Hz, H-2'); 7.44 (t, 1H, ^{3}J=7.9 Hz, H-5'); 7.08 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.8 Hz, H-4'); 4.12 (q, 2H, ^{3}J=7.0 Hz, CH_2); 1.37 (t, 3H, ^{3}J=7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.5 (CO_2H); 161.3 (C-2); 158.8 (C-3'); 155.5 (C-3a); 152.2 (C-5); 148.3 (C-7); 131.0 (C-1'); 130.2 (C-5'); 119.0 (C-6'); 117.1 (C-4'); 111.9 (C-2'); 102.0 (C-6); 63.2 (CH_2); 14.6 (CH_3).

2-(4-Ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3o).

Compound **2o** was reacted as per the general method 3.6.6 to yield **3o** (73%); Purity 95.0%; mp 301 - 303°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.65 (s, 1H, H-5); 8.06 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.07 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.11 (q, 2H, ^{3}J=7.0 Hz, CH_2); 1.36 (t, 3H, ^{3}J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.0 (CO_2H); 161.4 (C-2); 160.3 (C-4'), 156.6 (C-3a); 153.7 (C-5); 149.9 (C-7); 128.3 (C-2' and C-6'); 122.2 (C-1'); 114.7 (C-3' and C-5'); 101.0 (C-6); 63.3 (CH_2); 14.6 (CH_3).

2-(3-*iso*Propyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]-pyrimidine-6-carboxylic acid (3p).

Compound **2p** was reacted as per the general method 3.6.6 to yield **3p** (72%); Purity 97.5%; mp 279 - 281°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H, H-5); 7.69 (ddd, 1H, ^{3}J=7.8 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.61 (dd, 1H, ^{4}J=2.4 Hz, ^{4}J=1.5 Hz, H-2'); 7.44 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.07 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 4.70 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.32 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.6 (CO_2H); 161.3 (C-2); 157.8 (C-3'); 155.5 (C-3a); 152.1 (C-5); 148.3 (C-7); 131.1 (C-1'); 130.4 (C-5'); 119.0 (C-6'); 118.3 (C-4'); 113.2 (C-2'); 102.2 (C-6); 69.6 (<u>C</u>H$(CH_3)_2$); 21.9 (2C, CH(<u>C</u>$H_3)_2$).

2-(4-*iso*Propyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylic acid (3q).

Compound **2q** was reacted as per the general method 3.6.6 to yield **3q** (87%); Purity 95.0%; mp 310 - 312°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.65 (s, 1H, H-5); 8.04 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.06 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.70 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.30 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.8 (CO_2H); 161.4 (C-2); 159.6 (C-4'), 156.0 (C-3a); 152.6 (C-5); 148.8 (C-7); 128.5 (C-2' and C-6'); 121.7 (C-1'); 115.8 (C-3' and C-5'); 101.9 (C-6); 69.6 (<u>C</u>H$(CH_3)_2$); 21.9 (CH(<u>C</u>$H_3)_2$).

2-(3-*iso*Butyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3r).

Compound **2r** was reacted as per the general method 3.6.6 to yield **3r** (74%); Purity 97.7%; mp 271 - 273°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.66 (s, 1H, H-5); 7.70 (ddd, 1H, ^{3}J=7.7 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.63 (dd, 1H, ^{4}J=2.4 Hz, ^{4}J=1.5 Hz, H-2'); 7.43 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.08 (ddd, 1H, ^{3}J=8.1 Hz, ^{4}J=2.4 Hz, ^{4}J=0.6 Hz, H-4'); 3.83 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.04 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 1.00 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.3 (CO_2H); 161.7 (C-2); 159.2 (C-3'); 156.8 (C-3a); 154.1 (C-5); 150.3 (C-7); 131.5 (C-1'); 130.4 (C-5'); 119.2 (C-6'); 117.2 (C-4'); 112.2 (C-2'); 101.2 (C-6); 74.1 (CH_2); 27.9 (<u>C</u>H$(CH_3)_2$); 19.2 (2C, CH(<u>C</u>$H_3)_2$).

2-(4-*iso*Butyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3s).

Compound **2s** was reacted as per the general method 3.6.6 to yield **3s** (80%); Purity 95.3%; mp >400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.64 (s, 1H, NH); 8.62 (s, 1H, H-5); 8.07 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.05 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 3.82 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.04 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 1.00 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.8 (CO_2H); 162.4 (C-2); 160.4 (C-3a); 160.2 (C-4'), 159.4 (C-5); 155.6 (C-7); 128.2 (C-2' and C-6'); 123.5 (C-1'); 114.6 (C-3' and C-5'); 97.7 (C-6); 73.8 (CH_2); 27.7 (<u>C</u>H$(CH_3)_2$); 19.1 (CH(<u>C</u>H$_3)_2$).

2-(3-Trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3t).

Compound **2t** was reacted as per the general method 3.6.6 to yield **3t** (69%); Purity 97.1%; mp 325 - 328°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.69 (s, 1H, H-5); 8.45 (d, 1H, ^{3}J=7.7 Hz, H-4'); 8.39 (s, 1H, H-2'); 7.89 (d, 1H, ^{3}J=7.8 Hz, H-6'); 7.80 (t, 1H, ^{3}J=7.8 Hz, H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.6 (CO_2H); 160.7 (C-2); 157.8 (C-3a); 156.0 (C-5); 152.2 (C-7); 131.6 (C-1'); 130.5 (C-5'); 130.4 (C-6'); 129.7 (q, $^{2}J_{CF}$=32.0 Hz, C-3'); 126.8 (q, $^{3}J_{CF}$=3.3 Hz, C-4'); 124.1 (q, $^{1}J_{CF}$=272.4 Hz, CF_3); 122.8 (q, $^{3}J_{CF}$=3.9 Hz, C-2'); 100.2 (C-6).

2-(4-Trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylic acid (3u).

Compound **2u** was reacted as per the general method 3.6.6 to yield **3u** (68%); Purity 96.5%; mp 379 - 381°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H, H-5); 8.36 (d, 2H, ^{3}J=8.1 Hz, H-3' and H-5'); 7.90 (d, 2H, ^{3}J=8.1 Hz, H-2' and H-6'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.9 (CO_2H); 160.9 (C-2); 158.6 (C-3a); 157.2 (C-5); 153.5 (C-7); 134.7 (C-1'); 130.1 (q, $^{2}J_{CF}$=31.8 Hz, C-4'); 127.4 (C-2' and C-6'); 125.8 (q, $^{3}J_{CF}$=3.7 Hz, C-3' and C-5'); 124.2 (q, $^{1}J_{CF}$=272.1 Hz, CF_3); 99.5 (C-6).

2-(3-Trifluoromethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*] pyrimidine-6-carboxylic acid (3v).

Compound **2v** was reacted as per the general method 3.6.6 to yield **3v** (92%); Purity 98.7%; mp 325 - 328°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (s, 1H,

H-5); 8.16 (ddd, 1H, ^{3}J=7.9 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 8.01 – 7.97 (m, 1H, H-2'); 7.70 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.57 – 7.51 (m, 1H, H-4'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.8 (CO_2H); 160.4 (C-2); 155.7 (C-3a); 153.0 (C-5); 148.9 (q, $^{3}J_{CF}$=1.3 Hz, C-3'); 132.3 (C-1'); 131.5 (C-5'); 125.8 (C-6'); 123.1 (C-2'); 120.2 (q, $^{1}J_{CF}$=256.8 Hz, CF_3); 118.7 (C-4'); 102.0 (C-6).

2-(Pyridin-3-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3w).

Compound **2w** was reacted as per the general method 3.6.6 to yield **3w** (74%); Purity 96.9%; mp 292 - 294°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.4 (br, 1H, NH); 9.30 (d, 1H, ^{4}J=1.4 Hz, H-2'); 8.69 (dd, 1H, ^{3}J=4.7 Hz, ^{4}J=1.4 Hz, H-6'); 8.67 (s, 1H, H-5); 8.48 (dt, 1H, ^{3}J=7.9 Hz, ^{4}J=1.9 Hz, H-4'); 7.57 (ddd, 1H, ^{3}J=7.9 Hz, ^{3}J=4.7 Hz, ^{5}J=0.6 Hz, H-5').

2-(Pyridin-4-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3x).

Compound **2x** was reacted as per the general method 3.6.6 to yield **3x** (20%); Purity 99.1%; mp > 400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.4 (s, 1H, NH); 8.73 (d, 2H, ^{3}J=6.0 Hz, H-2' and H-6'); 8.07 (d, 2H, ^{3}J=6.1 Hz, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.7 (CO_2H); 160.62 (C-2); 160.59 (C-3a); 160.0 (C-5); 156.8 (C-7); 150.5 (C-2' and C-6'); 138.5 (C-4'); 120.8 (C-3' and C-5'); 98.0 (C-6).

2-(Thien-2-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (3y).

Compound **2y** was reacted as per the general method 3.6.6 to yield **3y** (77%); Purity 97.3%; mp > 400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.53 (s, 1H, NH); 8.63 (s, 1H, H-5); 7.76 (dd, 1H, ^{3}J=3.6 Hz, ^{4}J=1.2 Hz, H-5'); 7.69 (dd, 1H, ^{3}J=5.0 Hz, ^{4}J=1.2 Hz, H-3'); 7.19 (dd, 1H, ^{3}J=5.0 Hz, ^{3}J=3.6 Hz, H-4'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.8 (CO_2H); 160.5 (C-2); 159.7 (C3a); 158.9 (C-5); 156.4 (C-7); 134.3 (C-2'); 128.3 , 128.1, 127.2 (C-3', C-4' and C-5'); 97.9 (C-6).

3.6.7 General method for the synthesis of 2-substituted-1,2,4-triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**1a-y**)

The appropriate 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid (**3**, 1 mmol) in 2 mL of 5% aqueous solution of potassium dihydrogen phosphate was heated at 190°C for 30 min in a microwave reactor. After cooling to room temperature, the precipitate was filtered and washed with water before drying it in a vacuum oven at 60°C overnight. Copies of NMR spectra are presented in Appendix 4.

1,2,4-Triazolo[1,5-*a*]pyrimidin-7-one (1a).

Compound **3a** was reacted as per the general method 3.6.7 to yield **1a** (23%); Purity 98.5%; mp 293 - 295°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.22 (s, 1H, H-2); 7.99 (d, 1H, ^{3}J=7.5 Hz, H-5); 5.93 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.4 (C-2); 151.9 (C-3a); 150.9 (C-5); 140.7 (C-7); 99.1 (C-6).

2-Methylthio-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1b).

Compound **3b** was reacted as per the general method 3.6.7 to yield **1b** (54%); Purity 97.2%; mp 327 - 329°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.90 (d, 1H, ^{3}J=7.6 Hz, H-5); 5.90 (d, 1H, ^{3}J=7.6 Hz, H-6); 2.58 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.3 (C-2); 155.4 (C-3a); 151.5 (C-5); 139.7 (C-7); 99.6 (C-6); 13.5 (CH_3).

2-Phenyl-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1c).

Compound **3c** was reacted as per the general method 3.6.7 to yield **1c** (85%); Purity 98.6%; mp 365 - 368°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.33 (br, 1H, NH); 8.20 – 8.06 (m, 2H, H-2' and H-6'); 8.00 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.60 – 7.47 (m, 3H, H-3', H-4' and H-5'); 5.97 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.8 (C-2); 156.2 (C-3a); 151.5 (C-5); 140.2 (C-7); 130.3 (C-4'); 130.0 (C-1'); 128.9 (C-3' and C-5'); 126.6 (C-2' and C-6'); 99.4 (C-6).

2-(2-Fluorophenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1d).

Compound **3d** was reacted as per the general method 3.6.7 to yield **1d** (33%); Purity 97.9%; mp 349 - 352°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.41 (br, 1H, NH); 8.09 (ddd, 1H, ^{3}J=8.1 Hz, $^{4}J_{HF}$=7.4 Hz, ^{4}J=1.8 Hz, H-6'); 8.02 (d, 1H,

^{3}J=7.5 Hz, H-5); 7.57 (dddd, 1H, ^{3}J=8.3 Hz, ^{3}J=7.4 Hz, $^{4}J_{HF}$=5.1 Hz, ^{4}J=1.9 Hz, H-4'); 7.45 – 7.31 (m, 2H, H-3' and H-5'); 5.98 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 159.9 (d, $^{1}J_{CF}$=255.0 Hz, C-2'); 157.6 (d, $^{3}J_{CF}$=5.0 Hz, C-2); 156.2 (C-5); 151.2 (d, $^{5}J_{CF}$=1.1 Hz, C-3a), 140.4 (C-7); 132.2 (d, $^{3}J_{CF}$=8.5 Hz, C-4'); 130.5 (d, $^{4}J_{CF}$=2.3 Hz, C-5'); 124.7 (d, $^{3}J_{CF}$=3.6 Hz, C-6'); 118.1 (d, $^{2}J_{CF}$=11.1 Hz, C-1'); 116.8 (d, $^{2}J_{CF}$=21.2 Hz, C-3'); 99.4 (C-6).

2-(3-Fluorophenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1e).

Compound **3e** was reacted as per the general method 3.6.7 to yield **1e** (69%); Purity 99.3%; mp 356 - 357°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 13.41 (br, 1H, NH); 8.01 (d, 1H, ^{3}J=7.6 Hz, H-5); 7.99 (ddd, 1H, ^{3}J=7.8 Hz, ^{4}J=1.3 Hz, ^{4}J=1.1 Hz, H-6'); 7.82 (ddd, 1H, $^{3}J_{HF}$=10.0 Hz, ^{4}J=2.6 Hz, ^{4}J=1.4 Hz, H-2'); 7.60 (td, 1H, ^{3}J=8.0 Hz, $^{4}J_{HF}$=6.0 Hz, H-5'); 7.37 (dddd, 1H, $^{3}J_{HF}$=8.8 Hz, ^{3}J=8.4 Hz, ^{4}J=2.6 Hz, ^{4}J=0.6 Hz, H-4'); 5.98 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 162.3 (d, $^{1}J_{CF}$=243.8 Hz, C-3'); 159.7 (d, $^{4}J_{CF}$=3.0 Hz, C-2); 156.2 (C-3a); 151.6 (C-5); 140.4 (C-7); 132.4 (d, $^{3}J_{CF}$=8.4 Hz, C-1'); 131.2 (d, $^{3}J_{CF}$=8.3 Hz, C-5'); 122.8 (d, $^{4}J_{CF}$=2.7 Hz, C-6'); 117.2 (d, $^{2}J_{CF}$=21.1 Hz, C-4'); 113.1 (d, $^{2}J_{CF}$=23.3 Hz, C-2'); 99.6 (C-6).

2-(4-Fluorophenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1f).

Compound **3f** was reacted as per the general method 3.6.7 to yield **1f** (65%); Purity 96.8%; mp 379 - 381°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.15 (dd, 2H, ^{3}J=8.9 Hz, $^{4}J_{HF}$=5.6 Hz, H-2' and H-6'); 7.96 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.36 (dd, 2H, ^{3}J=8.9 Hz, $^{3}J_{HF}$=8.9 Hz, H-3' and H-5'); 5.96 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.6 (d, $^{1}J_{CF}$=247.5, Hz C-4'); 160.2 (C-2); 156.5 (C-3a); 151.8 (C-5); 140.6 (C-7); 129.2 (d, $^{3}J_{CF}$=8.8 Hz C-2' and C-6',); 126.7 (d, $^{4}J_{CF}$=3.0 Hz, C-1'); 116.2 (d, $^{2}J_{CF}$=21.9 Hz, C-3' and C-5'); 99.7 (C-6).

2-(2-Chlorophenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1g).

Compound **3g** was reacted as per the general method 3.6.7 to yield **1g** (65%); Purity 95.3%; mp 293 - 295°C; *Anal* ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 13.44 (br, 1H, NH); 8.04 (d, 1H, ^{3}J=7.6 Hz, H-5); 7.93 (dd, 1H, ^{3}J=7.5 Hz, ^{4}J=2.0 Hz, H-6'); 7.63 (dd, 1H, ^{3}J=7.5 Hz, ^{4}J=1.7 Hz, H-3'); 7.58 – 7.46 (m, 2H, H-4' and H-5'); 5.99 (d, 1H, ^{3}J=7.6 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 159.8 (C2); 156.2 (C-

3a); 150.9 (C-5); 140.4 (C-7); 132.0 (C-1'); 131.9 (C-4'); 131.5 (C-2'); 130.7 (C-6'); 129.3 (C-3'); 127.4 (C-5'); 99.5 (C-6).

2-(3-Chlorophenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1h).

Compound **3h** was reacted as per the general method 3.6.7 to yield **1h** (59%); Purity 97.6%; mp 359 - 360°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.41 (br, 1H, NH); 8.14 – 8.04 (m, 2H, H-2' and H-4'); 8.02 (d, 1H, ^{3}J=7.6 Hz, H-5); 7.65 – 7.54 (m, 2H, H-5' and H-6'); 5.98 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.5 (C-2); 156.2 (C-3a); 151.7 (C-5); 140.6 (C-7); 133.7 (C-1'); 132.2 (C-3'); 131.0 (C-4'); 130.2 (C-5'); 126.1 (C-2'); 125.2 (C-6'); 99.5 (C-6).

2-(4-Chlorophenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1i).

Compound **3i** was reacted as per the general method 3.6.7 to yield **1i** (72%); Purity 98.7%; mp 390 - 393°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.12 (d, 2H, ^{3}J=8.7 Hz, H-2' and H-6'); 8.00 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.60 (d, 2H, ^{3}J=8.7 Hz, H-3' and H-5'); 5.96 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.8 (C-2); 156.2 (C-3a); 151.7 (C-5); 140.5 (C-7); 135.0 (C-4'); 129.1 (C-2' and C-6'); 129.0 (C-1'); 128.4 (C-3' and C-5'); 99.5 (C-6).

2-(3-Methylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1j).

Compound **3j** was reacted as per the general method 3.6.7 to yield **1j** (58%); Purity 97.4%; mp 324 - 327°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.95 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.94 (s, 1H, H-2'); 7.90 (d, 1H, ^{3}J=7.7 Hz, H-6'); 7.41 (t, 1H, ^{3}J=7.6 Hz, H-5'); 7.32 (d, 1H, ^{3}J=7.6 Hz, H-4'); 5.95 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.1 (C-2); 156.6 (C-3a); 151.9 (C-5); 140.8 (C-7); 138.4 (C-3'); 131.3 (C-1'); 130.1 (C-4'); 129.1 (C-2'); 127.3 (C-5'); 124.1 (C-6'); 99.5 (C-6); 21.2 (CH_3).

2-(4-Methylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1k).

Compound **3k** was reacted as per the general method 3.6.7 to yield **1k** (72%); Purity 96.7%; mp > 400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.29 (br, 1H, NH); 8.01 (d, 2H, ^{3}J=8.2 Hz, H-2' and H-6'); 7.98 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.34 (d, 2H, ^{3}J=8.0 Hz, H-3' and H-5'); 5.95 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.8 (C-2); 156.2 (C-3a); 151.4 (C-5); 140.1 (C-7); 129.5 (C-3' and C-5'); 127.3 (C-4'); 126.6 (C1', C-2' and C-6'); 99.4 (C-6); 21.0 (CH_3).

2-(3-Methoxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1l).

Compound **3l** was reacted as per the general method 3.6.7 to yield **1l** (72%); Purity 98.8%; mp 313 - 315°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.00 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.71 (ddd, 1H, ^{3}J=7.8 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.63 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.4 Hz, H-2'); 7.45 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.09 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.7 Hz, ^{4}J=0.9 Hz, H-4'); 5.96 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.6 (C-2); 159.5 (C-3'); 156.3 (C-3a); 151.5 (C-5); 140.3 (C-7); 131.4 (C-1'); 130.2 (C-5'); 119.1 (C-6'); 116.4 (C-4'); 111.4 (C-2'); 99.4 (C-6); 55.2 (CH_3).

2-(4-Methoxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1m).

Compound **3m** was reacted as per the general method 3.6.7 to yield **1m** (79%); Purity 95.7%; mp 350 - 352°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.05 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.97 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.08 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 5.94 (d, 1H, ^{3}J=7.5 Hz, H-6); 3.83 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.0 (C-2); 160.7 (C-4'), 156.2 (C-3a); 151.4 (C-5); 139.9 (C-7); 128.3 (C-2' and C-6'); 122.4 (C-1'); 114.3 (C-3' and C-5'); 99.4 (C-6); 55.3 (CH_3).

2-(3-Ethoxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1n).

Compound **3n** was reacted as per the general method 3.6.7 to yield **1n** (91%); Purity 99.4%; mp 308 - 310°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.33 (br, 1H, NH); 8.00 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.69 (ddd, 1H, ^{3}J=7.7 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.61 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.4 Hz, H-2'); 7.43 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.07 (ddd, 1H, ^{3}J=8.2 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 5.97 (d, 1H, ^{3}J=7.6 Hz, H-6); 4.12 (q, 2H, ^{3}J=7.0 Hz, CH_2); 1.37 (t, 3H, ^{3}J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.7 (C-2); 158.8 (C-3'); 156.2 (C-3a); 151.4 (C-5); 140.1 (C-7); 131.4 (C-1'); 130.1 (C-5'); 118.9 (C-6'); 116.9 (C-4'); 111.8 (C-2'); 99.5 (C-6); 63.2 (CH_2); 14.7 (CH_3).

2-(4-Ethoxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1o).

Compound **3o** was reacted as per the general method 3.6.7 to yield **1o** (92%); Purity 99.1%; mp 327 - 329°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.27 (br, 1H, NH); 8.03 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.97 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.06

(d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 5.93 (d, 1H, ^{3}J=7.5 Hz, H-6); 4.10 (q, 2H, ^{3}J=7.0 Hz, CH_2); 1.36 (t, 3H, ^{3}J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.8 (C-2); 160.3 (C-4'), 156.2 (C-3a); 151.4 (C-5); 140.0 (C-7); 128.3 (C-2' and C-6'); 122.3 (C-1'); 114.7 (C-3' and C-5'); 99.4 (C-6); 63.3 (CH_2); 14.6 (CH_3).

2-(3-*iso*Propyloxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1p).

Compound **3p** was reacted as per the general method 3.6.7 to yield **1p** (96%); Purity 98.6%; mp 280 - 282°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.31 (br, 1H, NH); 7.99 (d, 1H, ^{3}J=7.4 Hz, H-5); 7.67 (ddd, 1H, ^{3}J=7.9 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.60 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.5 Hz, H-2'); 7.42 (t, 1H, ^{3}J=7.9 Hz, H-5'); 7.05 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 5.94 (d, 1H, ^{3}J=7.5 Hz, H-6); 4.69 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.31 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.6 (C-2); 157.7 (C-3'); 156.3 (C-3a); 151.9 (C-5); 140.9 (C-7); 131.6 (C-1'); 130.2 (C-5'); 118.8 (C-6'); 118.0 (C-4'); 113.1 (C-2'); 99.2 (C-6); 69.5 (<u>C</u>H$(CH_3)_2$); 21.8 (2C, CH(<u>C</u>H$_3)_2$).

2-(4-*iso*Propyloxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1q).

Compound **3q** was reacted as per the general method 3.6.7 to yield **1q** (78%); Purity 98.2%; mp 314 - 316°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.27 (br, 1H, NH); 8.02 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.96 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.05 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 5.93 (d, 1H, ^{3}J=7.5 Hz, H-6); 4.71 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.30 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.8 (C-2); 159.3 (C-4'), 156.2 (C-3a); 151.4 (C-5); 140.0 (C-7); 128.3 (C-2' and C-6'); 122.1 (C-1'); 115.7 (C-3' and C-5'); 99.4 (C-6); 69.4 (<u>C</u>H$(CH_3)_2$); 21.8 (2C, CH(<u>C</u>H$_3)_2$).

2-(3-*iso*Butyloxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1r).

Compound **3r** was reacted as per the general method 3.6.7 to yield **1r** (93%); Purity 98.3%; mp 306 - 309°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.31 (br, 1H, NH); 8.00 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.69 (ddd, 1H, ^{3}J=7.8 Hz, ^{4}J=1.3 Hz, ^{4}J=1.3 Hz, H-6'); 7.62 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.5 Hz, H-2'); 7.43 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.08 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.7 Hz, ^{4}J=0.9 Hz, H-4'); 5.95 (d, 1H, ^{3}J=7.5 Hz, H-6); 3.83 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.06 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 1.01 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.6 (C-2); 159.0 (C-3'); 156.2 (C-3a); 151.4 (C-5); 140.2 (C-7); 131.3 (C-1'); 130.1 (C-5'); 118.9 (C-

6'); 117.0 (C-4'); 111.9 (C-2'); 99.4 (C-6); 73.8 (CH_2); 27.7 ($\underline{C}H(CH_3)_2$); 19.0 (2C, $CH(\underline{C}H_3)_2$).

2-(4-*iso*Butyloxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1s).

Compound **3s** was reacted as per the general method 3.6.7 to yield **1s** (76%); Purity 99.1%; mp 333 - 334°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.03 (d, 2H, ^3J=8.9 Hz, H-2' and H-6'); 7.96 (d, 1H, ^3J=7.5 Hz, H-5); 7.07 (d, 2H, ^3J=8.9 Hz, H-3' and H-5'); 5.93 (d, 1H, ^3J=7.5 Hz, H-6); 3.82 (d, 2H, ^3J=6.6 Hz, CH_2); 2.04 (m, 1H, ^3J=6.6 Hz, $C\underline{H}(CH_3)_2$); 1.00 (d, 6H, ^3J=6.7 Hz, $CH(C\underline{H_3})_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.8 (C-2); 160.6 (C-4'), 156.3 (C-3a); 151.6 (C-5); 140.3 (C-7); 128.3 (C-2' and C-6'); 122.4 (C-1'); 114.8 (C-3' and C-5'); 99.4 (C-6); 73.9 (CH_2); 27.7 ($\underline{C}H(CH_3)_2$); 19.1 (2C, $CH(\underline{C}H_3)_2$).

2-(3-Trifluoromethylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1t).

Compound **3t** was reacted as per the general method 3.6.7 to yield **1t** (98%); Purity 98.1%; mp 343 - 345°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.43 (br, 1H, NH); 8.41 (d, 1H, ^3J=7.8 Hz, H-4'); 8.36 (s, 1H, H-2'); 8.03 (d, 1H, ^3J=7.6 Hz, H-5); 7.91 (d, 1H, ^3J=7.8 Hz, H-6'); 7.80 (t, 1H, ^3J=7.8 Hz, H-5'); 6.00 (d, 1H, ^3J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.5 (C-2); 156.2 (C-3a); 151.7 (C-5); 140.4 (C-7); 131.1 (C-1'); 130.5 (C-5'); 130.4 (C-6'); 129.7 (q, $^2J_{CF}$=32.0 Hz, C-3'); 126.9 (q, $^3J_{CF}$=1.2 Hz, C-4'); 124.0 (q, $^1J_{CF}$=272.3 Hz, CF_3); 122.8 (q, $^3J_{CF}$=1.3 Hz, C-2'); 99.7 (C-6).

2-(4-Trifluoromethylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1u).

Compound **3u** was reacted as per the general method 3.6.7 to yield **1u** (92%); Purity 98.6%; mp 358 - 360°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.42 (br, 1H, NH); 8.33 (d, 2H, ^3J=8.0 Hz, H-2' and H-6'); 8.03 (d, 1H, ^3J=7.6 Hz, H-5); 7.91 (d, 2H, ^3J=8.2 Hz, H-3' and H-5'); 6.00 (d, 1H, ^3J=7.6 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.6 (C-2), 156.3 (C-3a); 151.7 (C-5); 140.5 (C-7); 134.0 (q, $^5J_{CF}$=1.6 Hz, C-1'); 130.4 (q, $^2J_{CF}$=32.0 Hz, C-4'); 127.4 (C-2' and C-6'); 126.0 (q, $^3J_{CF}$=3.7 Hz, C-3' and C-5'); 124.1 (q, $^1J_{CF}$=272.3 Hz, CF_3); 99.7 (C-6).

2-(3-Trifluoromethoxylphenyl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1v).

Compound **3v** was reacted as per the general method 3.6.7 to yield **1v** (58%); Purity 96.6%; mp 321 - 323°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.40 (br,

1H, NH); 8.15 (ddd, 1H, ^{3}J=7.8 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 8.03 (d, 1H, ^{3}J=7.6 Hz, H-5); 8.00 – 7.96 (m, 1H, H-2'); 7.70 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.57 – 7.50 (m, 1H, H-4'); 5.99 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.4 (C-2); 156.2 (C-3a); 151.7 (C-5); 148.7 (q, ^{3}J=1.7 Hz, C-3'); 140.5 (C-7); 132.3 (C-1'); 131.3 (C-5'); 125.6 (C-6'); 122.9 (C-4'); 120.1 (q, $^{1}J_{CF}$=256.7 Hz, CF_3); 118.5 (C-2'); 99.6 (C-6).

2-(Pyridin-3-yl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1w).

Compound **3w** was reacted as per the general method 3.6.7 to yield **1w** (77%); Purity 99.4%; mp 354 - 356°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.43 (br, 1H, NH); 9.27 (br, 1H, H-2'); 8.72 (d, 1H, ^{3}J=5.5 Hz, H-6'); 8.44 (dt, 1H, ^{3}J=8.0 Hz, ^{4}J=1.9 Hz, H-4'); 8.02 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.58 (dd, 1H, ^{3}J=7.9 Hz, ^{3}J=4.8 Hz, H-5'); 5.99 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.8 (C-2); 156.2 (C-3a); 151.7 (C-5); 151.2 (C-6'); 147.6 (C-4'); 140.5 (C-7); 134.1 (C-5'); 126.1 (C-3'); 124.1 (C-2'); 99.6 (C-6).

2-(Pyridin-4-yl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1x).

Compound **3x** was reacted as per the general method 3.6.7 to yield **1x** (43%); Purity 98.6%; mp 368 - 370°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.5 (br, 1H, NH); 8.76 (br, 2H, H-2' and H-6'); 8.04 (d, 1H, ^{3}J=7.6 Hz, H-5); 8.03 (d, 2H, ^{3}J=4.6 Hz, H-3' and H-5'); 6.00 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.9 (C-2); 156.2 (C-3a); 151.8 (C-5); 150.6 (C-2' and C-6'); 140.6 (C-7); 137.3 (C-3' and C-5'); 120.8 (C-4'); 99.6 (C-6).

2-(Thien-2-yl)-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one (1y).

Compound **3y** was reacted as per the general method 3.6.7 to yield **1y** (57%); Purity 99.3%; mp > 400°C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.98 (d, 1H, ^{3}J=7.5 Hz, H-5); 7.76 (dd, 1H, ^{3}J=3.6 Hz, ^{4}J=1.1 Hz, H-5'); 7.75 (dd, 1H, ^{3}J=5.1 Hz, ^{4}J=1.1 Hz, H-3'); 7.22 (dd, 1H, ^{3}J=5.0 Hz, ^{3}J=3.6 Hz, H-4'); 5.95 (d, 1H, ^{3}J=7.5 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 157.2 (C-2); 156.1 (C-3a); 151.4 (C-5); 140.2 (C-7); 132.8 (C-2'); 129.1 (C-3'); 128.3 (C-5'); 128.0 (C-4'); 99.6 (C6).

3.6.8 Xanthine oxidase inhibitory assay

The effect of each compound on XO activity was evaluated spectrophotometrically monitoring absorbance of uric acid at 295 nm. Bovine milk XO was reconstituted with 55 mM phosphate buffer at pH 7.5 to obtain a suitable concentration that gave a final enzyme concentration of 0.01 IU/mL during each assay. Xanthine was used as substrate at a final concentration of 40 μM and Allopurinol was used as positive control. Each compound and allopurinol were dissolved in DMSO and diluted to a suitable concentration using the phosphate buffer. The final DMSO concentration was not more than 1% v/v. A blank solution with DMSO at 1% v/v was shown no effect on XO enzymatic activity. All tests were performed at 37°C by recording UV absorbance due to the formation of uric acid for 60 seconds at 4 second intervals. Each compound was tested in triplicate at five different concentrations. The slope of the curve of absorbance vs time and resulting IC_{50} values for each compound were calculated using GraphPad Prism 9.2.0 (GraphPad Software Inc.).

The enzyme kinetics assay with two most active compounds (**1t** and **3q**) was also used to identify their mechanism of inhibition. Five concentrations of both **1t** and **3q** were tested using xanthine at five different concentrations (5, 10, 20, 30 and 40 μM).

GraphPad Prism 9.2.0 (GraphPad Software Inc.) was used to perform a non-linear regression using the Michaelis-Menten equation and a Lineweaver-Burk plot was used to identify the mechanism of inhibition.

3.6.9 HPLC purity characterisation

HPLC was used to assess the purity of each compound. The experiments were performed using Agilent HPLC 1260 Infinity II instrument with a PDA detector and an Apollo C18, 5 μ, 150 x ID 4.6 mm column.

The flow rate was 1 mL/min applying a 27 minute gradient, with a mobile phase A of 20 mM phosphate buffer pH 6.9 and a mobile phase B of methanol.

Time (min)	A %	B %
0	90	10
2	90	10
15	20	80
22	20	80
24	90	10
27	90	10

Data were collected at UV wavelength of 254 nm. Each sample was prepared at an approximate concentration of 0.5 mg/mL in DMSO. Duplicate injection was performed using a 5 µL injection volume.

A modification of the above gradient was used to analyse compounds **1a**, **2a** and **3a** to move the retention time of the compounds further from the solvent front.

Time (min)	A %	B %
0	100	0
3	100	0
15	20	80
22	20	80
24	100	0
27	100	0

3.6.10 Molecular docking

Molecular docking simulations were performed using Autodock 4.0[210] with an interaction grid with 0.375 Å spacing. The crystal structure of bovine milk XO in complex with hypoxanthine (PDB entry 3nrz) was used to study the interactions between the inhibitor and the enzyme. The internal energy of each molecule and the enzyme was minimised using Biovia Discovery Studio 2021 (Dassault Systèmes). The structure of the enzyme was prepared by removing the ligand hypoxanthine and all of the water molecules. CHARMM charges were assigned to both the enzyme and the inhibitors. The grid box for docking was centered using the coordinates X center = 89.606, Y center = 9.758 and Z center = 17.716. The grid box had the following dimensions: x = 52 Å, y = 50 Å and z = 58 Å.

The final predicted docked poses were visualised using Biovia Discovery Studio 2021 (Dassault Systèmes).

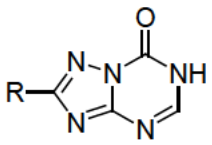
Chapter 4

4 Synthesis, in-vitro testing and molecular modelling of 2-substituted-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6H)-one derivatives

4.1 Introduction

Based on the results obtained with the 2-substituted-1,2,4-triazolo[1,5-*a*]-pyrimidin-7-one derivatives as inhibitors of XO, described in Chapter 3, the effect of an extra nitrogen on the two fused heterocycle rings was investigated. Derivatives of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine have already demonstrated to have a wide range of biological activities such as inhibitors of adenosine receptor[211, 212] for the treatment of various neurological disorders, inhibitors of eosinophilia for the treatment of asthma[213], and inhibitors of thymidine phosphorylase for the treatment of cancer[214]. They have also demonstrated anti-inflammatory, anti-oxidant, anti-bacterial and anti-fungal properties[215, 216]. Robins et al.[8] investigated the ability of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives to inhibit XO. In their work the authors synthesised six 5-substituted-[1,2,4]-triazolo-[1,5-*a*][1,3,5]triazin-7-one compounds and reported their *in vitro* IC_{50} values, ranging from 1.4 to 100 μM (Allopurinol was reported with IC_{50} = 5.9 μM). None of the [1,2,4]-triazolo-[1,5-*a*][1,3,5]triazin-7-one analogues reported by these authors had a substitution in position 2.

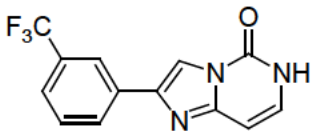
To exploit the results obtained with the molecules described in Chapter 3, it was decided to synthesise analogues of 2-substituted-[1,2,4]-triazolo-[1,5-*a*][1,3,5]triazin-7-one (**1**), test their inhibitory activity and identify the enzyme-inhibitor interaction via molecular docking.



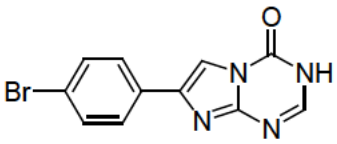
1

R = a)Ph, b)2-FC_6H_4, c)3-FC_6H_4, d)4-FC_6H_4, e)3-ClC_6H_4, f)4-ClC_6H_4, g)3-MeC_6H_4, h)4-MeC_6H_4, i)3-$MeOC_6H_4$, j)4-$MeOC_6H_4$, k)3-$EtOC_6H_4$, l)4-$EtOC_6H_4$, m)3-*i*$PrOC_6H_4$, n)4-*i*$PrOC_6H_4$, o)3-*i*$BuOC_6H_4$, p)4-*i*$BuOC_6H_4$, q)Pyridin-3-yl, r)Thien-2-yl, s)Indolin-1-yl, t)Morpholin-1-yl.

The positive effect of a substituent in position 2 was also supported by the work published in 2014 by Evenas et al[176]. In their search for new XO inhibitors they identified two hypoxanthine analogues (**2** and **3**) with IC_{50} values lower than 1 μM and with substitution pattern similar to that of the proposed [1,2,4]-triazolo-[1,5-*a*][1,3,5]triazin-7-ones.



2

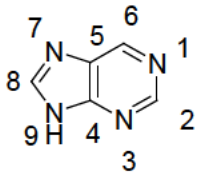


3

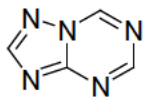
A library of 20 compounds in the [1,2,4]-triazolo-[1,5-*a*][1,3,5]triazin-7-one (**1**) series and substituted in position 2, by either aromatic or non-aromatic substituents, was synthesised. Their IC_{50} values were determined using xanthine as substrate and compared with the enzyme inhibition produced be a reference drug, Allopurinol. The structure of the most active molecule was docked into the active site of XO.

4.2 Chemistry

Azolo triazines and, more specifically, 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives have a skeleton structure almost identical to purine, with only one extra nitrogen at position 5 of the purine ring (Figure 1). This similarity with the heterocycle, which re-occurs in nature, makes them promising compounds for the discovery of new drugs.



Purine



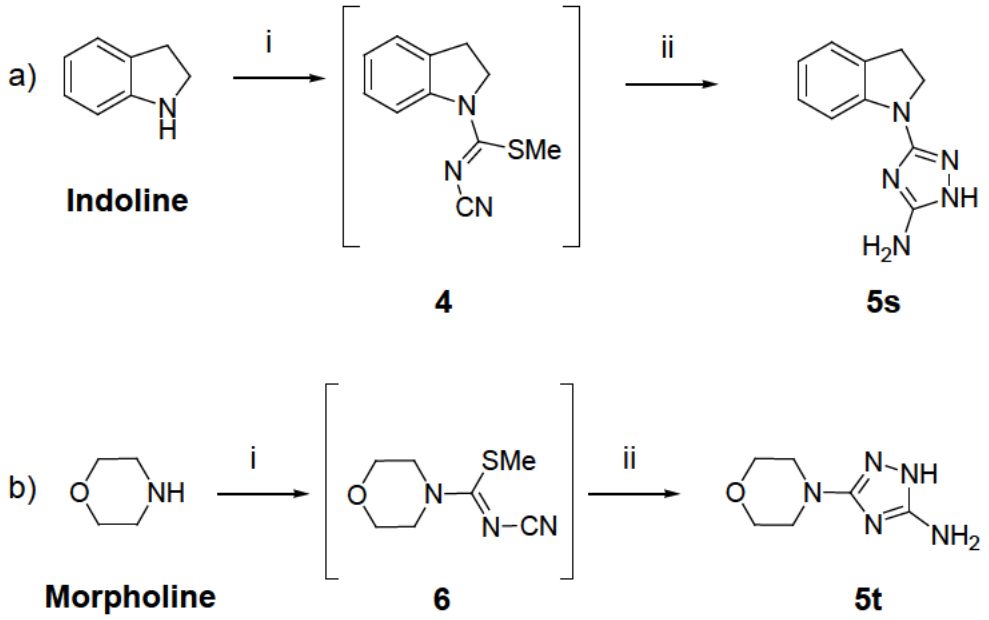
1,2,4-triazolo[1,5-*a*][1,3,5]triazine

Fig 1.

The synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives described in the literature[215, 216] can be summarised into two main procedures: (1) triazine ring formation starting from 1,2,4-triazole derivatives, or (2) 1,2,4-triazole ring

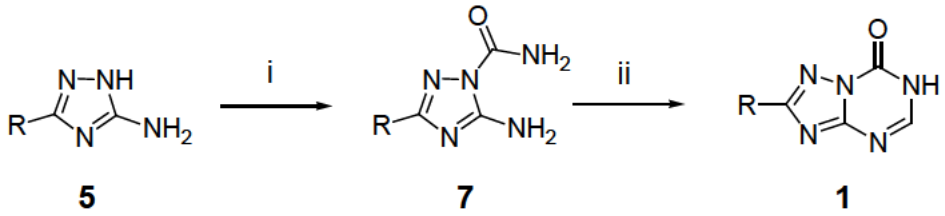
formation starting from 1,3,5-triazine derivatives. The first procedure was chosen because it would use a selection of the same starting 5-amino-1,2,4-triazoles prepared during the work described in Chapter 3. A library of 18 triazole derivatives of type **5** was selected based on the results obtained with the compounds described in Chapter 3, and two more new analogues were added to explore a bicycle system (indoline) and a non-aromatic ring (morpholine).

The synthesis of these two new triazoles derivatives was performed as per Scheme 1:



Scheme 1: i) $(CH_3S)_2C{=}NCN$, MeOH, reflux ii) N_2H_4, MeOH, reflux

A total of 20 derivatives of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine-7-one (**1**) were synthesised as shown in Scheme 2:



R = a)Ph, b)2-FC_6H_4, c)3-FC_6H_4, d)4-FC_6H_4, e)3-ClC_6H_4, f)4-ClC_6H_4, g)3-MeC_6H_4, h)4-MeC_6H_4, i)3-$MeOC_6H_4$, j)4-$MeOC_6H_4$, k)3-$EtOC_6H_4$, l)4-$EtOC_6H_4$, m)3-*i*$PrOC_6H_4$, n)4-*i*$PrOC_6H_4$, o)3-*i*$BuOC_6H_4$, p)4-*i*$BuOC_6H_4$, q)Pyridin-3-yl, r)Thien-2-yl, s)Indolin-1-yl, t)Morpholin-1-yl.

Scheme 2: i) KCNO, HCl, 40% EtOH, room temp. ii) $HC(OEt)_3$, toluene, μW, 30 min, 180°C

A modification of the reaction conditions described by Akakoshi et al.[217] was developed and optimised for the synthesis of intermediate **7**. Ethanol was added

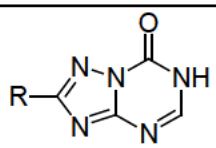
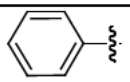
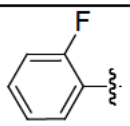
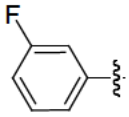
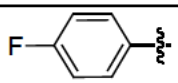
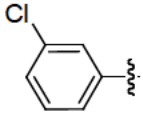
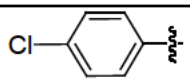
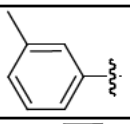
to water as co-solvent for the reaction to improve the solubility of the starting material (**5**). Using 40% ethanol in water also helped to increase the reaction yield and facilitated the purification of the reaction product **7**.

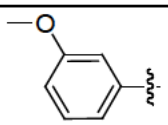
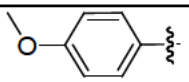
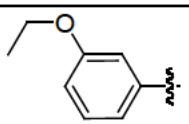
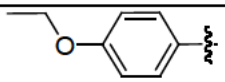
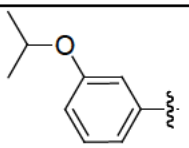
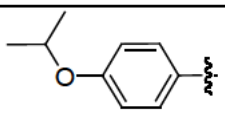
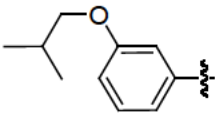
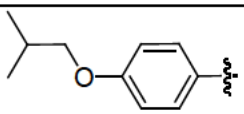
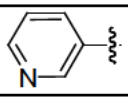
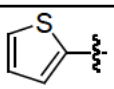
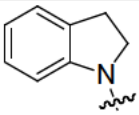
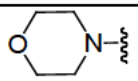
Optimised microwave-assisted reaction conditions were used to prepare the final analogues **1**.

4.3 *In vitro* inhibition of XO

The ability of compounds to prevent oxidation of xanthine by bovine milk XO was assessed at five different concentrations to determine IC_{50} values (i.e. the concentration of the inhibitor required to inhibit 50% of the enzyme activity. The enzyme activity was measured spectrophotometrically at 295 nm by recording the absorbance of uric acid formed over the time from xanthine. Every sample was tested in triplicate and its average IC_{50} is reported in Table 1. Allopurinol was used as a reference and its IC_{50} value was estimated to be 16.42 µM.

Table 1. *In vitro* inhibition data of XO for all synthesised inhibitors.[a]

 1	R	IC_{50} (µM)
a		0.474 ± 0.066
b		0.917 ± 0.134
c		0.308 ± 0.050
d		0.346 ± 0.024
e		0.152 ± 0.022
f		0.235 ± 0.005
g		0.218 ± 0.006
h		0.548 ± 0.081

i		0.044 ± 0.001
j		0.139 ± 0.015
k		0.045 ± 0.001
l		0.138 ± 0.012
m		0.024 ± 0.001
n		0.106 ± 0.008
o		0.0178 ± 0.0003
p		0.055 ± 0.002
q		0.551 ± 0.112
r		0.276 ± 0.006
s		0.225 ± 0.024
t		>100
Allopurinol[b]		16.42 ± 2.46

[a] Experiments were conducted at 37°C in 55 mM phosphate buffer pH 7.5, 40 μM xanthine solution, 0.01 U/mL solution of XO and < 1% of DMSO.

[b] Positive control.

With the exception of the analogue carrying the non-aromatic morpholino group (**1t**), all the others showed better inhibitory activity than the control Allopurinol, suggesting that the more "flat" aromatic substituent at position 2 of compounds of type **1** was a better fit for the active site of the enzyme. Derivatives with a phenyl substituent carrying a halogen atom (**1b-f**) resulted in worse inhibitory activity than derivatives with phenyl substituents carrying an alkyloxy group (**1i-p**). Amongst this subgroup of compounds, analogues with the alkyoxy group at

position 3 of the phenyl ring (**1i**, **1k**, **1m** and **1o**) demonstrated to be 3-4 times more potent than analogues with an alkyloxy group at position 4 of the phenyl ring (**1j**, **1l**, **1n** and **1p**). This behaviour was also observed with analogues bearing a methyl group on the phenyl ring (**1g** compared to **1h**). Interestingly, the piridyn-3-yl (**1q**) and phenyl (**1a**) derivatives exhibited comparable inhibitory activity, but it was more than twice higher for the indolin-1-yl derivative (**1s**). The five-membered heteroaromatic ring of thiophene (**1r**) also demonstrated to be a better substituent than the phenyl (**1a**) and pyridine (**1q**) ring. The most active compounds were the 3-alkyloxyphenyl derivatives, and the order of potency was found to be 3-*i*BuOC$_6$H$_4$>3-*i*PrOC$_6$H$_4$>3-MeOC$_6$H$_4$, 3-EtOC$_6$H$_4$. The most effective compound **1o** was more than 900-fold stronger inhibitor of XO compared to the standard drug Allopurinol.

Enzyme kinetic studies performed using the lead compound **1o** revealed a mix type inhibition mechanism as reflected in the Lineweaver-Burk plot (Figure 2) obtained in experiments at different concentrations of the substrate (xanthine).

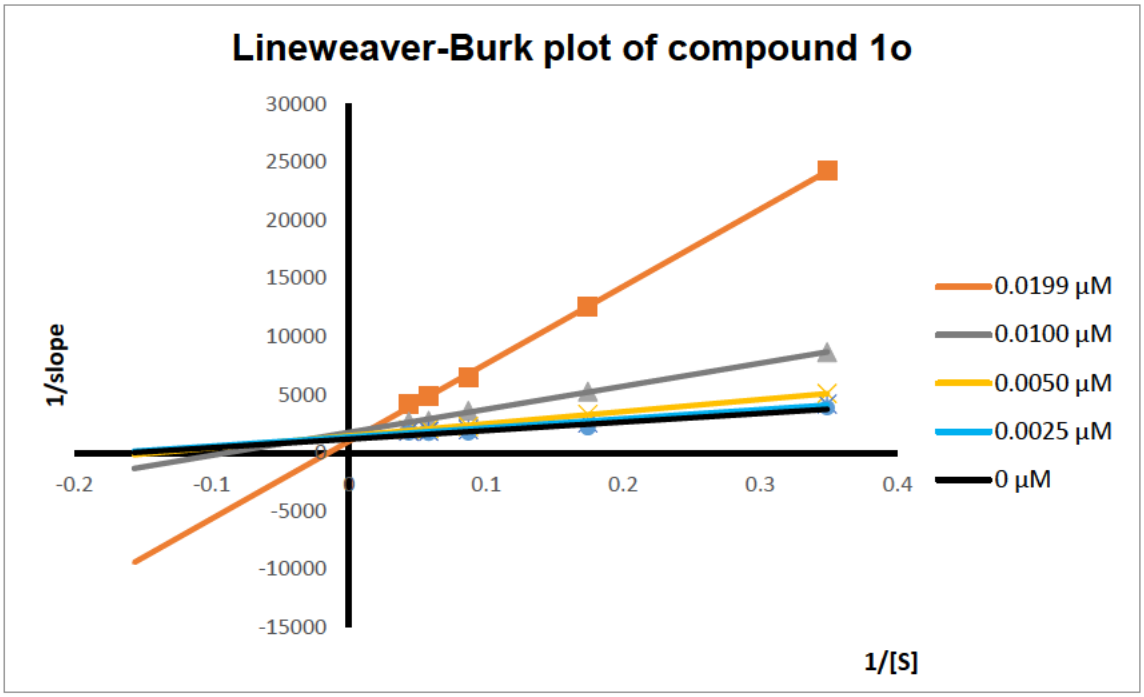


Fig 2: Lineweaver-Burk plot of compounds **1o**.

4.4 Molecular docking

The crystal structure of bovine milk XO in complex with hypoxanthine (PDB entry 3nrz) was used to study the interactions between the inhibitor and the enzyme. The internal energy of each molecule was minimised using Biovia Discovery

Studio 2021 (Dassault Systèmes). The structure of the enzyme was prepared by removing the ligand hypoxanthine and all of the water molecules. CHARMM charges were assigned to both the enzyme and the inhibitors. Molecular docking simulations were then performed using Autodock 4.0[210] with an interaction grid spacing of 0.375 Å. The final predicted docked poses were visualised using Biovia Discovery Studio 2021 (Dassault Systèmes).

The predicted poses of all the 20 analogues had the triazinone ring located towards the inside of the enzyme active site and were predicted to overlap with each other. All the structures showed similar interactions with the amino acid residues within the enzyme active site. The carbonylic oxygen of the inhibitors was predicted for the majority of analogues to form hydrogen bonds with both the Thr1010 amidic hydrogen and the Arg880 guanidinic hydrogen. Residues Phe914 and Phe1009 were predicted to stabilise the enzyme-inhibitor complex in the same way it has been reported in literature for the natural substrate of the enzyme: a π-π interaction that results in the purine analogue ring being sandwiched between Phe914 and Phe1009. Figure 3 shows the predicted interactions between the most active compound **1o** and the residues in the enzyme active site.

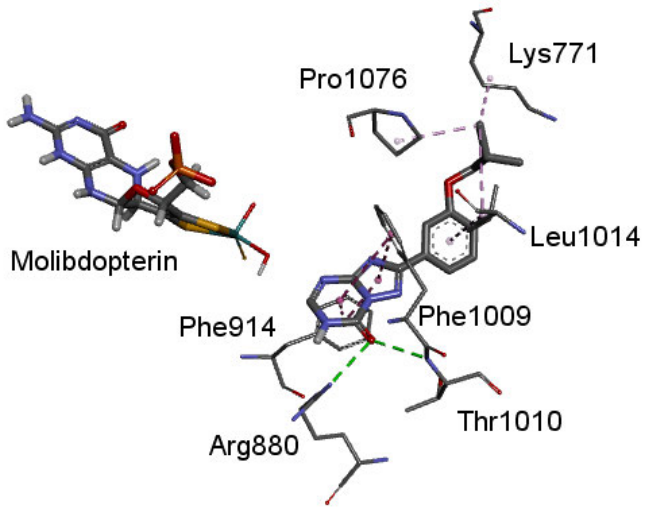


Fig 3: Predicted interactions between amino acid residues in the XO active site and the most active compound **1o**. Hydrogen bonds are shown in green, π-π interactions in dark purple and hydrophobic interactions in light purple.

The flexibility of the alkyloxy side chain on the phenyl ring allowed a better fit within the active site when compared to the less flexible indoline ring. The triazolo triazinone ring of compound **1s** with an indoline ring as substituent was predicted

to be located in a more retrograde position compared to that of the triazolo triazinone ring of all the alkyloxyphenyl analogues (**1i-p**). As a result, compound **1s** was predicted to lose one of the stabilising hydrogen bonds between the Arg880 side chain hydrogen and the carbonylic oxygen on the inhibitor, as shown in Figure 4. This could explain the loss of inhibitory activity.

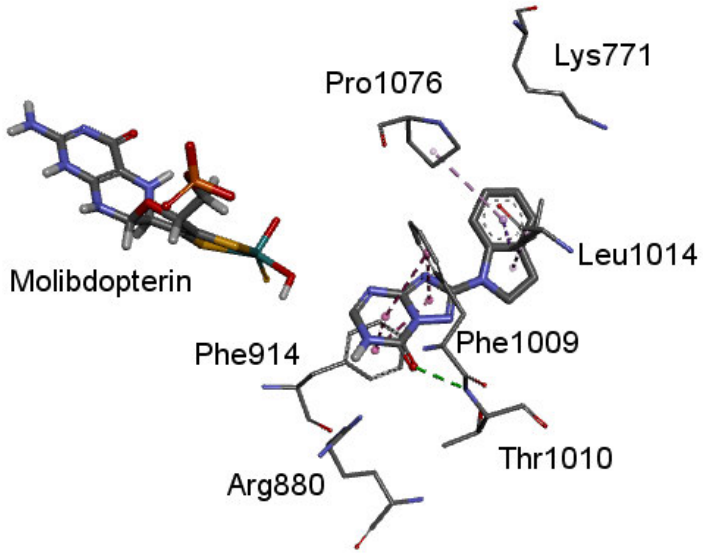


Fig 4: Predicted interactions between the amino acid residues in the XO active site and the most active compound **1s**. Hydrogen bonds are shown in green, π-π interactions in dark purple and hydrophobic interactions in light purple.

The very low inhibitory activity exhibited by compound **1t**, with a morpholine ring as substituent at position 2 of the triazolo triazinone rings, could be explained, when compared to the most potent compound **1o**, by inspecting the hydrophobic surfaces of the enzyme active site, as shown in Figure 5.

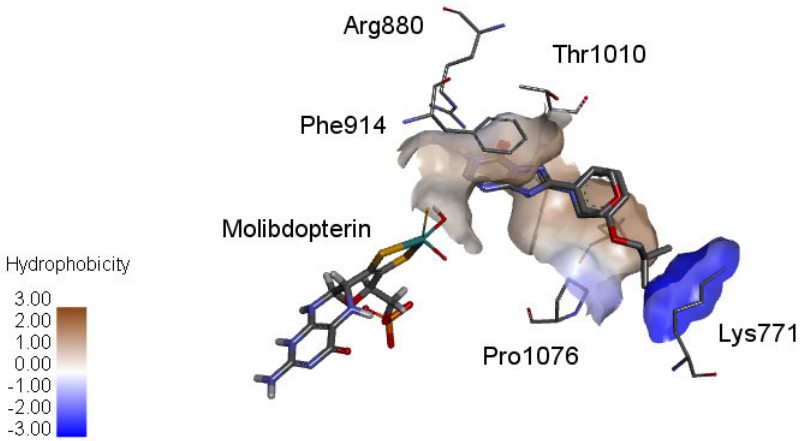


Fig 5: Representation of the hydrophobic surfaces of amino acid residues within the active site of XO. The superimposed predicted poses of compound **1t** (morpholinic oxygen in red) and **1o** are shown.

The morpholine group of compound **1t** is predicted to be sandwiched between two hydrophobic regions, causing a non-favourable enzyme-inhibitor interaction, whilst the oxygen of the isobutyloxy in compound **1o,** due to the more flexible side chain, is directed towards the less hydrophobic region of Pro1076.

4.5 Conclusions

A total of 20 novel 2-substituted-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-one analogues were designed and prepared based on the inhibitory activity obtained with 2-substituted-7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidinones described in Chapter 3. *In vitro* testing was performed with each compound to determine their inhibitory activity against XO (IC_{50}), whilst enzyme inhibition kinetics studies of the most active compound (**1o**) demonstrated a mixed type inhibition mechanism. All the newly synthesised analogues exhibited, with the exception of compound **1t**, better inhibitory activity than the control Allopurinol. The most active molecule inhibited the enzyme more than 900 times more than Allopurinol. Molecular docking simulations predicted that all the compounds were positioned inside the active site directing their triazinone ring towards the molibdopterin cofactor, with the substituent at position 2 oriented towards the entrance of the active site. Compound **1t** demonstrated to be more than 10 times weaker than Allopurinol. This decrease in potency could be explained by the fact that the morpholine ring is predicted to be sandwiched between two hydrophobic regions within the enzyme active site, resulting in a non-favourable enzyme-inhibitor interaction.

4.6 Experimental

4.6.1 Chemistry

Reagents were purchased either from Alfa Aesar or Sigma-Aldrich. Microwave-assisted reactions were conducted using a CEM Discover SP instrument. Melting points were measured using an Electrothermal Digital melting point apparatus (IA9100). ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker Avance III 400 MHz spectrometer using DMSO-d_6 as solvent. Enzyme inhibition was evaluated using a Shimadzu UV-1280 spectrophotometer with a 1 mL quartz cuvette. Bovine milk XO was purchased from Sigma-Aldrich. Statistical analysis was performed using GraphPad Prism 9.2.0.

4.6.2 General method for the synthesis of 3-substituted-5-amino-1,2,4-triazoles (**5s** and **5t**)

A mixture of the appropriate amine (morpholine or indoline) (16.8 mmol) and dimethyl *N*-cyanodithioiminocarbonate (14.6 mmol) was heated under reflux in methanol (30 mL) overnight. After this time, hydrazine (17.5 mmol) was added to the solution and heating continued for another four hours. The solvent was evaporated and the product was recrystallized from ethanol.

5-Amino-3-(indolin-1-yl)-1,2,4-triazole (5s)

Indoline was reacted as per the general method 4.6.2 to yield **5s** (49%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.31 (br, 1H, NH); 7.74 (d, 1H, ^{3}J=7.9 Hz, H-4'); 7.10 (d, 1H, ^{3}J=7.2 Hz, H-7'); 7.05 (t, 1H, ^{3}J=7.6 Hz, H-5'); 6.69 (t, 1H, ^{3}J=7.3 Hz, H-6'); 5.95 (br, 2H, NH_2); 3.90 (t, 2H, ^{3}J=8.8 Hz, H-2'); 3.08 (t, 2H, ^{3}J=8.7 Hz, H-3'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.4 (C-3); 155.8 (C-5); 145.3 (C-7'a); 130.1 (C-3'a); 126.9 (C-6'); 124.3 (C-4'); 118.8 (C-5'); 111.3 (C-7'); 48.7 (C-2'); 27.3 (C-3')

5-Amino-3-(morpholin-1-yl)-1,2,4-triazole (5t)

Morpholine was reacted as per the general method 4.6.2 to yield **5t** (89%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.63 (br, 2H, NH_2); 3.68 – 3.58 (m, 2H, H-3' and H-5'); 3.16 – 3.07 (m, 2H, H-2' and H-6'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.6 (C-3); 156.4 (C-5); 65.7 (CH_2OCH_2); 46.8 (CH_2NCH_2).

4.6.3 General method for the synthesis of 5-amino-3-substituted-1-carbamoyl-1,2,4-triazoles (**7**)

The appropriate 5-amino-3-substituted-1,2,4-triazole (5 mmol) was added portionwise to a solution of potassium cyanate (10 mmol) in aqueous ethanol (40%, 10 mL) while keeping the reaction flask in an ice bath. Hydrochloric acid (10 mmol) was added dropwise afterwards while maintaining the reaction flask in the ice bath. After 10 min, the reaction was left to stir at room temperature until no 5-amino-3-substituted-1,2,4-triazole could be observed by TLC. The mixture was filtered and the collected solid was washed with water before drying under vacuum at 50 °C.

5-Amino-3-(3-chlorophenyl)-1-carbamoyl-1,2,4-triazole (7e)

5-Amino-3-(3-chlorophenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7e** (89%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.01 – 7.97 (m, 1H, H-2'); 7.93 – 7.89 (m, 1H, H-6'); 7.84 (br, 1H, NHCO); 7.70 (br, 1H, NHCO); 7.54 – 7.49 (m, 2H, H-4' and H-5'); 7.33 (br, 2H, NH_2).

5-Amino-3-(3-methylphenyl)-1-carbamoyl-1,2,4-triazole (7g)

5-Amino-3-(3-methylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7g** (98%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.84 – 7.81 (m, 1H, H-2'); 7.79 (br, 1H, NHCO); 7.77 (d, 1H, ^{3}J=7.7 Hz, H-6'); 7.60 (br, 1H, NHCO); 7.34 (t, 1H, ^{3}J=7.6 Hz, H-5'); 7.30 – 7.21 (m, 3H, H-4' and NH_2); 2.36 (s, 3H, CH_3).

5-Amino-3-(3-ethoxylphenyl)-1-carbamoyl-1,2,4-triazole (7k)

5-Amino-3-(3-ethoxylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7k** (99%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.80 (br, 1H, NHCO); 7.62 (br, 1H, NHCO); 7.54 (dt, 1H, ^{3}J=7.6 Hz, ^{4}J=1.2 Hz, H-6'); 7.52 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.3 Hz, H-2'); 7.35 (t, 1H, ^{3}J=7.9 Hz, H-5'); 7.27 (br, 2H, NH_2); 6.99 (ddd, 1H, ^{3}J=8.2 Hz, ^{4}J=2.6 Hz, ^{4}J=1.0 Hz, H-4'); 4.07 (q, 2H, ^{3}J=7.0 Hz, CH_2); 1.35 (t, 3H, ^{3}J=7.0 Hz, CH_3).

5-Amino-3-(4-ethoxylphenyl)-1-carbamoyl-1,2,4-triazole (7l)

5-Amino-3-(4-ethoxylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7l** (93%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.89 (d, 2H, ^{3}J=8.6 Hz, H-2' and H-6'); 7.76 (br, 1H, NHCO); 7.53 (br, 1H, NHCO); 7.23 (br, 2H, NH_2); 6.99 (d, 2H, ^{3}J=8.7 Hz, H-3' and H-5'); 4.07 (q, 2H, ^{3}J=6.9 Hz, CH_2); 1.34 (t, 3H, ^{3}J=6.9 Hz, CH_3).

5-Amino-3-(3-*iso*propyloxylphenyl)-1-carbamoyl-1,2,4-triazole (7m)

5-Amino-3-(3-*iso*propyloxylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7m** (99%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.80 (br, 1H, NHCO); 7.63 (br, 1H, NHCO); 7.55 – 7.48 (m, 2H, H-2' and H-6'); 7.34 (t, 1H, ^{3}J=7.9 Hz, H-5'); 7.27 (br, 2H, NH_2); 6.98 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.5 Hz, ^{4}J=1.0 Hz, H-4'); 4.64 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.29 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3$)$_2$).

5-Amino-3-(4-*iso*propyloxylphenyl)-1-carbamoyl-1,2,4-triazole (7n)

5-Amino-3-(4-*iso*propyloxylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7n** (91%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.87 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.76 (br, 1H, NHCO); 7.53 (br, 1H, NHCO); 7.23 (br, 2H, NH_2); 6.98 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.67 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.28 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$).

5-Amino-3-(3-*iso*butyloxylphenyl)-1-carbamoyl-1,2,4-triazole (7o)

5-Amino-3-(3-*iso*butyloxylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7o** (95%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.80 (br, 1H, NHCO); 7.64 (br, 1H, NHCO); 7.57 – 7.50 (m, 2H, H-2' and H-6'); 7.35 (t, 1H, ^{3}J=7.9 Hz, H-5'); 7.26 (br, 2H, NH_2); 7.00 (ddd, 1H, ^{3}J=8.2 Hz, ^{4}J=2.5 Hz, ^{4}J=0.9 Hz, H-4'); 3.78 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.03 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 1.00 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$).

5-Amino-3-(4-*iso*butyloxylphenyl)-1-carbamoyl-1,2,4-triazole (7p)

5-Amino-3-(4-*iso*butyloxylphenyl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7p** (90%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.89 (d, 2H, ^{3}J=8.9 Hz, H-2' and H-6'); 7.75 (br, 1H, NHCO); 7.54 (br, 1H, NHCO); 7.23 (br, 2H, NH_2); 7.00 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 3.79 (d, 2H, ^{3}J=6.5 Hz, CH_2); 2.03 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 0.99 (d, 6H, ^{3}J=6.7 Hz, CH(C<u>H</u>$_3)_2$).

5-Amino-3-(pyridin-3-yl)-1-carbamoyl-1,2,4-triazole (7q)

5-Amino-3-(pyridin-3-yl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7q** (85%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.14 (dd, 1H, ^{4}J=2.1 Hz, ^{4}J=0.7 Hz, H-2'); 8.64 (dd, 1H, ^{3}J=4.8 Hz, ^{4}J=1.7 Hz, H-6'), 8.26 (dt, 1H, ^{3}J=7.9 Hz, ^{4}J=1.9 Hz, H-4'); 7.86 (br, 1H, NHCO); 7.68 (br, 1H, NHCO); 7.51 (ddd, 1H, ^{3}J=7.9 Hz, ^{3}J=4.8 Hz, ^{4}J=0.8 Hz, H-5'); 7.36 (br, 2H, NH_2).

5-Amino-3-(indolin-1-yl)-1-carbamoyl-1,2,4-triazole (7s)

5-Amino-3-(indolin-1-yl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7s** (87%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.93 (d, 1H, ^{3}J=7.9 Hz, H-4'); 7.59 (br, 1H, NHCO); 7.29 (br, 3H, NHCO and NH_2); 7.15 (dd, 1H, ^{3}J=7.2

Hz, ^4J=0.8 Hz, H-7'); 7.10 (td, 1H, ^3J=7.7 Hz, ^4J=1.0 Hz, H-6'); 6.80 (td, 1H, ^3J=7.4 Hz, ^4J=0.9 Hz, H-5'); 3.99 (t, 2H, ^3J=8.8 Hz, H-2'); 3.13 (t, 2H, ^3J=8.7 Hz, H-3').

5-Amino-3-(morpholin-1-yl)-1-carbamoyl-1,2,4-triazole (7t)

5-Amino-3-(morpholin-1-yl)-1,2,4-triazole was reacted as per general method 4.6.3 to yield **7t** (90%). *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.94 (br, 1H, NH); 5.63 (br, 2H, NH_2); 3.62 (dd, 4H, ^3J=5.3 Hz, ^3J=4.2 Hz, H-3' and H-5'); 3.12 (dd, 4H, ^3J=5.2 Hz, ^3J=4.4 Hz, H-2' and H-6').

4.6.4 General method for the synthesis of 2-substituted-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-ones (**1**)

A mixture of the appropriate 5-amino-3-substituted-1-carbamoyl-1,2,4-triazole (1 mmol) and triethyl orthoformate (1.5 mmol) in 2 mL of toluene was heated at 180°C for 30 min in a microwave reactor. After cooling to room temperature, the precipitate was filtered and recrystallized from an appropriate solvent. Copies of NMR spectra are reported in Appendix 5.

2-Phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1a)

Compound **7a** was reacted as per the general method 4.6.4 to yield **1a** (68%). Final product was crystallized from ethanol. Mp 287 – 288 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.29 (br, 1H, NH); 8.42 (s, 1H, H-5); 8.19 – 8.12 (m, 2H, H-2' and H-6'); 7.59 - 7.50 (m, 3H, H-3', H-4' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 (C-2); 158.7 (C-3a); 151.1 (C-5); 143.9 (C-7); 130.7 (C-4'); 129.8 (C-1'); 129.0 (C-3' and C-5'); 126.8 (C-2' and C-6'). Anal. Calcd for $C_{10}H_7N_5O$: C, 56.34; H, 3.31; N, 32.85. Found: C, 56.48; H, 3.40; N, 32.64.

2-(2-Fluorophenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1b)

Compound **7b** was reacted as per the general method 4.6.4 to yield **1b** (35%). Final product was crystallized from methanol. Mp 257 – 258 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.33 (br, 1H, NH); 8.44 (s, 1H, H-5); 8.13 (td, 1H, ^3J=7.7 Hz, ^4J=1.7 Hz, H-6'); 7.66 - 7.54 (m, 1H, H-4'); 7.47 – 7.34 (m, 2H, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.0 (d, $^1J_{CF}$=255.3 Hz, C-2'); 159.8 (d, $^3J_{CF}$=4.9 Hz, C-2); 158.2 (br, C-3a); 151.3 (C-5); 143.8 (C-7); 132.6 (d, $^3J_{CF}$=8.4 Hz, C-4'); 130.7 (d, $^4J_{CF}$=2.2 Hz, C-5'); 124.9 (d, $^3J_{CF}$=3.5 Hz, C-6'); 117.8 (d,

$^{2}J_{CF}$=11.1 Hz, C-1'); 116.9 (d, $^{2}J_{CF}$=21.1 Hz, C-3'). Anal. Calcd for $C_{10}H_6FN_5O$: C, 51.95; H, 2.62; N, 30.29. Found: C, 52.06; H, 2.76; N, 30.14.

2-(3-Fluorophenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1c)

Compound **7c** was reacted as per the general method 4.6.4 to yield **1c** (40%). Mp 253 – 254 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.34 (br, 1H, NH); 8.44 (s, 1H, H-5); 8.01 (ddd, 1H, ^{3}J=7.9 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.86 (ddd, 1H, $^{3}J_{HF}$=9.9 Hz, ^{4}J=2.6 Hz, ^{4}J=1.4 Hz, H-2'); 7.61 (td,1H, ^{3}J=8.0 Hz, $^{4}J_{HF}$=6.0 Hz, H-5'); 7.40 (dddd, 1H, $^{3}J_{HF}$=8.5 Hz, ^{3}J=8.5 Hz, ^{4}J=2.6 Hz, ^{4}J=0.5 Hz, H-4'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4 (d, $^{1}J_{CF}$=244.0 Hz, C-3'); 161.8 (d, $^{4}J_{CF}$=3.1 Hz, C-2); 158.8 (C-3a); 151.4 (C-5); 143.8 (C-7); 132.2 (d, $^{3}J_{CF}$=8.4 Hz, C-1'); 131.3 (d, $^{3}J_{CF}$=8.3 Hz, C-5'); 122.9 (d, $^{4}J_{CF}$=2.7 Hz, C-6'); 117.6 (d, $^{2}J_{CF}$=21.1 Hz, C-4'); 113.3 (d, $^{2}J_{CF}$=23.4 Hz, C-2'). Anal. Calcd for $C_{10}H_6FN_5O$: C, 51.95; H, 2.62; N, 30.29. Found: C, 52.12; H, 2.78; N, 30.07.

2-(4-Fluorophenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1d)

Compound **7d** was reacted as per the general method 4.6.4 to yield **1d** (60%). Mp 279 – 280 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.30 (br, 1H, NH); 8.42 (s, 1H, H-5); 8.20 (dd, 2H, ^{3}J=8.9 Hz, $^{4}J_{HF}$=5.5 Hz, H-2' and H-6'); 7.39 (dd, 2H, $^{3}J_{HF}$=8.9 Hz, ^{3}J=8.9 Hz, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.6 (d, $^{1}J_{CF}$=247.9 Hz, C-4'); 162.1 (C-2); 158.8 (C-3a); 151.4 (C-5); 143.9 (C-7); 129.1 (d, $^{3}J_{CF}$=8.8 Hz, C-2' and C-6'); 126.4 (d, $^{4}J_{CF}$=3.0 Hz, C-1'); 116.1 (d, $^{2}J_{CF}$=22.0 Hz, C-3' and C-5'). Anal. Calcd for $C_{10}H_6FN_5O$: C, 51.95; H, 2.62; N, 30.29. Found: C, 52.09; H, 2.77; N, 30.02.

2-(3-Chlorophenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1e)

Compound **7e** was reacted as per the general method 4.6.4 to yield **1e** (62%). Final product was crystallized from ethanol. Mp 301 – 302 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.35 (br, 1H, NH); 8.44 (s, 1H, H-5); 8.16 – 8.07 (m, 2H, H-2' and H-6'); 7.65 – 7.56 (m, 2H, H-4' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.6 (C-2); 158.8 (C-3a); 151.4 (C-5); 143.8 (C-7); 133.8 (C-3'); 131.9 (C-1'); 131.1 (C-4'); 130.5 (C-5'); 126.2 (C-2'); 125.3 (C-6'). Anal. Calcd for $C_{10}H_6ClN_5O$: C, 48.50; H, 2.44; N, 28.28. Found: C, 48.55; H, 2.50; N, 28.12.

2-(4-Chlorophenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1f)

Compound **7f** was reacted as per the general method 4.6.4 to yield **1f** (49%). Final product was crystallized from ethanol 95%. Mp 265 – 266 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.33 (br, 1H, NH); 8.43 (s, 1H, H-5); 8.16 (d, 2H, ^{3}J=8.6 Hz, H-2' and H-6'); 7.62 (d, 2H, ^{3}J=8.6 Hz, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.0 (C-2); 158.7 (C-3a); 151.3 (C-5); 143.8 (C-7); 135.4 (C-4'); 129.2 (C-3' and C-5'); 128.7 (C-1'); 128.5 (C-2' and C-6'). Anal. Calcd for $C_{10}H_6ClN_5O$: C, 48.50; H, 2.44; N, 28.28. Found: C, 48.61; H, 2.52; N, 28.20.

2-(3-Methylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1g)

Compound **7g** was reacted as per the general method 4.6.4 to yield **1g** (53%). Final product was crystallized from methanol. Mp 295 – 297 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.26 (br, 1H, NH); 8.41 (s, 1H, H-5); 8.02 – 7.97 (m, 1H, H-2'); 7.97 – 7.90 (m, 1 H, H-6'); 7.43 (t, 1H, ^{3}J=7.6 Hz, H-5'); 7.38 – 7.31 (m, 1H, H-4'); 2.41 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 (C-2); 158.6 (C-3a); 151.1 (C-5); 143.8 (C-7); 138.2 (C-3'); 131.3 (C-4'); 129.8 (C-1'); 128.9 (C-5'); 127.2 (C-2'); 123.9 (C-6'); 21.0 (CH_3). Anal. Calcd for $C_{11}H_9N_5O$: C, 58.14; H, 3.99; N, 30.82. Found: C, 58.23; H, 4.06; N, 30.70.

2-(4-Methylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1h)

Compound **7h** was reacted as per the general method 4.6.4 to yield **1h** (60%). Final product was crystallized from ethanol. Mp 266 – 267 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.26 (br, 1H, NH); 8.40 (s, 1H, H-5); 8.04 (d, 2H, ^{3}J=8.1 Hz, H-2' and H-6'); 7.35 (d, 2H, ^{3}J=8.1 Hz, H-3' and H-5'); 2.39 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.0 (C-2); 158.6 (C-3a); 151.0 (C-5); 143.8 (C-7); 140.5 (C-4'); 129.6 (C-2' and C-6'); 127.1 (C-1'); 126.7 (C-3' and C-5'); 21.1 (CH_3). Anal. Calcd for $C_{11}H_9N_5O$: C, 58.14; H, 3.99; N, 30.82. Found: C, 58.19; H, 4.03; N, 30.75.

2-(3-Methoxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1i)

Compound **7i** was reacted as per the general method 4.6.4 to yield **1i** (65%). Final product was crystallized from methanol. Mp 267 – 268 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.29 (br, 1H, NH); 8.42 (s, 1H, H-5); 7.74 (dt, 1H, ^{3}J=7.6 Hz, ^{4}J=1.3 Hz, H-6'); 7.66 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.4 Hz, H-2'); 7.47 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.11 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.7 Hz, ^{4}J=0.9 Hz, H-4'); 3.86 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.8 (C-3'); 159.6 (C-2); 158.6 (C-3a);

151.2 (C-5); 143.8 (C-7); 131.2 (C-1'); 130.2 (C-5'); 119.1 (C-6'); 116.7 (C-4'); 111.5 (C-2'); 55.3 (OCH_3). Anal. Calcd for $C_{11}H_9N_5O_2$: C, 54.32; H, 3.73; N, 28.79. Found: C, 54.40; H, 3.81; N, 28.67.

2-(4-Methoxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1j)

Compound **7j** was reacted as per the general method 4.6.4 to yield **1j** (69%). Final product was crystallized from ethanol. Mp 295 – 296 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.24 (br, 1H, NH); 8.39 (s, 1H, H-5); 8.09 (d, 2H, ^3J=8.9 Hz, H-2' and H-6'); 7.10 (d, 2H, ^3J=8.9 Hz, H-3' and H-5'); 3.83 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.9 (C-4'); 161.2 (C-2); 158.5 (C-3a); 151.0 (C-5); 143.8 (C-7); 128.4 (C-2' and C-6'); 122.2 (C-1'); 114.4 (C-3' and C-5'); 55.3 (CH_3). Anal. Calcd for $C_{11}H_9N_5O_2$: C, 54.32; H, 3.73; N, 28.79. Found: C, 54.46; H, 3.82; N, 28.65.

2-(3-Ethoxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1k)

Compound **7k** was reacted as per the general method 4.6.4 to yield **1k** (31%). Final product was crystallized from methanol. Mp 279 – 281 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.27 (br, 1H, NH); 8.41 (s, 1H, H-5); 7.72 (dt, 1H, ^3J=7.6 Hz, ^4J=1.2 Hz, H-6'); 7.64 (dd, 1H, ^4J=2.5 Hz, ^4J=1.5 Hz, H-2'); 7.45 (t, 1H, ^3J=8.0 Hz, H-5'); 7.08 (ddd, 1H, ^3J=8.3 Hz, ^4J=2.6 Hz, ^4J=0.9 Hz, H-4'); 4.13 (q, 2H, ^3J=7.0 Hz, CH_2); 1.37 (t, 3H, ^3J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.7 (C-3'); 158.8 (C-2); 158.6 (C-3a); 151.3 (C-5); 143.9 (C-7); 131.2 (C-1'); 130.2 (C-5'); 119.0 (C-6'); 117.2 (C-4'); 111.8 (C-2'); 63.2 (CH_2); 14.6 (CH_3). Anal. Calcd for $C_{12}H_{11}N_5O_2$: C, 56.03; H, 4.31; N, 27.22. Found: C, 56.11; H, 4.40; N, 27.08.

2-(4-Ethoxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1l)

Compound **7l** was reacted as per the general method 4.6.4 to yield **1l** (72%). Final product was crystallized from methanol. Mp 305 – 307 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.24 (br, 1H, NH); 8.39 (s, 1H, H-5); 8.07 (d, 2H, ^3J=8.8 Hz, H-2' and H-6'); 7.07 (d, 2H, ^3J=8.9 Hz, H-3' and H-5'); 4.11 (q, 2H, ^3J=7.0 Hz, CH_2); 1.36 (t, 3H, ^3J=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.9 (C-4'); 160.5 (C-2); 158.5 (C-3a); 150.9 (C-5); 143.8 (C-7); 128.3 (C-2' and C-6'); 122.1 (C-1'); 114.8 (C-3' and C-5'); 63.3 (CH_2); 14.6 (CH_3). Anal. Calcd for $C_{12}H_{11}N_5O_2$: C, 56.03; H, 4.31; N, 27.22. Found: C, 56.08; H, 4.43; N, 27.07.

2-(3-*iso*Propyloxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1m)

Compound **7m** was reacted as per the general method 4.6.4 to yield **1m** (45%). Final product was crystallized from methanol. Mp 257 – 259 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.28 (br, 1H, NH); 8.41 (s, 1H, H-5); 7.70 (ddd, 1H, ^{3}J=7.7 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.63 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.5 Hz, H-2'); 7.44 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.07 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 4.71 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.31 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.7 (C-3'); 158.7 (C-2); 157.8 (C-3a); 151.4 (C-5); 144.0 (C-7); 131.2 (C-1'); 130.3 (C-5'); 118.9 (C-6'); 118.3 (C-4'); 113.2 (C-2'); 69.5 (<u>C</u>H$(CH_3)_2$); 21.8 (CH(<u>C</u>H$_3)_2$). Anal. Calcd for $C_{13}H_{13}N_5O_2$: C, 57.56; H, 4.83; N, 25.82. Found: C, 57.62; H, 4.90; N, 25.71.

2-(4-*iso*Propyloxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1n)

Compound **7n** was reacted as per the general method 4.6.4 to yield **1n** (49%). No further purification was required. Mp 288 – 290 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.23 (br, 1H, NH); 8.38 (s, 1H, H-5); 8.05 (d, 2H, ^{3}J=8.8 Hz, H-2' and H-6'); 7.06 (d, 2H, ^{3}J=8.9 Hz, H-3' and H-5'); 4.71 (m, 1H, ^{3}J=6.0 Hz, C<u>H</u>$(CH_3)_2$); 1.31 (d, 6H, ^{3}J=6.0 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.9 (C-4'); 159.5 (C-2); 158.5 (C-3a); 150.9 (C-5); 143.8 (C-7); 128.4 (C-2' and C-6'); 121.9 (C-1'); 115.7 (C-3' and C-5'); 69.4 (<u>C</u>H$(CH_3)_2$); 21.8 (CH(<u>C</u>H$_3)_2$). Anal. Calcd for $C_{13}H_{13}N_5O_2$: C, 57.56; H, 4.83; N, 25.82. Found: C, 57.42; H, 5.02; N, 25.58.

2-(3-*iso*Butyloxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1o)

Compound **7o** was reacted as per the general method 4.6.4 to yield **1o** (44%). Final product was crystallized from methanol. Mp 277 – 279 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.29 (br, 1H, NH); 8.41 (s, 1H, H-5); 7.73 (ddd, 1H, ^{3}J=7.8 Hz, ^{4}J=1.2 Hz, ^{4}J=1.2 Hz, H-6'); 7.65 (dd, 1H, ^{4}J=2.5 Hz, ^{4}J=1.5 Hz, H-2'); 7.44 (t, 1H, ^{3}J=8.0 Hz, H-5'); 7.10 (ddd, 1H, ^{3}J=8.3 Hz, ^{4}J=2.6 Hz, ^{4}J=0.9 Hz, H-4'); 3.84 (d, 2H, ^{3}J= 6.5 Hz, CH_2); 2.06 (m, 1H, ^{3}J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 1.02 (d, 6H, ^{3}J= 6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.7 (C-3'); 159.1 (C-2); 158.6 (C-3a); 151.2 (C-5); 143.9 (C-7); 131.1 (C-1'); 130.2 (C-5'); 119.0 (C-6'); 117.2 (C-4'); 112.0 (C-2'); 73.9 (CH_2); 27.7 (<u>C</u>H$(CH_3)_2$); 19.0 (CH(<u>C</u>H$_3)_2$).

Anal. Calcd for $C_{14}H_{15}N_5O_2$: C, 58.94; H, 5.30; N, 24.55. Found: C, 59.01; H, 5.35; N, 24.64.

2-(4-*iso*Butyloxylphenyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1p)

Compound **7p** was reacted as per the general method 4.6.4 to yield **1p** (63%). No further purification was required. Mp 317 – 319 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.23 (br, 1H, NH); 8.39 (s, 1H, H-5); 8.07 (d, 2H, ^3J=8.9 Hz, H-2' and H-6'); 7.09 (d, 2H, ^3J=8.9 Hz, H-3' and H-5'); 3.83 (d, 2H, ^3J= 6.5 Hz, CH_2); 2.06 (m, 1H, ^3J=6.6 Hz, C<u>H</u>$(CH_3)_2$); 1.00 (d, 6H, ^3J=6.7 Hz, CH(C<u>H</u>$_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.9 (C-4'); 160.7 (C-2); 158.5 (C-3a); 151.0 (C-5); 143.8 (C-7); 128.3 (C-2' and C-6'); 122.1 (C-1'); 114.9 (C-3' and C-5'); 73.9 (CH_2); 27.7 (<u>C</u>H$(CH_3)_2$); 19.0 (CH(<u>C</u>H$_3)_2$). Anal. Calcd for $C_{14}H_{15}N_5O_2$: C, 58.94; H, 5.30; N, 24.55. Found: C, 59.27; H, 5.46; N, 24.31.

2-(Pyridin-3-yl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1q)

Compound **7q** was reacted as per the general method 4.6.4 to yield **1q** (30%). No further purification was required. Mp 310 – 312 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.36 (br, 1H, NH); 9.30 (d, 1H, ^4J=1.3 Hz, H-2'); 8.73 (dd, 1H, ^3J=4.7 Hz, ^4J=1.3 Hz, H-6'); 8.48 (dt, 1H, ^3J=7.9 Hz, ^4J=2.0 Hz, H-4'); 8.45 (s, 1H, H-5); 7.59 (ddd, 1H, ^3J=7.9 Hz, ^4J=4.8 Hz, ^4J=0.6 Hz, H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.9 (C-2); 158.9 (C-3a); 151.6 and 151.5 (C-5 and C-6'); 147.7 (C-4'); 144.0 (C-7); 134.3 (C-5'); 125.9 (C-3'); 124.3 (C-2'). Anal. Calcd for $C_9H_6N_6O$: C, 50.47; H, 2.82; N, 39.24. Found: C, 50.63; H, 3.06; N, 38.97.

2-(Thien-2-yl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1r)

Compound **7r** was reacted as per the general method 4.6.4 to yield **1r** (57%). Final product was crystallized from ethoxyethanol. Mp 309 – 310 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.30 (br, 1H, NH); 8.41 (s, 1H, H-5); 7.83 (dd, 1H, ^3J=3.6 Hz, ^4J=1.2 Hz, H-5'); 7.79 (dd, 1H, ^3J=5.0 Hz, ^4J=1.2 Hz, H-3'); 7.24 (dd, 1H, ^3J=5.0 Hz, ^3J=3.6 Hz, H-4'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.2 (C-2); 158.5 (C-3a); 151.4 (C-5); 143.6 (C-7); 132.5 (C-2'); 129.5 (C-3'); 128.4 (C-4' and C-5'). Anal. Calcd for $C_8H_5N_5OS$: C, 43.83; H, 2.30; N, 31.95. Found: C, 43.90; H, 2.44; N, 31.79.

2-(Indolin-1-yl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1s)

Compound **7s** was reacted as per the general method 4.6.4 to yield **1s** (67%). No further purification was required. Mp 302 – 303 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.15 (br, 1H, NH); 8.35 (s, 1H, H-5); 7.95 (d, 1H, ^{3}J=7.9 Hz, H-4'); 7.22 (td, 1H, ^{3}J=7.9 Hz, ^{4}J=1.3 Hz, H-6'); 7.19 (dd, 1H, ^{3}J=7.8 Hz, ^{4}J=0.9 Hz, H-7'); 6.88 (td, 1H, ^{3}J=7.4 Hz, ^{4}J=0.9 Hz, H-5'); 4.11 (t, 2H, ^{3}J=8.7 Hz, H-2'); 3.22 (t, 2H, ^{3}J=8.7 Hz, H-3'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.0 (C-2); 157.4 (C-3a); 150.9 (C-5); 143.3 and 143.2 (C-7 and C-7'a); 130.9 (C-3'a); 127.2 (C-6'); 124.7 (C-4'); 120.8 (C-5'); 112.4 (C-7'); 48.6 (C-2'); 27.2 (C-3'). Anal. Calcd for $C_{12}H_{10}N_6O$: C, 56.69; H, 3.96; N, 33.05. Found: C, 56.83; H, 4.05; N, 32.88.

2-(Morpholin-1-yl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7(6*H*)-one (1t)

Compound **7t** was reacted as per the general method 4.6.4 to yield **1t** (62%). Final product was crystallized from aqueous ethanol (90%). Mp 303 – 304 °C; *Anal* ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.03 (br, 1H, NH); 8.27 (s, 1H, H-5); 3.69 (dd, 4H, ^{3}J=5.3 Hz, ^{3}J=4.4 Hz, H-3' and H-5'); 3.42 (dd, 4H, ^{3}J=5.3 Hz, ^{3}J=4.4 Hz, H-3' and H-5'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.7 (C-2); 157.7 (C-3a); 150.6 (C-5); 143.0 (C-7); 65.6 (CH_2OCH_2); 45.6 (CH_2NCH_2). Anal. Calcd for $C_8H_{10}N_6O_2$: C, 43.24; H, 4.54; N, 37.82. Found: C, 43.32; H, 4.62; N, 37.68.

4.6.5 Xanthine oxidase inhibitory assay

The IC_{50} value of each compound was evaluated using a UV spectrophotometer with the wavelength set at 295 nm. Bovine milk XO was reconstituted with 55 mM phosphate buffer at pH 7.5 to obtain a suitable concentration that gave a final enzyme concentration of 0.01 IU/mL during each assay. Xanthine was used as substrate at a final concentration of 40 μM and Allopurinol was used as positive control. Each compound and allopurinol were dissolved in DMSO and diluted to a suitable concentration using the phosphate buffer. The final DMSO concentration was no more than 1% v/v. A blank solution with DMSO at 1% v/v was shown not to affect substrate oxidation. All tests were performed at 37°C by recording UV absorbance due to the formation of uric acid for 60 seconds at 4 second intervals. Each compound was tested in triplicate at five different concentrations. The slope of the curve of absorbance vs time and resulting IC_{50} values for each compound were obtained using GraphPad Prism 9.2.0 (GraphPad Software Inc.).

The most active compound **1o** was also used in the enzyme kinetics experiments to identify the mechanism of inhibition. Five concentrations of **1o** were tested using xanthine at five different concentrations (5, 10, 20, 30 and 40 μM).
GraphPad Prism 9.2.0 (GraphPad Software Inc.) was used to perform a non-linear regression using the Michaelis-Menten equation and a Lineweaver-Burk plot was used to identify the mechanism of inhibition.

4.6.6 Molecular docking

Molecular docking simulations were performed using Autodock 4.0[(210)] with an interaction grid with 0.375 Å spacing. The crystal structure of bovine milk XO in complex with hypoxanthine (PDB entry 3nrz) was used to study the interactions between the inhibitor and the enzyme. The internal energy of each molecule and the enzyme was minimised using Biovia Discovery Studio 2021 (Dassault Systèmes). The structure of the enzyme was prepared by removing the ligand hypoxanthine and all of the water molecules. CHARMM charges were assigned to both the enzyme and the inhibitors. The grid box for docking was centered using the coordinates X center = 89.606, Y center = 9.758 and Z center = 17.716. The grid box had the following dimensions: x = 52 Å, y = 50 Å and z = 58 Å.
The final predicted docked poses were visualised using Biovia Discovery Studio 2021 (Dassault Systèmes).

Chapter 5

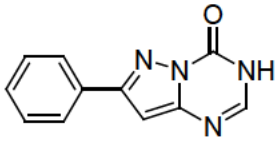
5 Conclusions and future directions

5.1 Conclusions

The work described in this thesis aimed to discover novel purine analogues as inhibitors of xanthine oxidase (XO). A comprehensive review of the available literature indicated that all published XO inhibitors could be classified into two main categories: purine-like and non purine-like inhibitors. The majority of research published in the last two decades has focussed on the identification of non purine-like inhibitors, resulting in the approval of two key drugs: Febuxostat and Topiroxostat. However, despite its known potential side effects, Allopurinol remains currently the drug of first choice in the treatment of hyperuricemia, regardless of the availability of non purine-like drug treatments.

The research described in this thesis aimed to find new purine analogues with better inhibitory activity than that of Allopurinol. Allopurinol has been used for more 50 years and its side effects - due to interactions with other metabolic pathways - are also well documented. The development of new purine-like drug XO inhibitors is designed to benefit from the existing foundation of Allopurinol use and decades of data collection on side effects.

In 1985 Robinson et al.[8] published the synthesis and *in-vitro* activity of more than 100 purine analogues. The most active compound was 7-Phenyl-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (**1**). In the same paper the authors synthesised and tested six derivatives of type **2** and five of type **3**.



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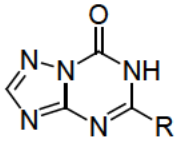

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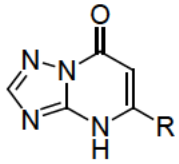
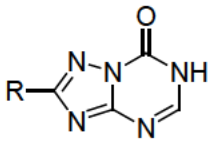


3

The authors did not report any structure of type **2** and **3** with substitution in position 2 that could simulate the substitution in the most active compound **1**.

The aim of this research was to expand the library of compounds published by Robins et al. and evaluate the inhibitory activity of analogues of type **2** and **3** with substituents in position 2 (**4** and **5**).



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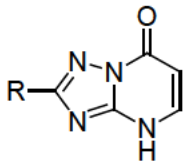
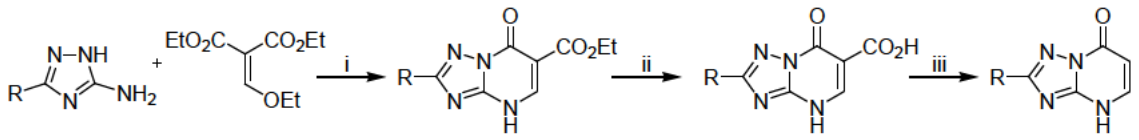


5

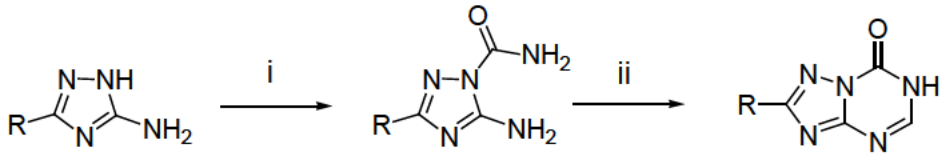
A total of four libraries (1 – 4) of compounds were designed and synthesised:

1. 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylates
2. 2-substituted-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acids
3. 2-substituted-1,2,4-triazolo[1,5-a] pyrimidin-7(4*H*)-ones
4. 2-substituted-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6*H*)-ones

New and efficient synthetic methods were developed for the synthesis of a total of 95 molecules. The common intermediate for all compounds was 2-substituted-5-amino-1,2,4-triazoles, which were prepared using known general methods. The reaction steps to produce final products were developed using a microwave reactor. The optimised reaction conditions resulted in very short reaction times (Schemes 1 and 2).



Scheme 1: i) AcOH, μW, 20 min, 150°C; ii) K_2CO_3 5%, μW, 15 min, 140°C; iii) NaH_2PO_4 5%, μW, 30 min, 190°C.



Scheme 2: i) KCNO, HCl, 40% EtOH, room temp. ii) $HC(OEt)_3$, toluene, μW, 30 min, 180°C

The optimised reaction conditions allowed to obtain final products with a purity of 95% or greater with minimal or non existent purification step.

All compounds were characterised by NMR analysis and tested for their XO inhibitory activity *in vitro*. A group of 24 compounds from libraries 2 and 3 demonstrated better inhibitory activity than the control Allopurinol, whilst compounds from library 1 showed lower activity than Allopurinol, with the exception of one compound exhibiting comparable activity. The majority of compounds from library 4 (19 out of 20) demonstrated better activity than Allopurinol. The most potent inhibitors from libraries 2 and 3 exhibited IC_{50} values 23 and 18 times lower, respectively, than that of Allopurinol, whilst the most active compound from library 4 was nearly three order more potent than Allopurinol.

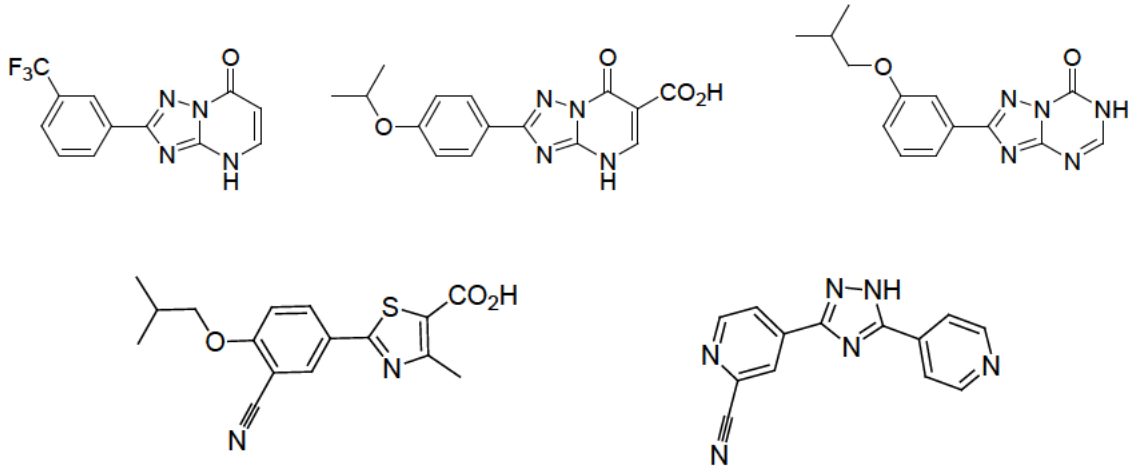
Enzyme kinetic studies were performed to identify the inhibition type for the most potent analogues. A mixed type of inhibition was observed for these compounds. This is consistent with the inhibition mechanism previously reported in the literature for purine analogues. The mixed type of inhibition, in the absence of evidence of an enzyme allosteric site, was rationalised by Tai and Hwang in 2004[31]. These authors hypothesised the presence of cooperative interactions between the two enzyme monomers: binding of the substrate to the active site of one subunit affects the catalytic rate of the other subunit.

The crystal structure of bovine milk XO in complex with hypoxanthine was used to study the interactions between the inhibitors and the enzyme. Molecular docking simulations were conducted with each of the 95 compounds to predict their interactions with the active site of the enzyme. The predicted poses showed that all the analogues were oriented with the triazolopyrimidinone or triazolotriazinone ring towards the molibdopterine cofactor inside the enzyme active site, whilst the substituent at position 2 was oriented towards the entrance of the active site.

5.2 Future directions

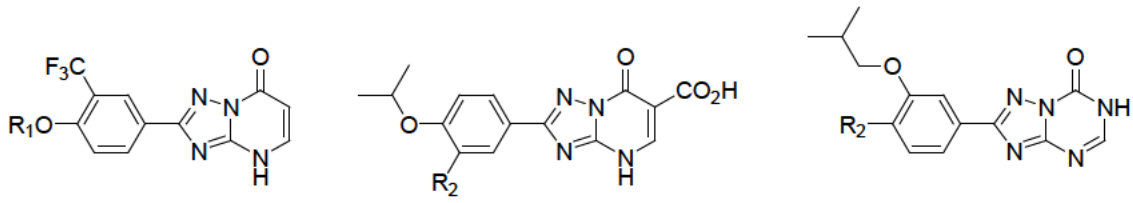
This research identified three libraries of novel purine analogue inhibitors of XO. The most active compounds of each library are potential leads for additional studies dedicated to the identification of more effective XO inhibitors.

Given the limited number of drugs presently available on the market for the treatment of hyperuricemia and gout, these new inhibitors of XO represent a good opportunity for the potential future expansion of available drug treatments.



Febuxostat Topiroxostat

By comparing the side chains of the three lead compounds identified in this project with the side chains of Febuxostat and Topiroxostat it seems that the alkyloxy substituent on the phenyl ring favours the interaction with the enzyme. The electron withdrawing group (EWG) in position 3 of the aromatic ring (CF_3 and CN) also seems to favour interaction with the enzyme. A useful future expansion on this work would be the synthesis and evaluation of the inhibitory potency of the purine analogues that have double substitution on the phenyl ring, for example compounds **6, 7** and **8**:



6 **7** **8**

R_1 = *iso*butyl or *iso*propyl

R_2 = CF_3 or CN

Ultimately, *in vivo* and toxicological studies will be required to confirm the potential "drugability" of all of these novel compounds prior to entering clinical trials.

Appendix 1

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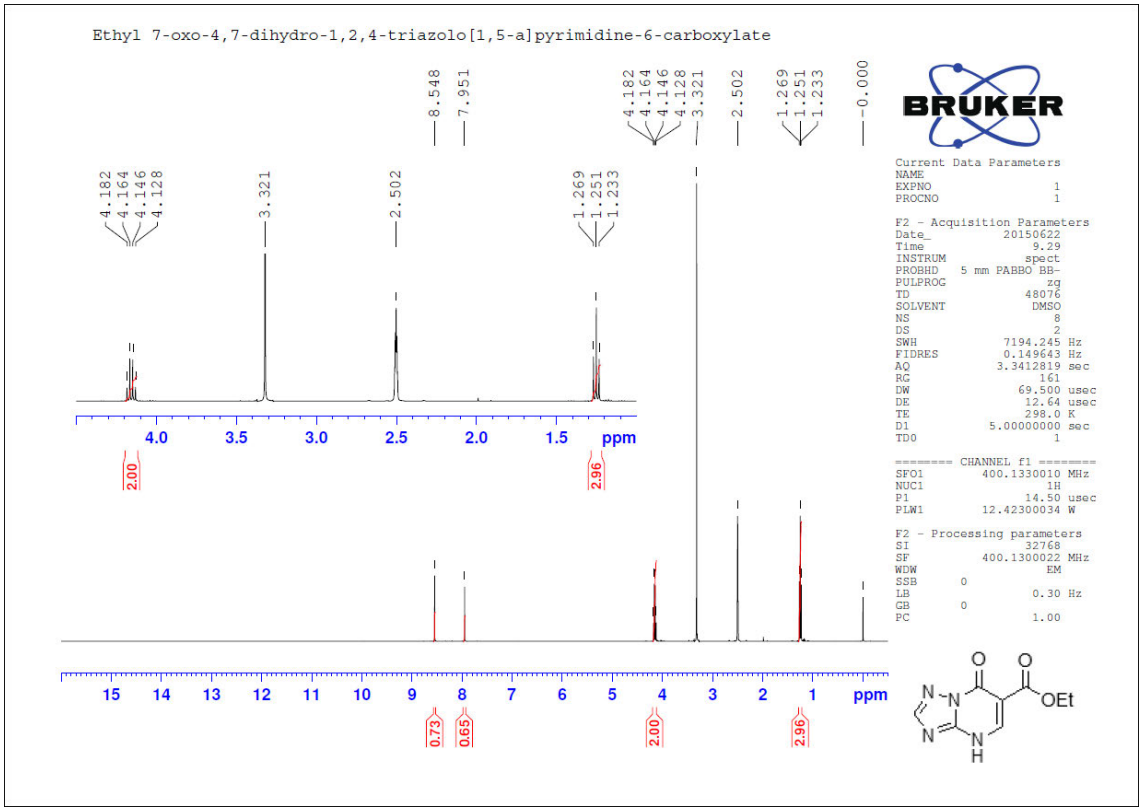
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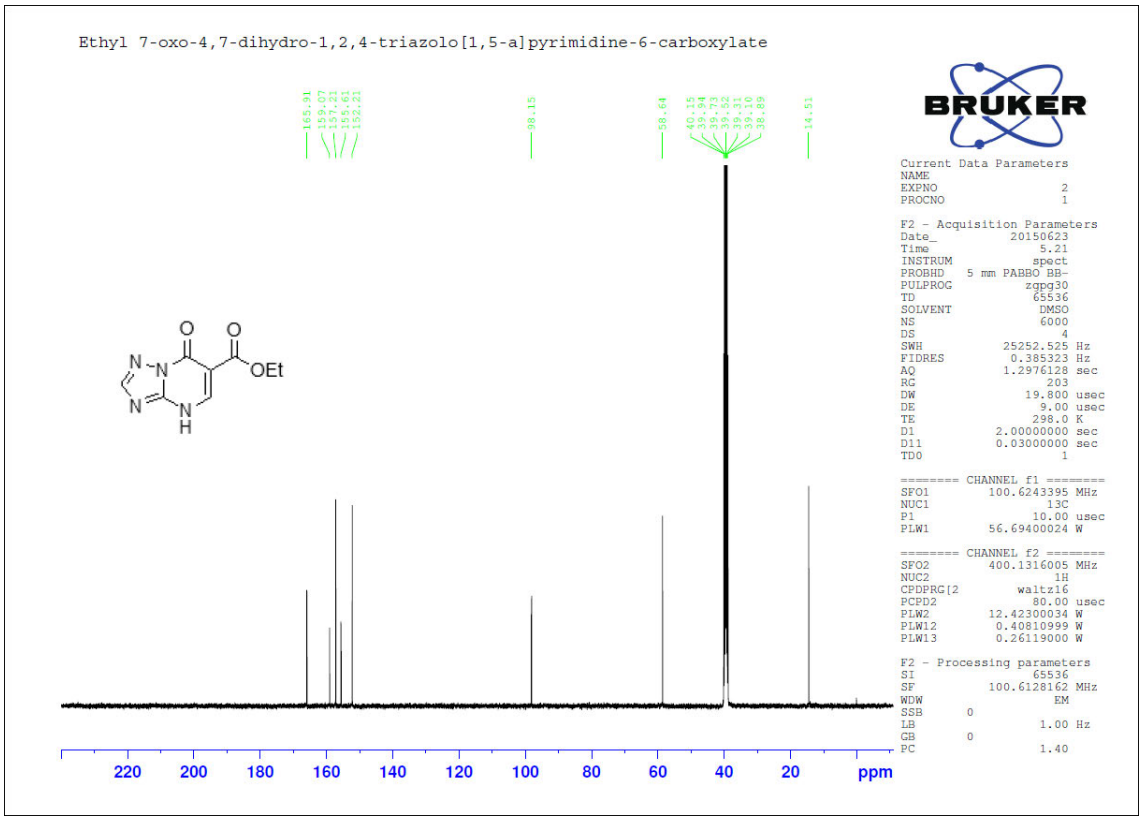
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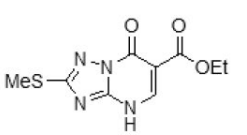

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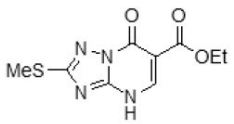

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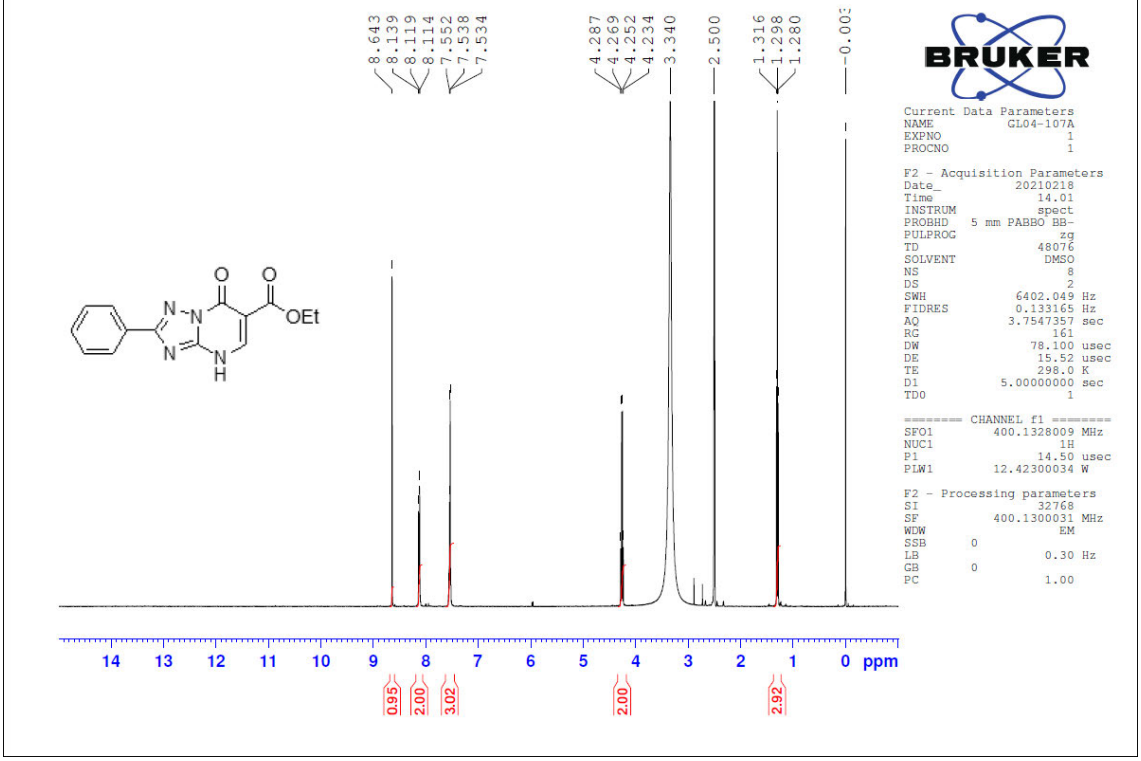


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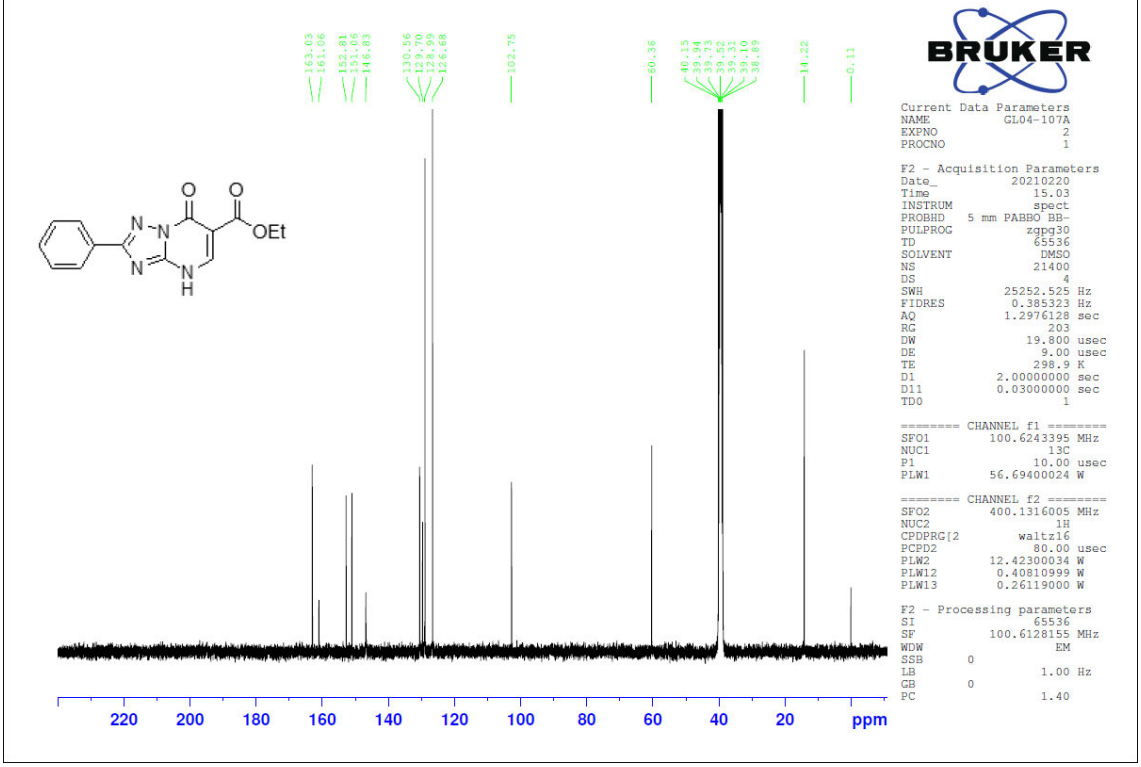


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F2 - Processing parameters
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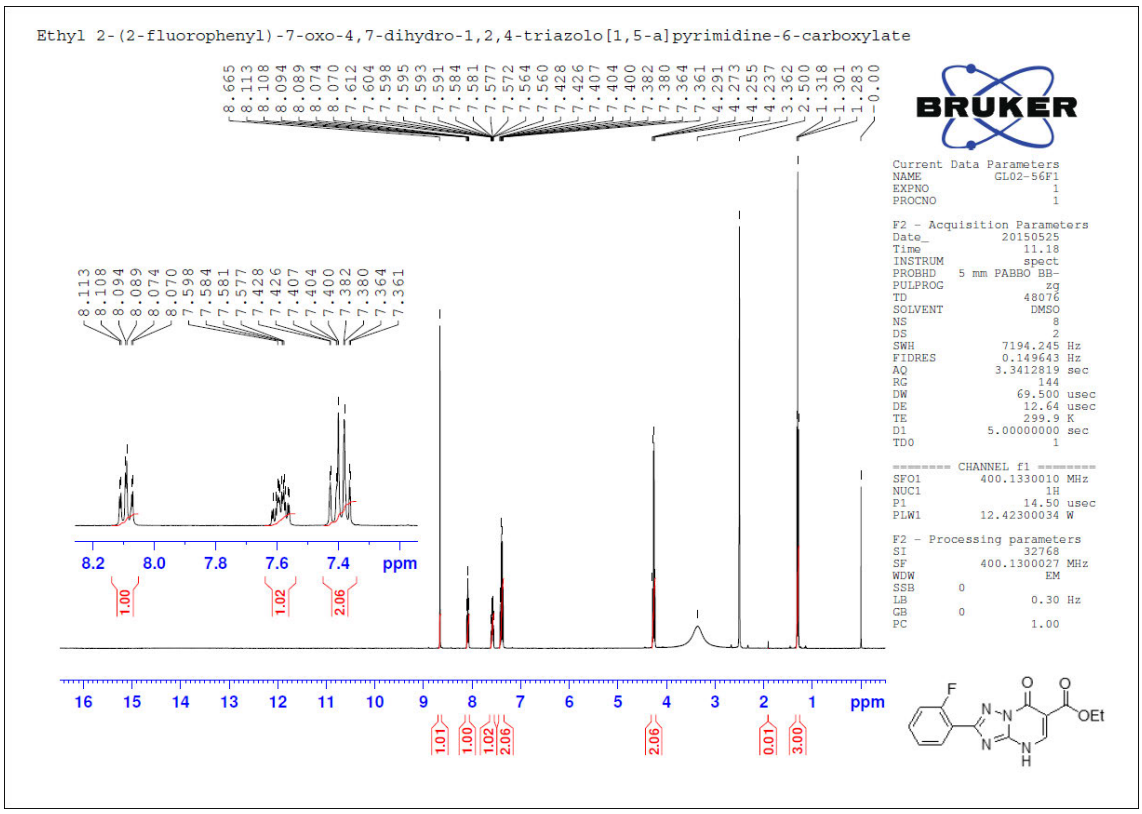
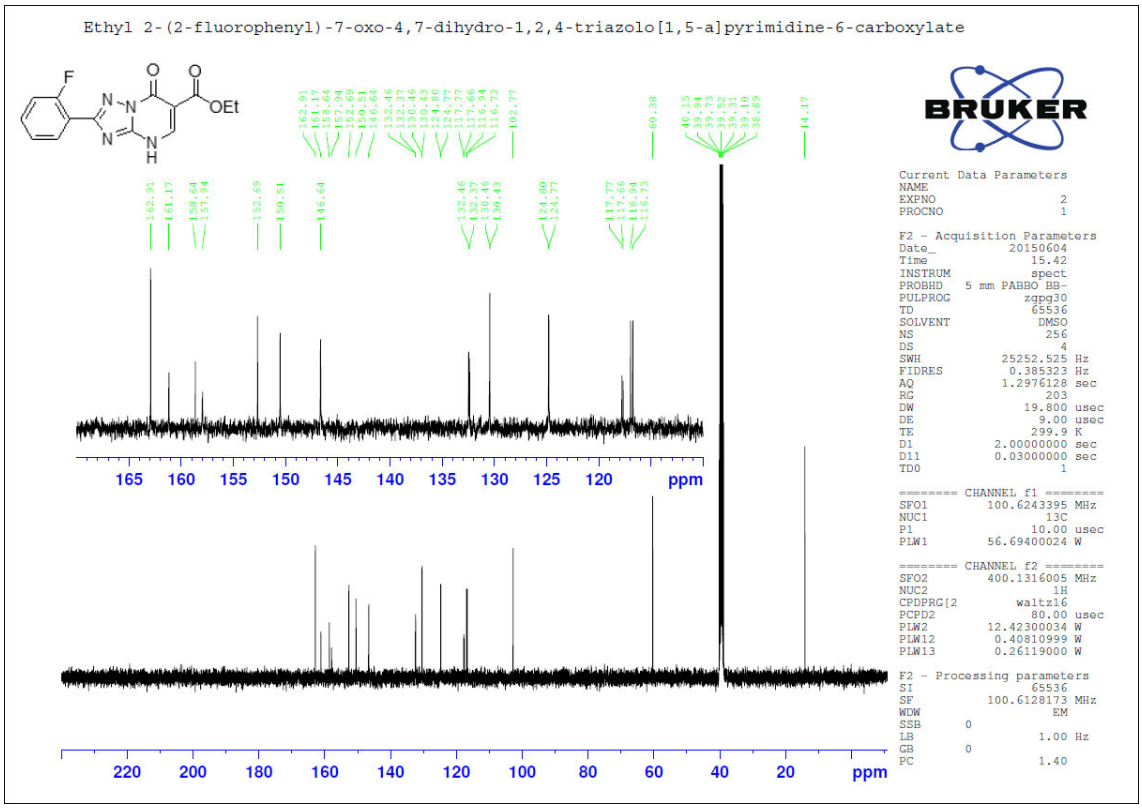
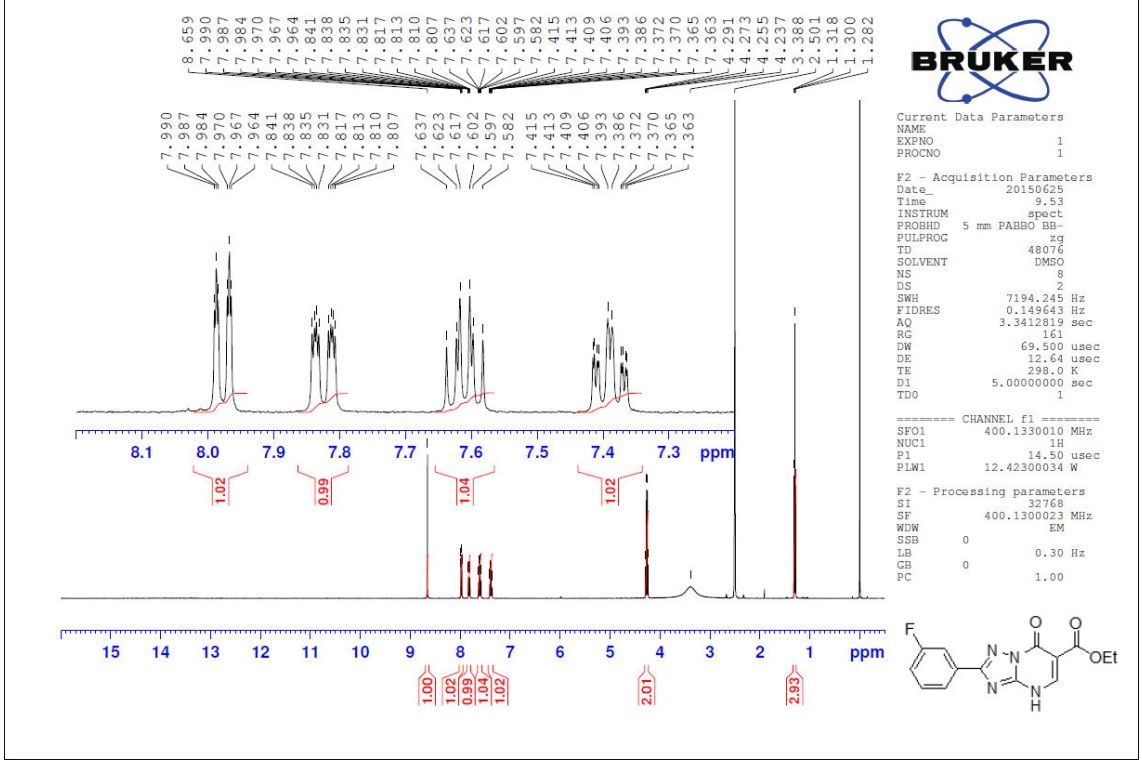
SF                  100.6128155 MHz
WDW                          EM
SSB                   0
LB                        1.00 Hz
GB                    0
PC                        1.40
```

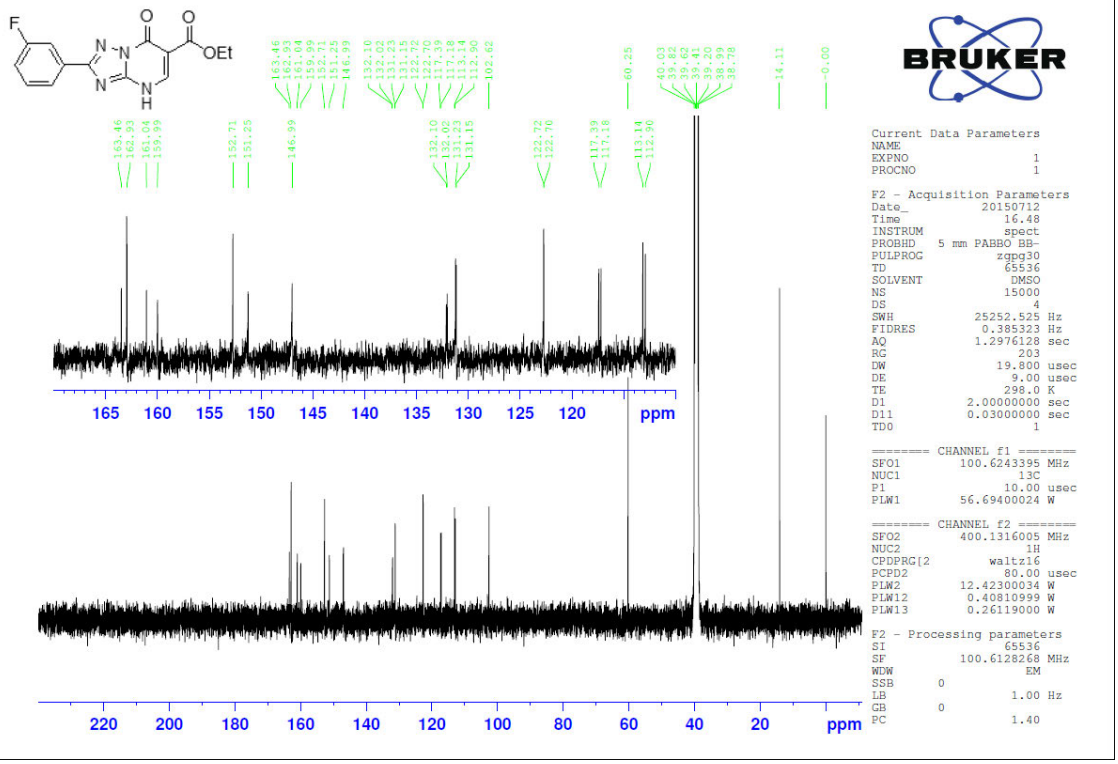

Ethyl 2-(2-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate
BRUKER
Current Data Parameters
NAME GL02-56F1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150525
Time 11.18
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 7194.245 Hz
FIDRES 0.149643 Hz
AQ 3.3412819 sec
RG 144
DW 69.500 usec
DE 12.64 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300027 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
OEt


Ethyl 2-(2-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150604
Time 15.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 256
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
CHANNEL f2
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128173 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
OEt

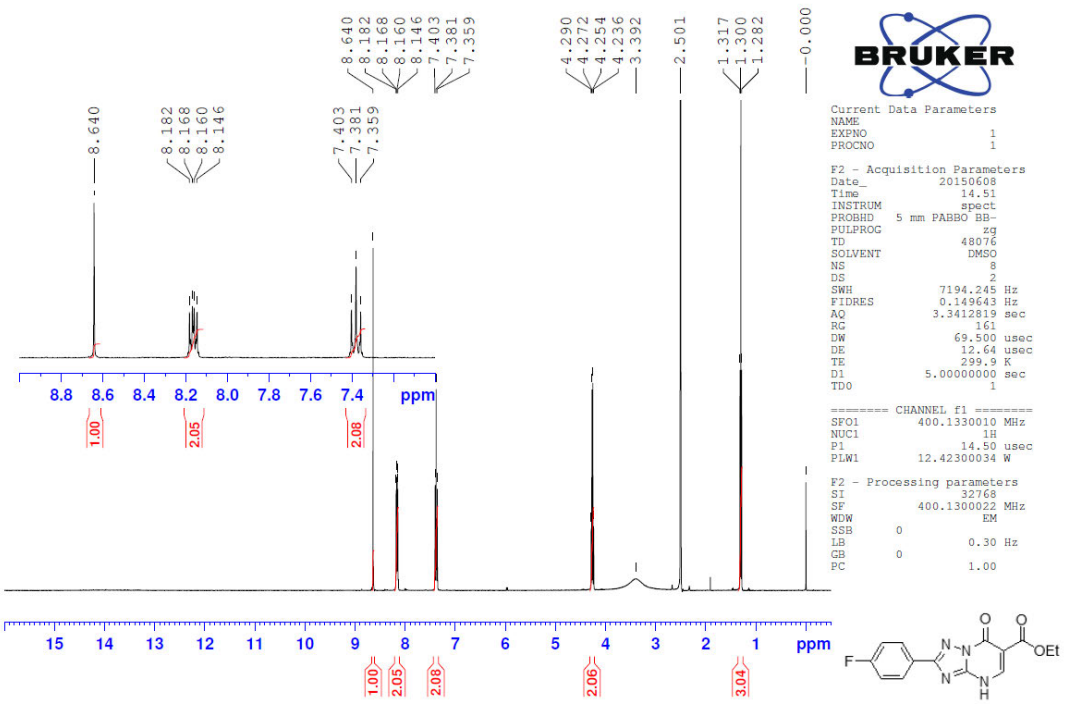
Ethyl 2-(3-fluoropheny)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Ethyl 2-(3-fluorophenyl)-7-oxo-4,5-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Ethyl 2-(4-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



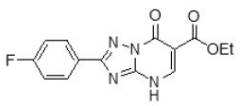
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150608
Time 14.51
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 7194.245 Hz
FIDRES 0.149643 Hz
AQ 3.3412819 sec
RG 161
DW 69.500 usec
DE 12.64 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300022 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ethyl 2-(4-fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Current Data Parameters
NAME
EXPNO 1
PROCNO 1

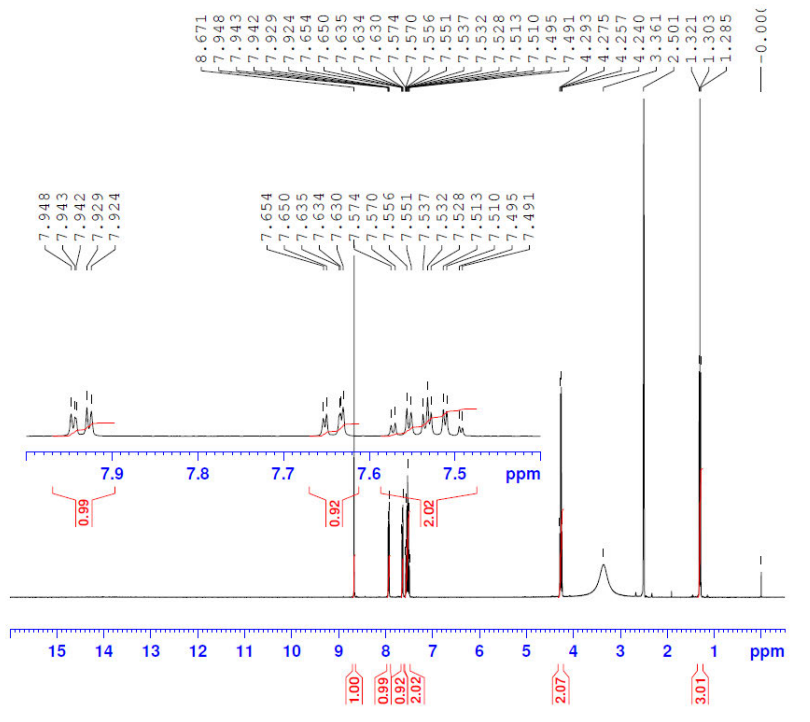
F2 - Acquisition Parameters
Date_ 20150621
Time 15.10
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 24000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128150 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Ethyl 2-(2-chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate

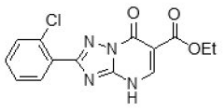


Current Data Parameters
NAME
EXPNO 1
PROCNO 1

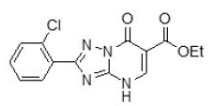
F2 - Acquisition Parameters
Date_ 20150615
Time 9.51
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 7194.245 Hz
FIDRES 0.149643 Hz
AQ 3.3412819 sec
RG 144
DW 69.500 usec
DE 12.64 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300021 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



Ethyl 2-(2chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Current Data Parameters
NAME
EXPNO 3
PROCNO 1

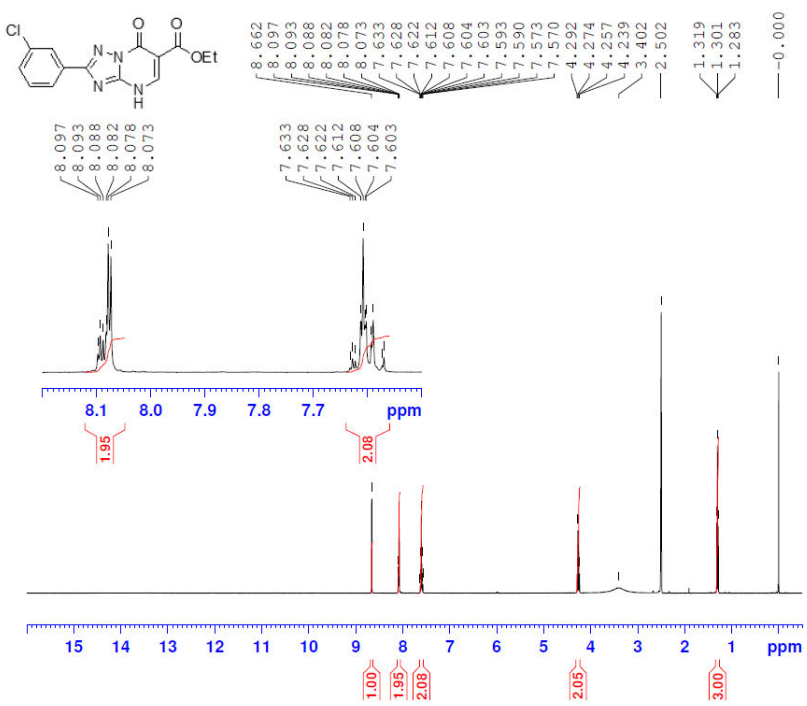
F2 - Acquisition Parameters
Date_ 20150622
Time 23.41
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128152 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Ethyl 2-(3-chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



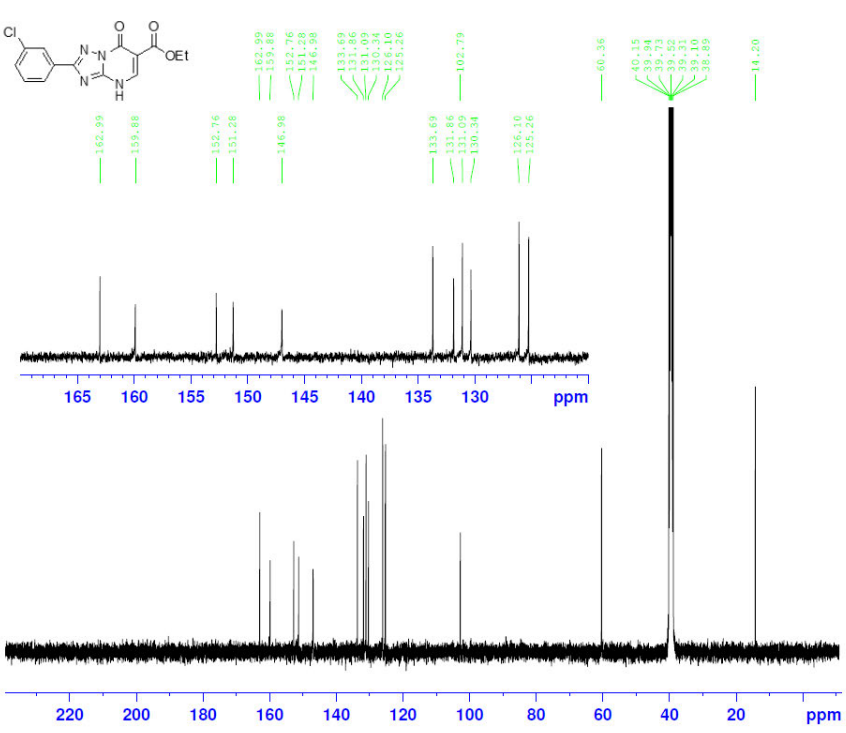
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150625
Time                9.47
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH           7194.245 Hz
FIDRES        0.149643 Hz
AQ            3.3412819 sec
RG                   161
DW             69.500 usec
DE             12.64 usec
TE                298.0 K
D1            5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        400.1330010 MHz
NUC1                   1H
P1                 14.50 usec
PLW1        12.42300034 W

F2 - Processing parameters
SI                 32768
SF          400.1300022 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Ethyl 2-(3-chlorophenyl)-7-oxo-4,5-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

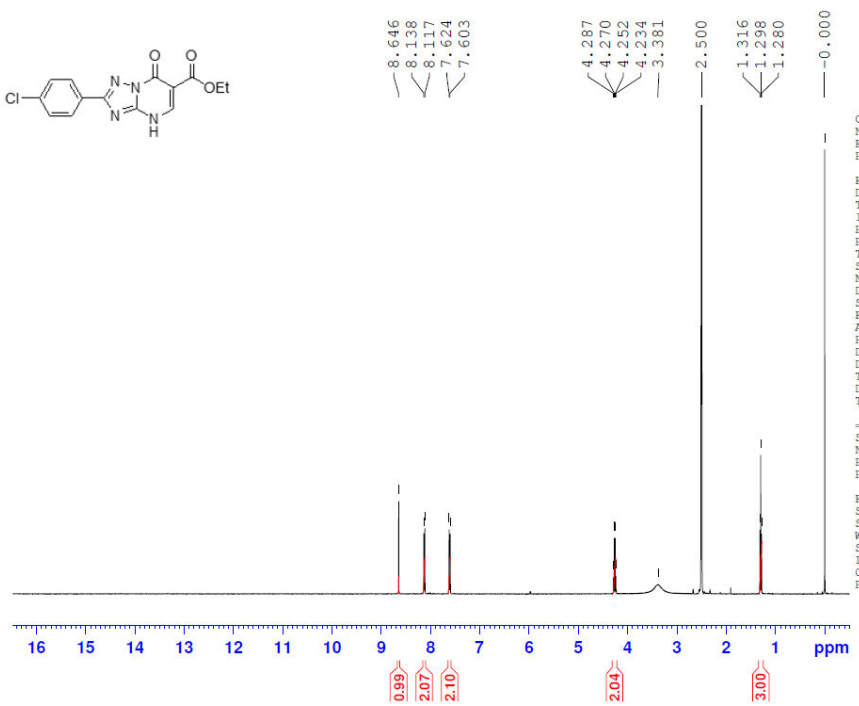
F2 - Acquisition Parameters
Date_           20160722
Time                6.31
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                 12000
DS                     4
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                   203
DW             19.800 usec
DE              9.00 usec
TE                299.8 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        100.6243395 MHz
NUC1                   13C
P1                10.00 usec
PLW1        56.69400024 W

======== CHANNEL f2 ========
SFO2        400.1316005 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2        12.42300034 W
PLW12        0.40810999 W
PLW13        0.26119000 W

F2 - Processing parameters
SI                 65536
SF          100.6128172 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Ethyl 2-(4-chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



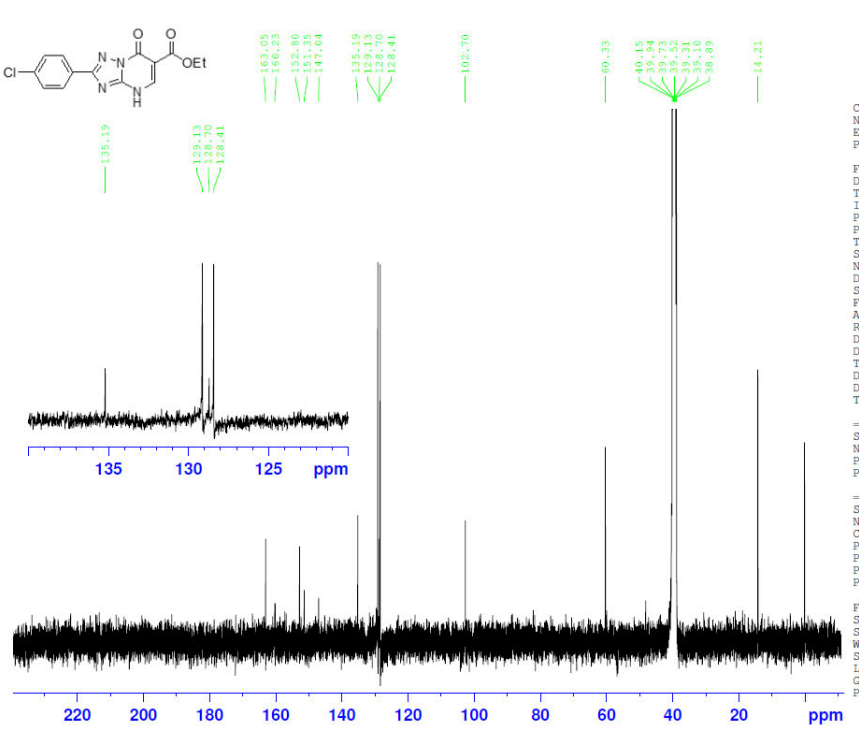

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_               20150527
Time                   11.10
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG                zg
TD                     48076
SOLVENT                DMSO
NS                         8
DS                         2
SWH                 7194.245 Hz
FIDRES              0.149643 Hz
AQ                  3.3412819 sec
RG                       181
DW                    69.500 usec
DE                    12.64 usec
TE                    299.9 K
D1                  5.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1           400.1330010 MHz
NUC1                      1H
P1                     14.50 usec
PLW1           12.42300034 W

F2 - Processing parameters
SI                     32768
SF                400.1300025 MHz
WDW                       EM
SSB                        0
LB                      0.30 Hz
GB                         0
PC                      1.00
```

Ethyl 2-(4-chlorophenyl)-7-oxo-4,5-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate




```
Current Data Parameters
NAME
EXPNO                  4
PROCNO                 1

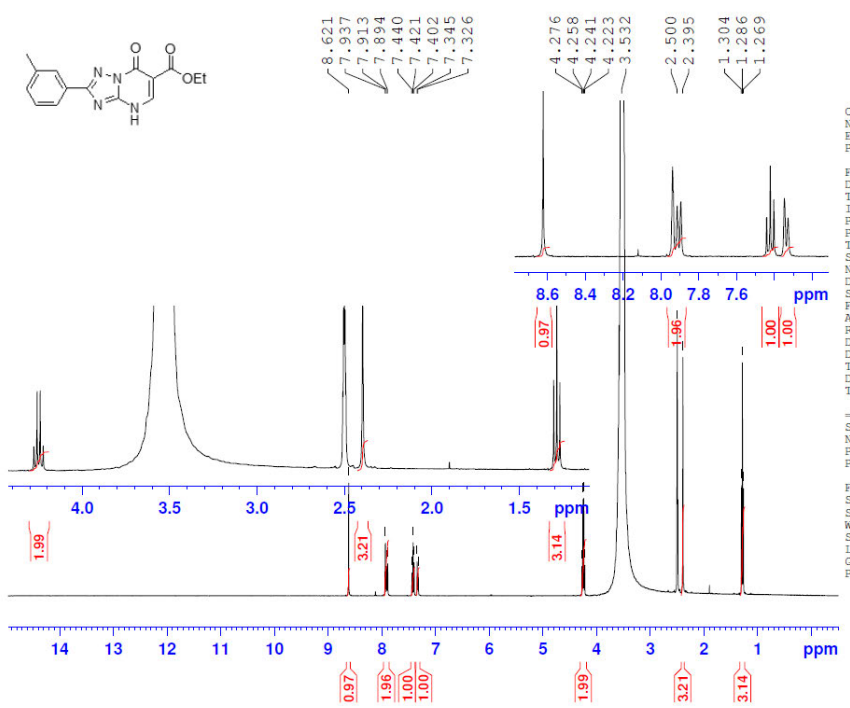
F2 - Acquisition Parameters
Date_               20150620
Time                   16.29
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                     65536
SOLVENT                DMSO
NS                     24000
DS                         4
SWH                25252.525 Hz
FIDRES              0.385323 Hz
AQ                  1.2976128 sec
RG                       203
DW                    19.800 usec
DE                     9.00 usec
TE                    298.2 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1           100.6243395 MHz
NUC1                      13C
P1                     10.00 usec
PLW1           56.69400024 W

======== CHANNEL f2 ========
SFO2           400.1316005 MHz
NUC2                      1H
CPDPRG[2             waltz16
PCPD2                  80.00 usec
PLW2           12.42300034 W
PLW12           0.40810999 W
PLW13           0.26119000 W

F2 - Processing parameters
SI                     65536
SF                100.6128170 MHz
WDW                       EM
SSB                        0
LB                      1.00 Hz
GB                         0
PC                      1.40
```

Ethyl 2-(3-methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

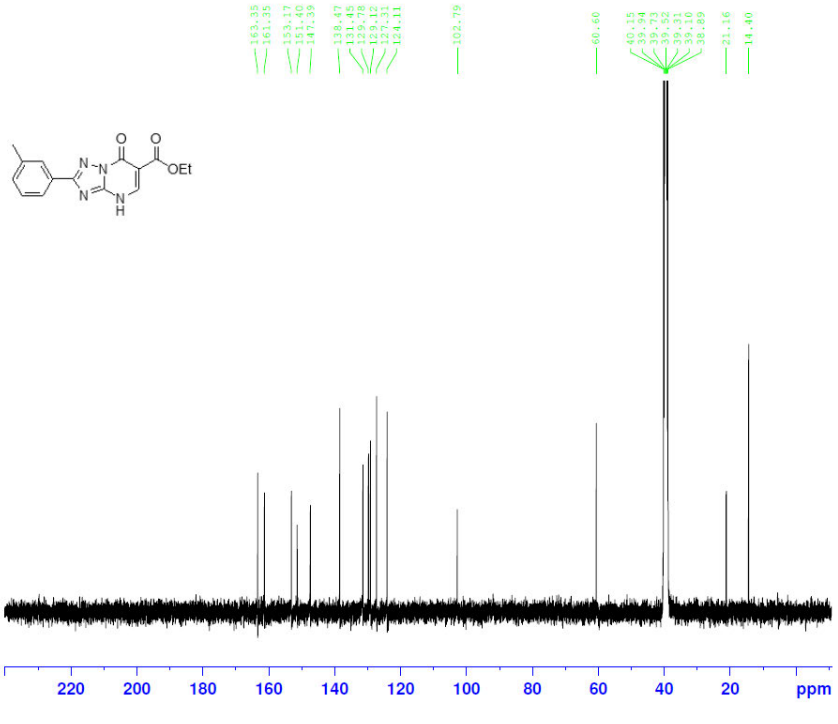
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210330
Time              11.21
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG           zg
TD               48076
SOLVENT           DMSO
NS                   8
DS                   2
SWH           6402.049 Hz
FIDRES        0.133165 Hz
AQ            3.7547357 sec
RG                  32
DW               78.100 usec
DE               15.52 usec
TE               298.3 K
D1            5.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        400.1328009 MHz
NUC1               1H
P1               14.50 usec
PLW1        12.42300034 W

F2 - Processing parameters
SI                 32768
SF          400.1300032 MHz
WDW                EM
SSB       0
LB               0.30 Hz
GB        0
PC                 1.00
```

Ethyl 2-(3-methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

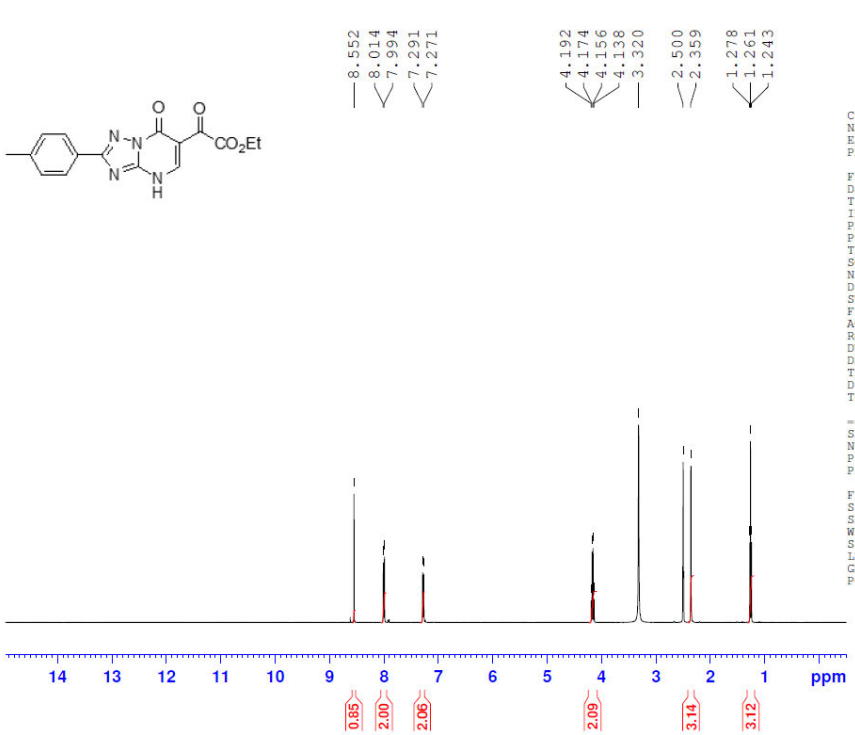
F2 - Acquisition Parameters
Date_           20210421
Time               8.03
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD              65536
SOLVENT           DMSO
NS                13900
DS                   4
SWH          25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                 203
DW               19.800 usec
DE                9.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        100.6243395 MHz
NUC1               13C
P1               10.00 usec
PLW1        56.69400024 W

======== CHANNEL f2 ========
SFO2        400.1316005 MHz
NUC2               1H
CPDPRG[2        waltz16
PCPD2            80.00 usec
PLW2        12.42300034 W
PLW12        0.40810999 W
PLW13        0.26119000 W

F2 - Processing parameters
SI              65536
SF          100.6127946 MHz
WDW                EM
SSB       0
LB               1.00 Hz
GB        0
PC                 1.40
```

Ethyl 2-(4-methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

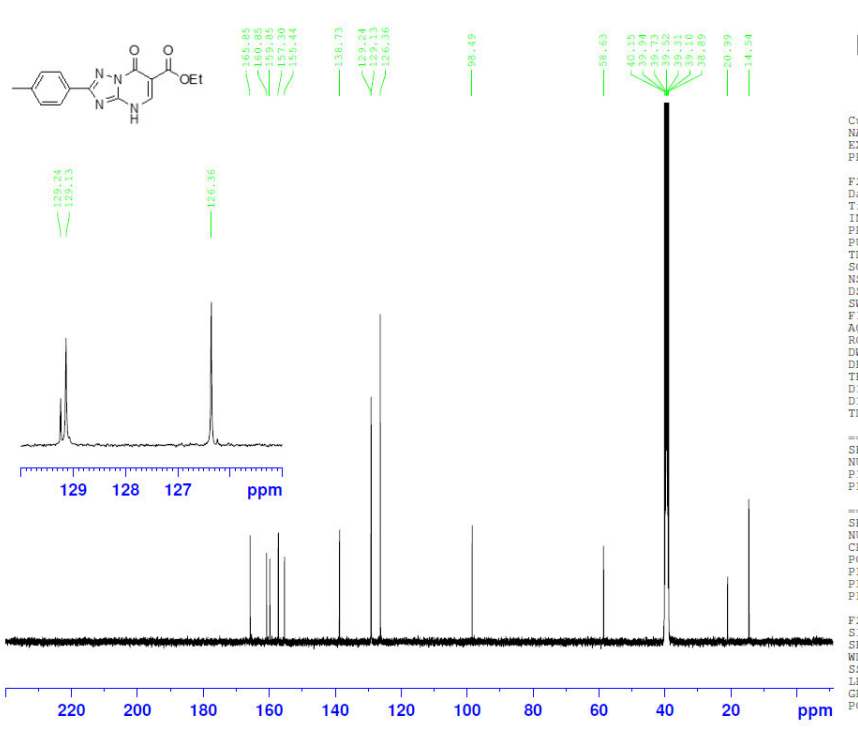
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210526
Time               10.04
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH             6402.049 Hz
FIDRES          0.133165 Hz
AQ              3.7547357 sec
RG                   203
DW                78.100 usec
DE                15.52 usec
TE                298.0 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1328009 MHz
NUC1                  1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                 32768
SF            400.1300031 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Ethyl 2-(4-methylphenyl)-7-oxo-4,5-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

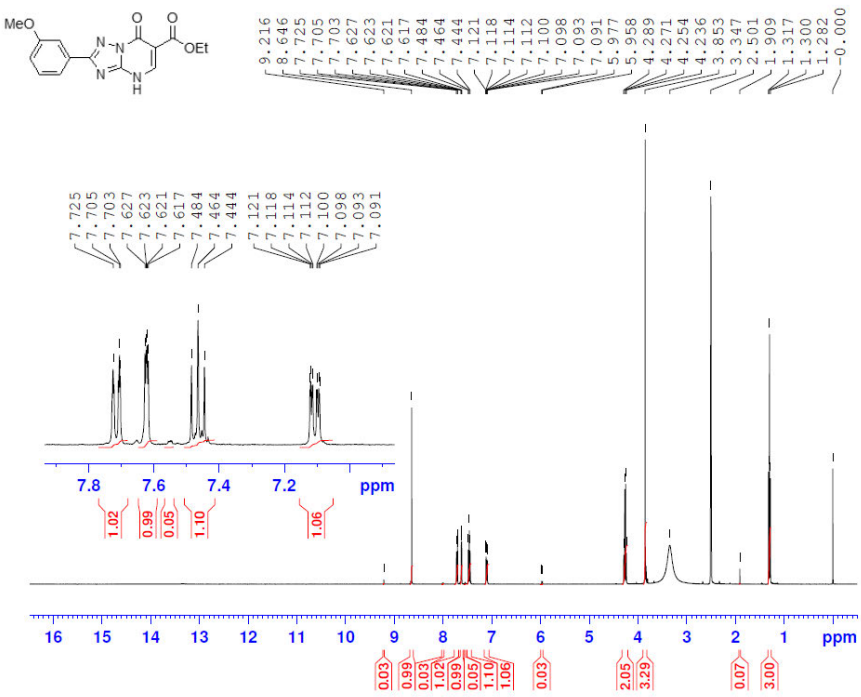
F2 - Acquisition Parameters
Date_           20210628
Time                6.56
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                 12700
DS                     4
SWH            25252.525 Hz
FIDRES          0.385323 Hz
AQ              1.2976128 sec
RG                   203
DW                19.800 usec
DE                 9.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                 10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

F2 - Processing parameters
SI                 65536
SF            100.6128151 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Ethyl 2-(3-methoxyphenyl)-7-oxo-4,5-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

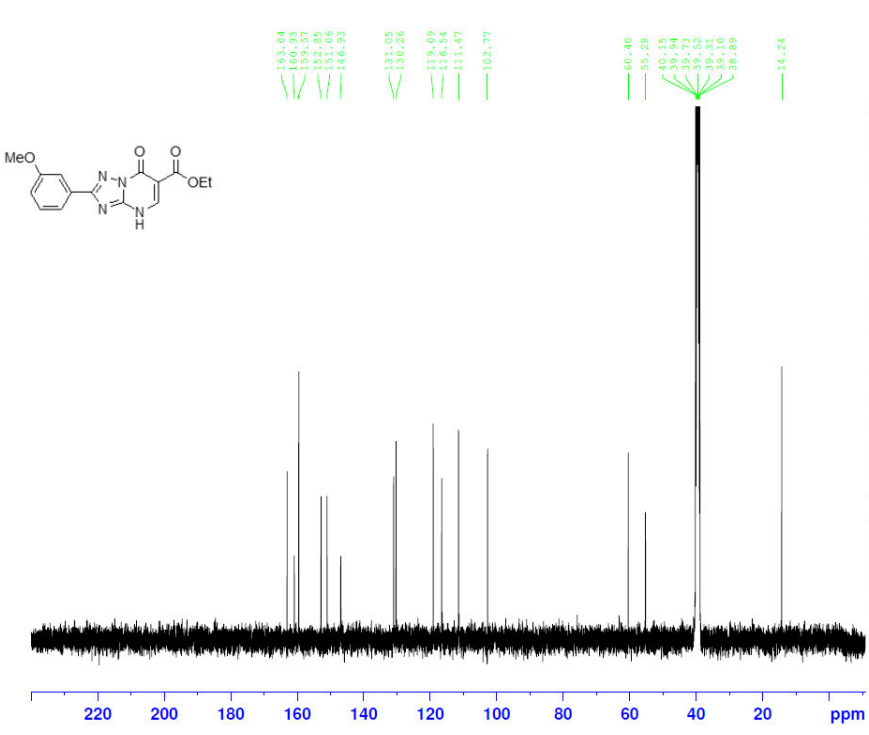
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150525
Time               11.24
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH             7194.245 Hz
FIDRES          0.149643 Hz
AQ              3.3412819 sec
RG                   144
DW                69.500 usec
DE                12.64 usec
TE                299.9 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1330010 MHz
NUC1                  1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                 32768
SF            400.1300022 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Ethyl 2-(3-methoxyphenyl)-7-oxo-4,5-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

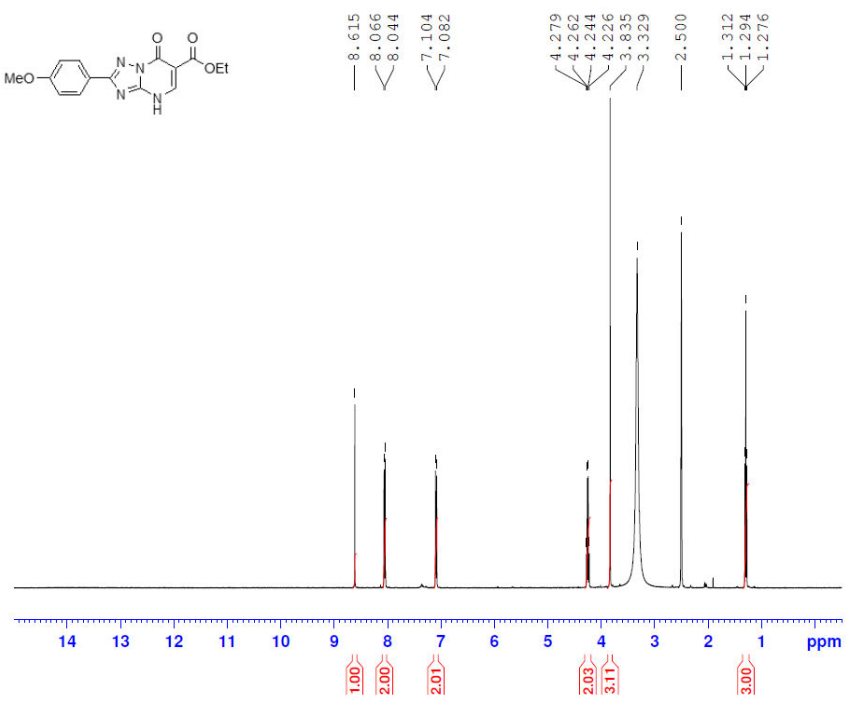
F2 - Acquisition Parameters
Date_           20210715
Time               1.36
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                  6950
DS                     4
SWH            25252.525 Hz
FIDRES          0.385323 Hz
AQ              1.2976128 sec
RG                   203
DW                19.800 usec
DE                 9.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

F2 - Processing parameters
SI                 65536
SF            100.6128122 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Ethyl 2-(4-methoxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

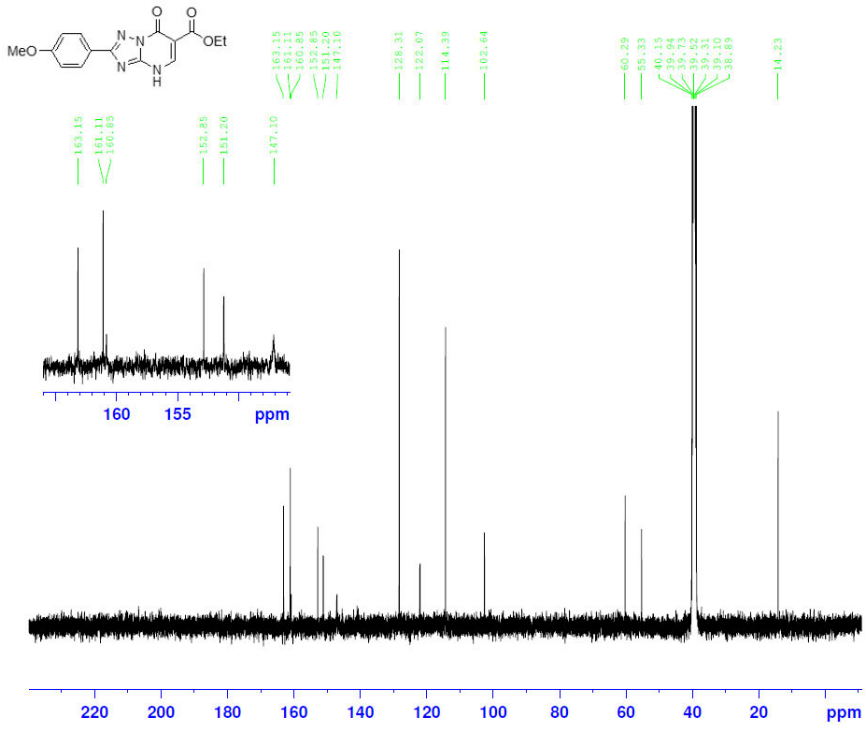
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210708
Time 10.49
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300026 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ethyl 2-(4-methoxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

Current Data Parameters
NAME
EXPNO 3
PROCNO 1

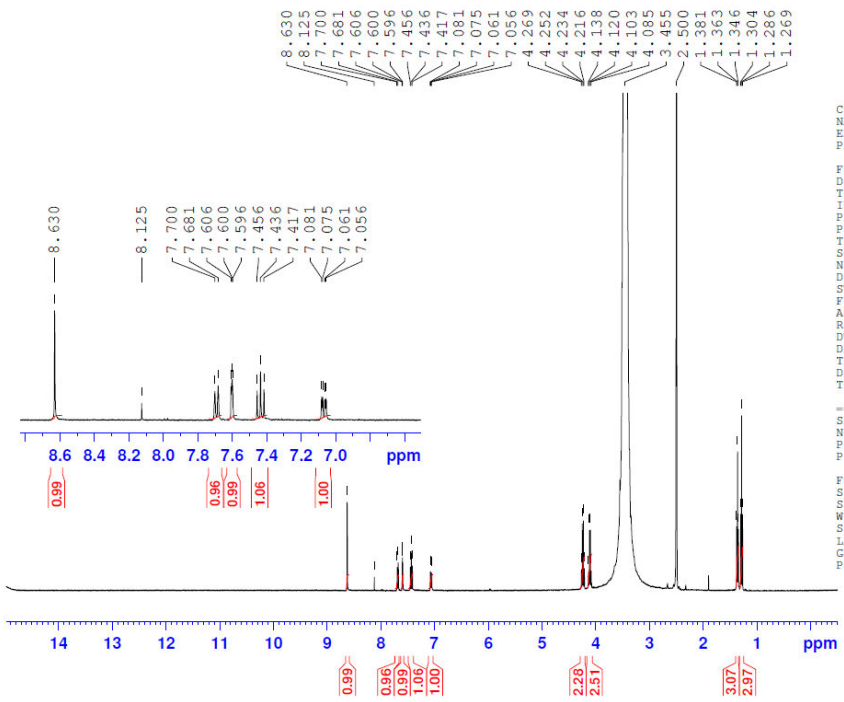
F2 - Acquisition Parameters
Date_ 20210927
Time 8.05
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128146 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Ethyl 2-(3-ethoxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



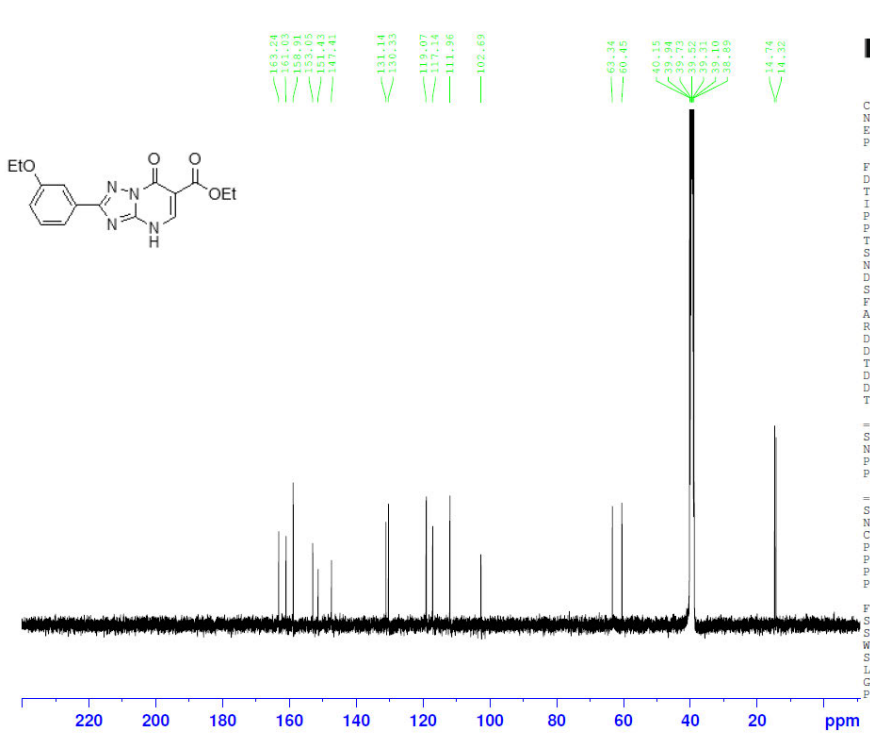
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210413
Time 9.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 45.2
DW 78.100 usec
DE 15.52 usec
TE 295.2 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ethyl 2-(3-ethoxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



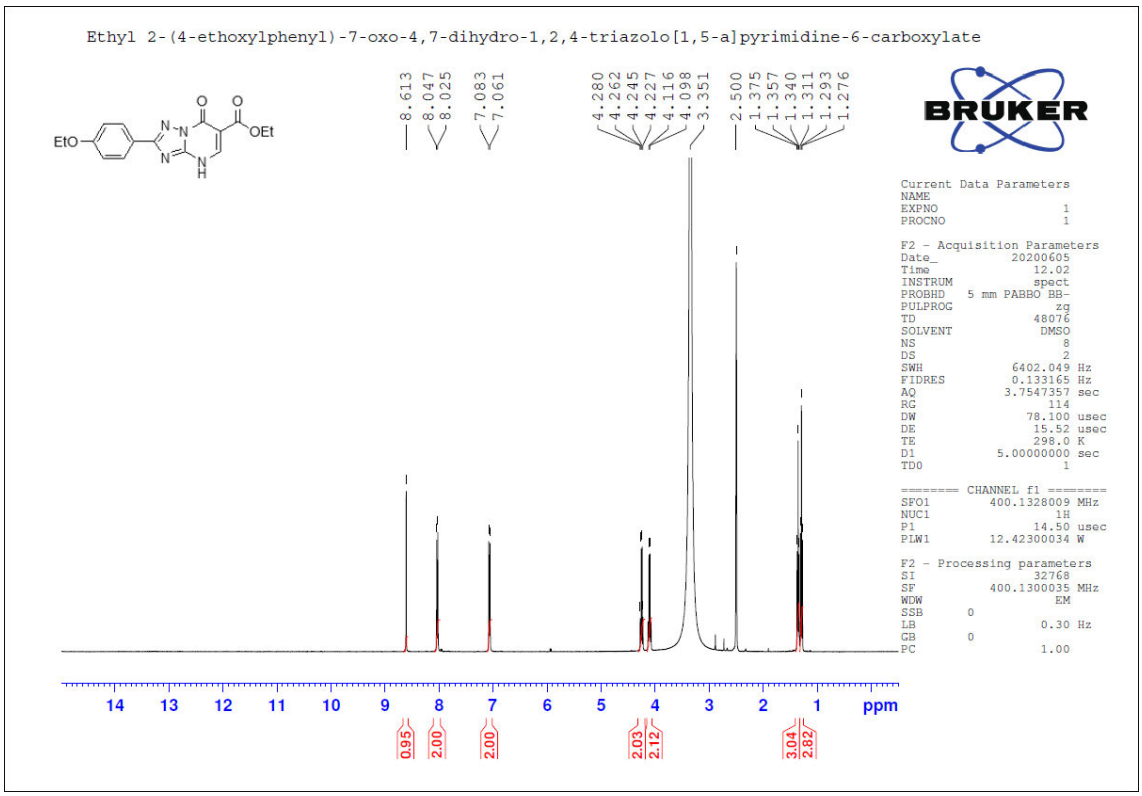
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

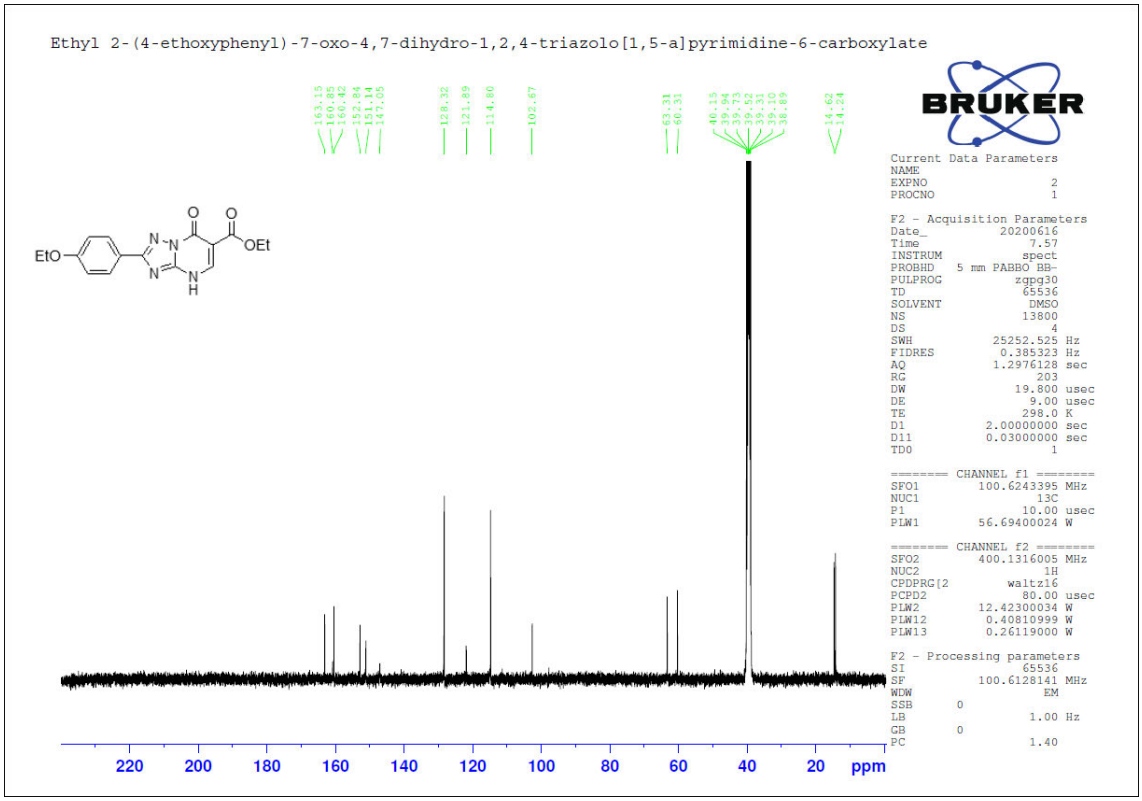
F2 - Acquisition Parameters
Date_ 20210418
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

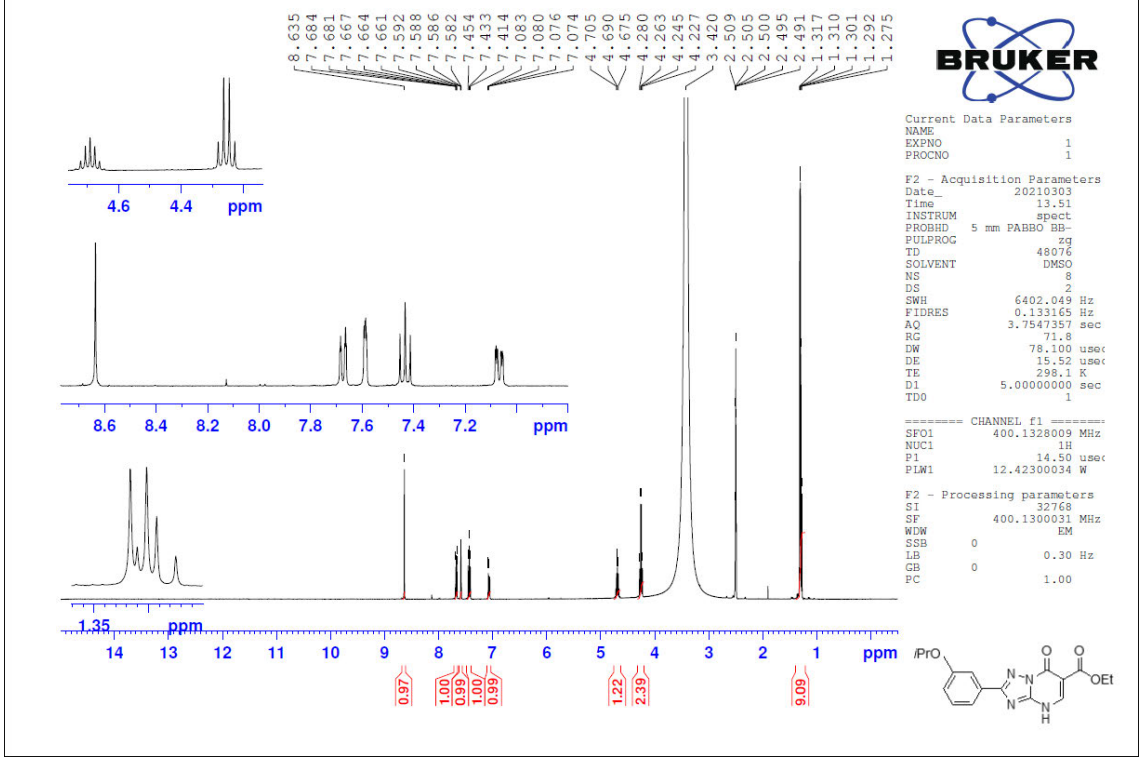
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128044 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

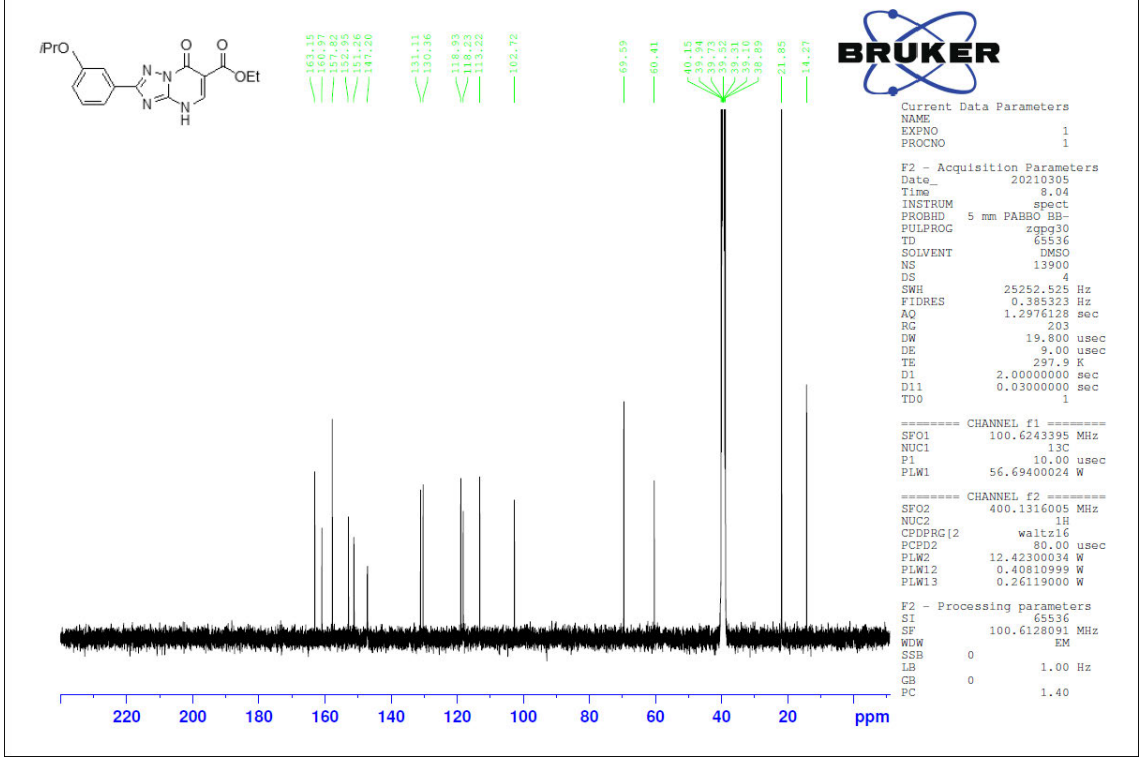

Ethyl 2-(4-ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate
EtO
OEt
8.613
8.047
8.025
7.083
7.061
4.280
4.262
4.245
4.227
4.116
4.098
3.351
2.500
1.375
1.357
1.340
1.311
1.293
1.276
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200605
Time 12.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 114
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300035 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.95 2.00 2.00 2.03 2.12 3.04 2.82


Ethyl 2-(4-ethoxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate
EtO
OEt
163.15
160.85
160.42
152.84
151.14
147.05
128.32
121.89
114.80
102.67
63.31
60.31
46.15
40.94
40.73
39.52
39.31
39.10
38.89
14.62
14.24
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200616
Time 7.57
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13800
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128141 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

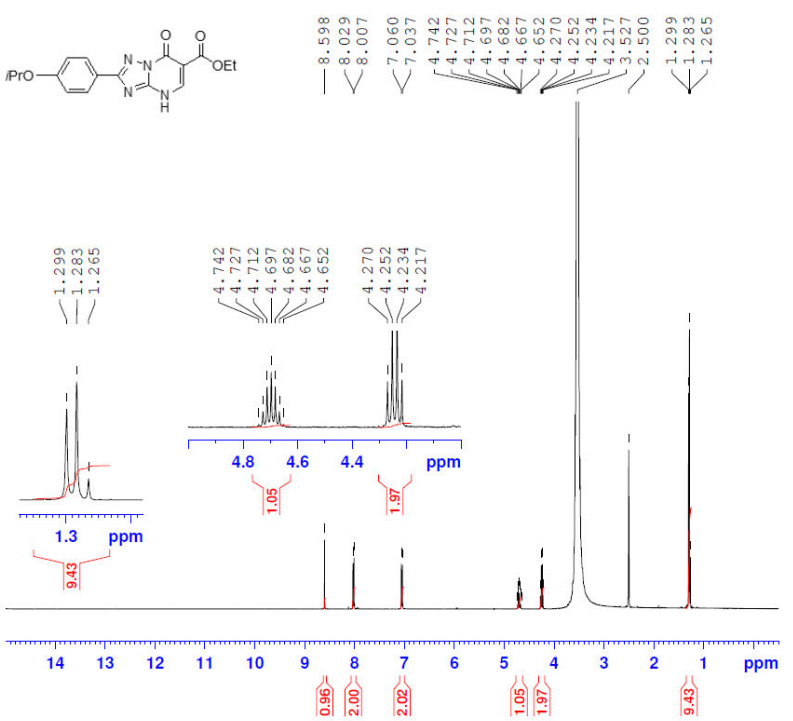
Ethyl 2-(3-*iso*propyloxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Ethyl 2-(3-isopropyloxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Ethyl 2-(4-isopropylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



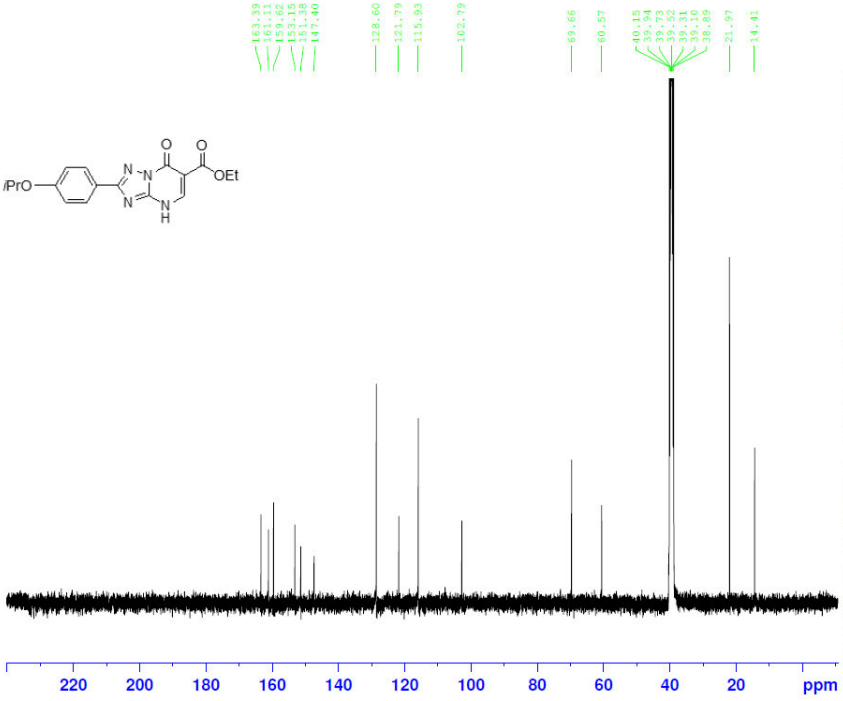
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210330
Time 11.26
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 36
DW 78.100 usec
DE 15.52 usec
TE 298.3 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ethyl 2-(4-isopropyloxyphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



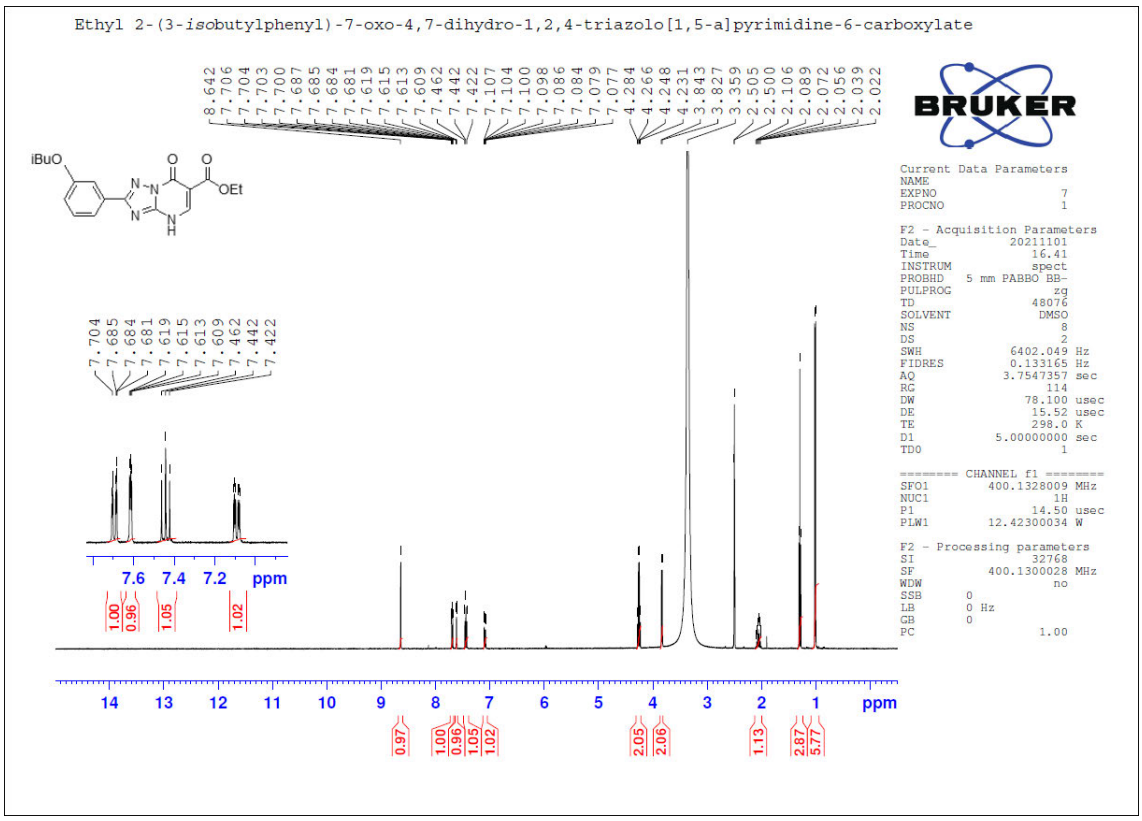
Current Data Parameters
NAME
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210413
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 295.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

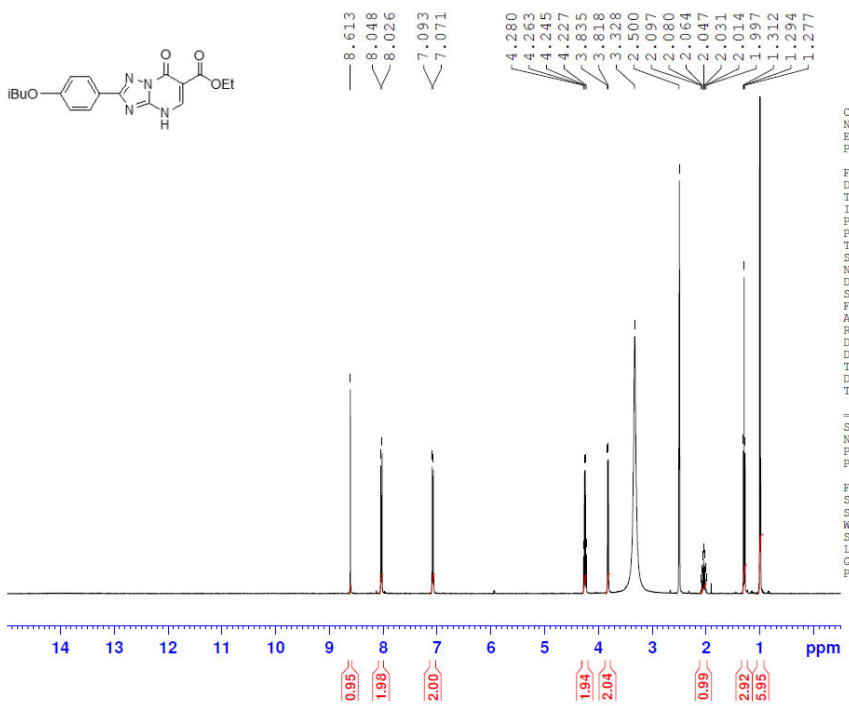
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6127941 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


Ethyl 2-(3-isobutylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate
iBuO
OEt
BRUKER
Current Data Parameters
NAME
EXPNO 7
PROCNO 1
F2 - Acquisition Parameters
Date_ 20211101
Time 16.41
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 114
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300028 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
ppm

Ethyl 2-(4-isobutyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



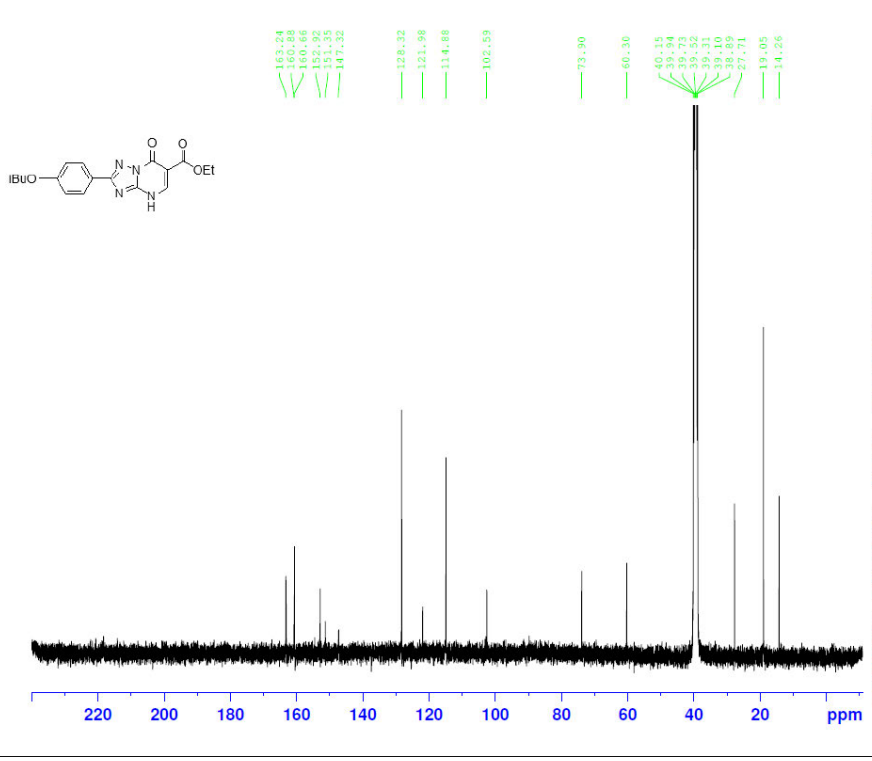

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_               20210706
Time                  10.11
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG                zg
TD                    48076
SOLVENT                DMSO
NS                        8
DS                        2
SWH                6402.049 Hz
FIDRES             0.133165 Hz
AQ                 3.7547357 sec
RG                      203
DW                   78.100 usec
DE                   15.52 usec
TE                   298.0 K
D1                5.00000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1             400.1328009 MHz
NUC1                     1H
P1                    14.50 usec
PLW1             12.42300034 W

F2 - Processing parameters
SI                    32768
SF               400.1300031 MHz
WDW                      EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.00
```

Ethyl 2-(4-*iso*butyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate




```
Current Data Parameters
NAME
EXPNO                  4
PROCNO                 1

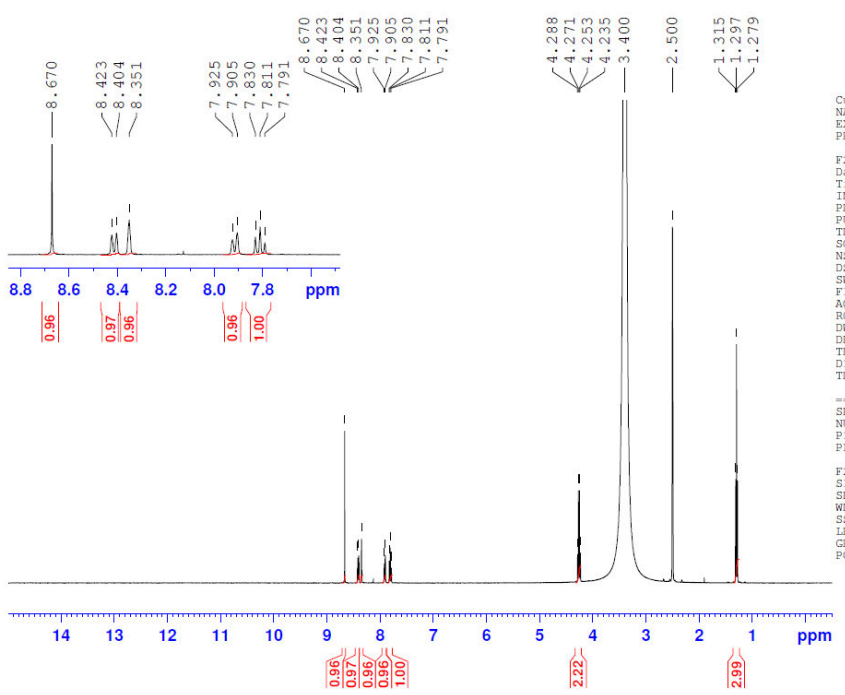
F2 - Acquisition Parameters
Date_               20211110
Time                  17.20
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                    65536
SOLVENT                DMSO
NS                    28236
DS                        4
SWH               25252.525 Hz
FIDRES             0.385323 Hz
AQ                 1.2976128 sec
RG                      203
DW                   19.800 usec
DE                    9.00 usec
TE                   298.1 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                      14

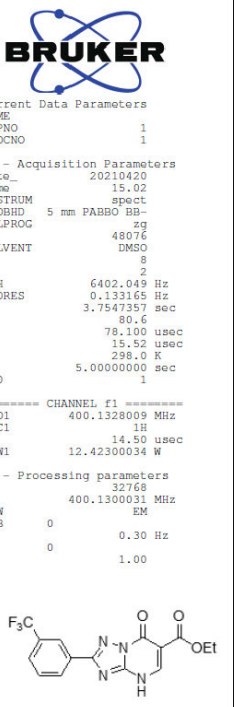
======== CHANNEL f1 ========
SFO1             100.6243395 MHz
NUC1                     13C
P1                    10.00 usec
PLW1             56.69400024 W

======== CHANNEL f2 ========
SFO2             400.1316005 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                 80.00 usec
PLW2             12.42300034 W
PLW12             0.40810999 W
PLW13             0.26119000 W

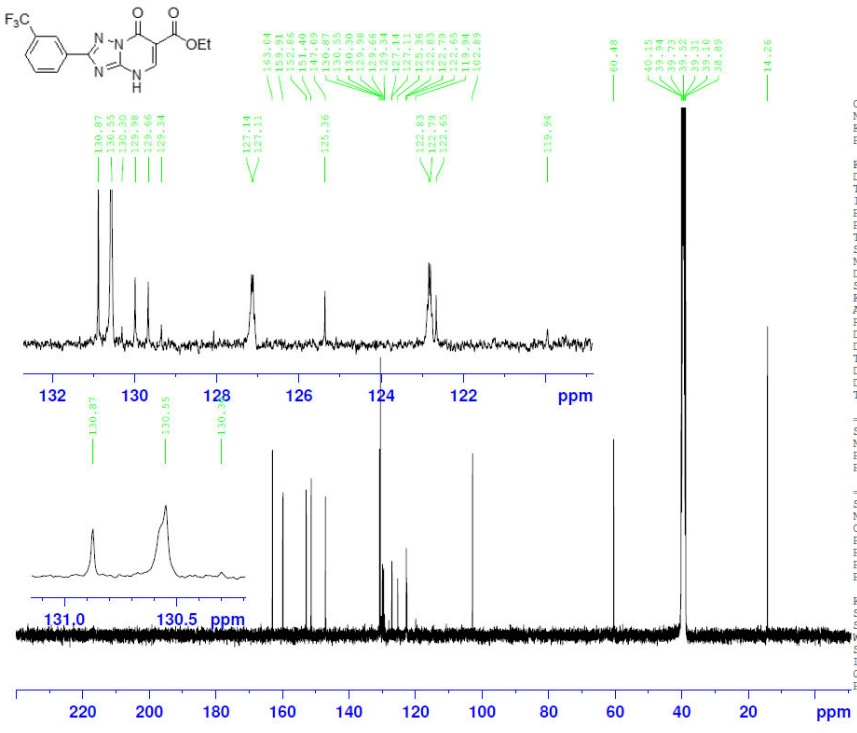
F2 - Processing parameters
SI                    65536
SF               100.6128123 MHz
WDW                      EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    1.40
```

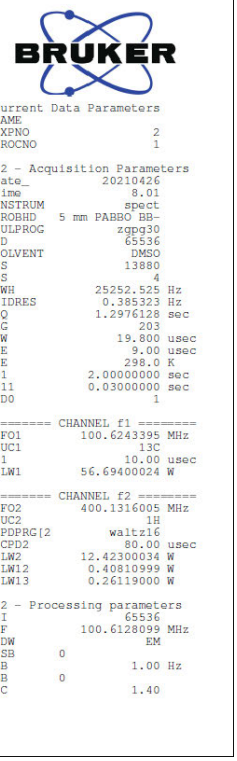
Ethyl 2-(3-trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



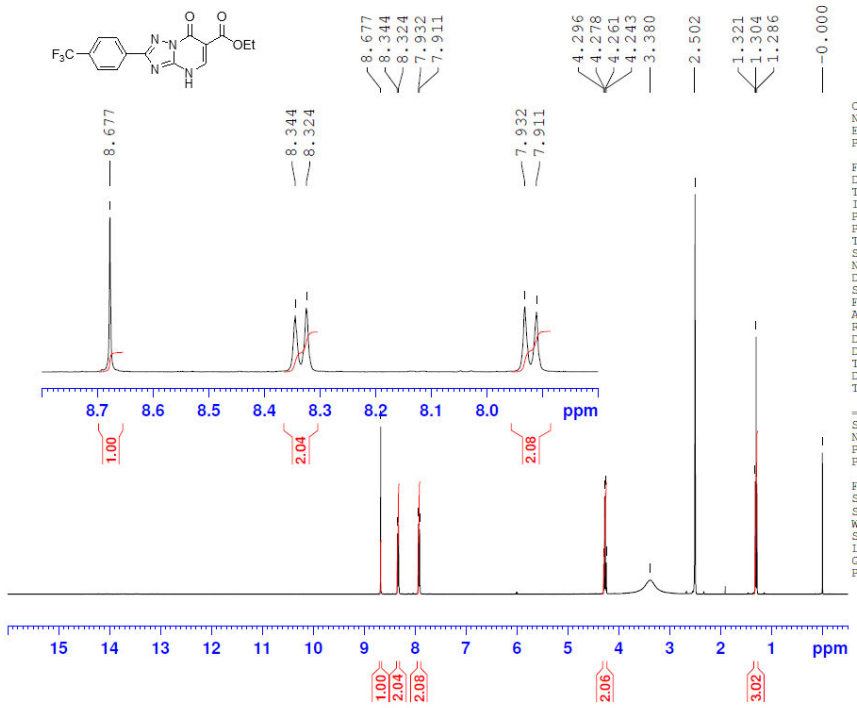


Ethyl 2-(3-trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate





Ethyl 2-(4-trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



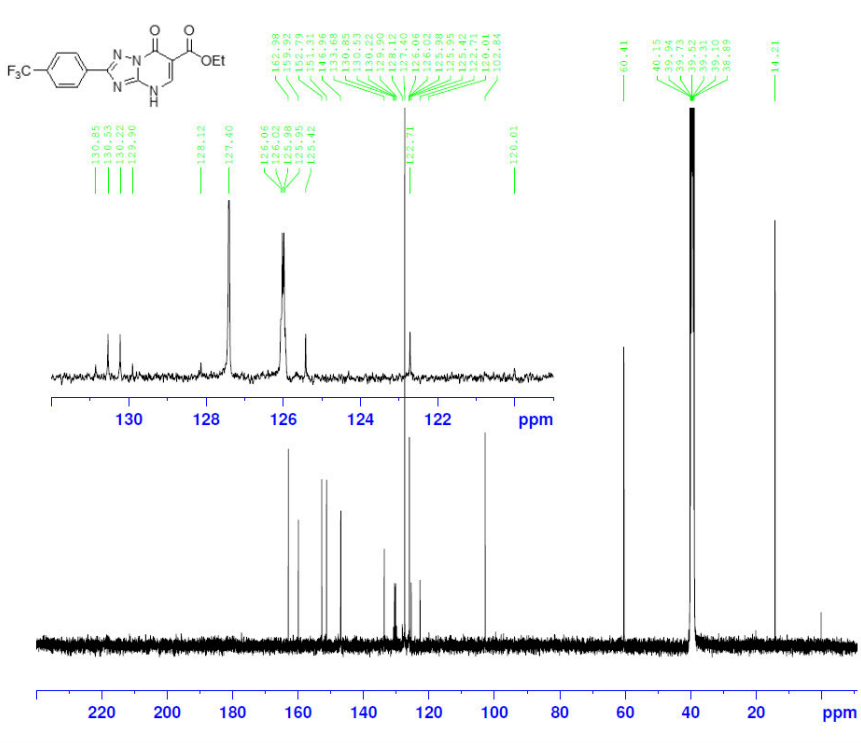
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150629
Time               13.04
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            7194.245 Hz
FIDRES        0.149643 Hz
AQ            3.3412819 sec
RG                  144
DW               69.500 usec
DE               12.64 usec
TE                298.0 K
D1            5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1330010 MHz
NUC1                 1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300021 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

Ethyl 2-(4-trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

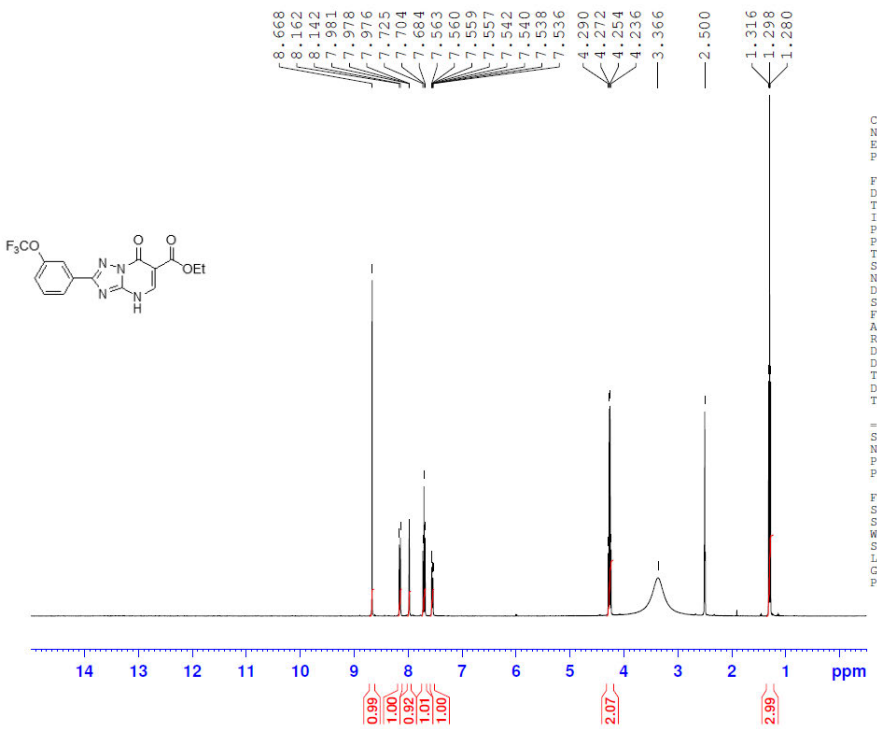
F2 - Acquisition Parameters
Date_           20150712
Time               2.44
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                20000
DS                    4
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                 13C
P1                10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128157 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

Ethyl 2-(3-trifluoromethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

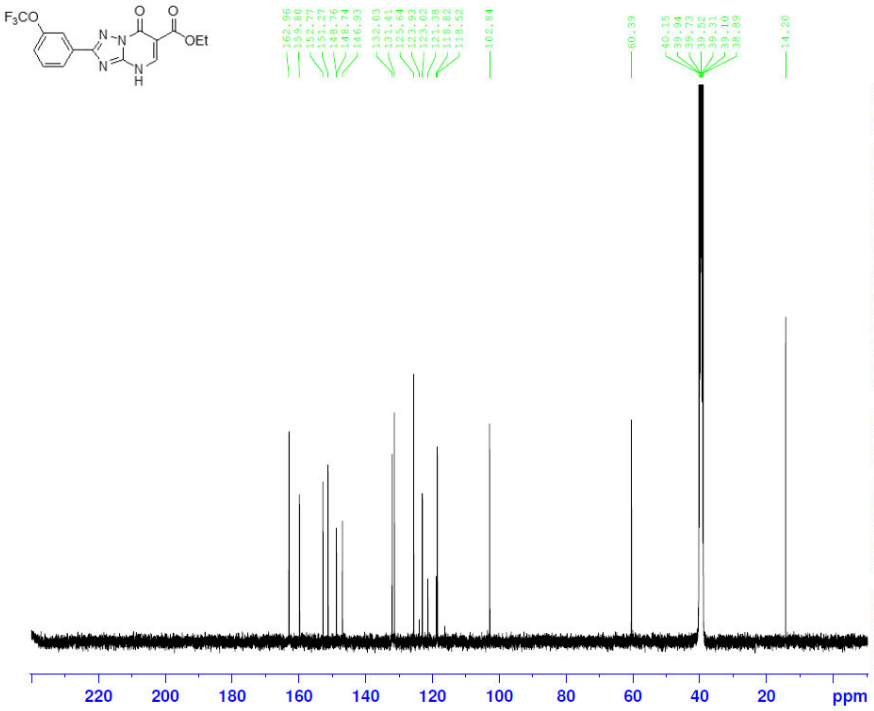
Current Data Parameters
NAME
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210604
Time 16.20
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ethyl 2-(3-trifluoromethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

Current Data Parameters
NAME
EXPNO 5
PROCNO 1

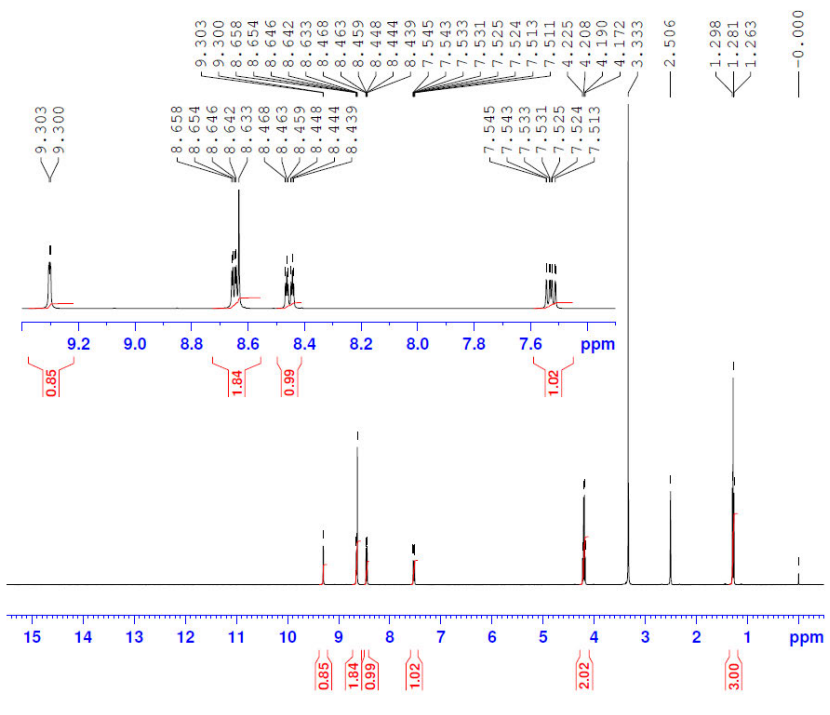
F2 - Acquisition Parameters
Date_ 20210605
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128159 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Ethyl 2-(pyridin-3-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

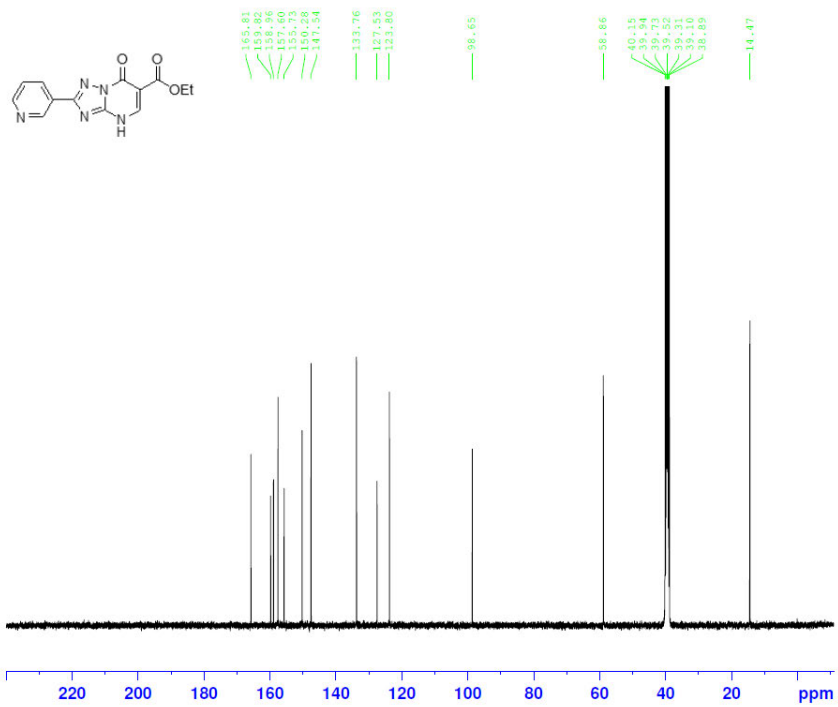
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160706
Time              15.58
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES         0.133165 Hz
AQ             3.7547357 sec
RG                  114
DW                78.100 usec
DE                15.52 usec
TE                299.8 K
D1             5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1          400.1330010 MHz
NUC1                  1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                32768
SF            400.1300011 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

Ethyl 2-(pyridin-3-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

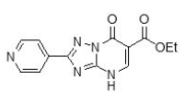
F2 - Acquisition Parameters
Date_           20160707
Time               7.16
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                12000
DS                    4
SWH           25252.525 Hz
FIDRES         0.385323 Hz
AQ             1.2976128 sec
RG                  203
DW                19.800 usec
DE                 9.00 usec
TE                299.8 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

F2 - Processing parameters
SI                65536
SF            100.6128173 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

Ethyl 2-(pyridin-4-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

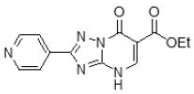
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210624
Time               10.53
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH             6402.049 Hz
FIDRES          0.133165 Hz
AQ              3.7547357 sec
RG                   203
DW                78.100 usec
DE                15.52 usec
TE                298.0 K
D1              5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1328009 MHz
NUC1                  1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                 32768
SF            400.1300031 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

Ethyl 2-(pyridin-4-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

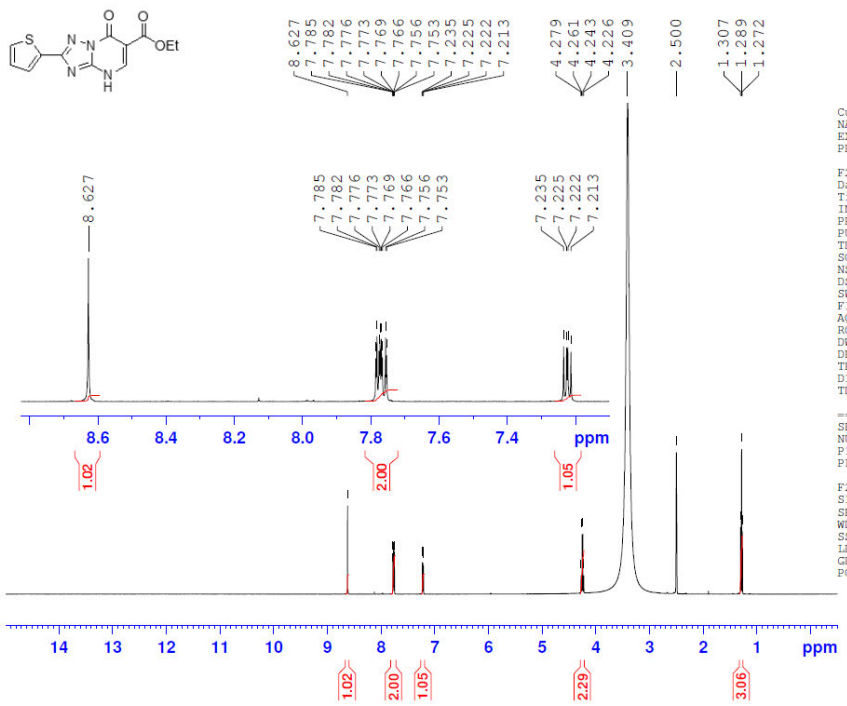
F2 - Acquisition Parameters
Date_           20210718
Time                8.06
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG            zgpg30
TD                 65536
SOLVENT             DMSO
NS                  6950
DS                     4
SWH            25252.525 Hz
FIDRES          0.385323 Hz
AQ              1.2976128 sec
RG                   203
DW                19.800 usec
DE                 9.00 usec
TE                298.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

F2 - Processing parameters
SI                 65536
SF            100.6128137 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

Ethyl 2-(thien-2-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



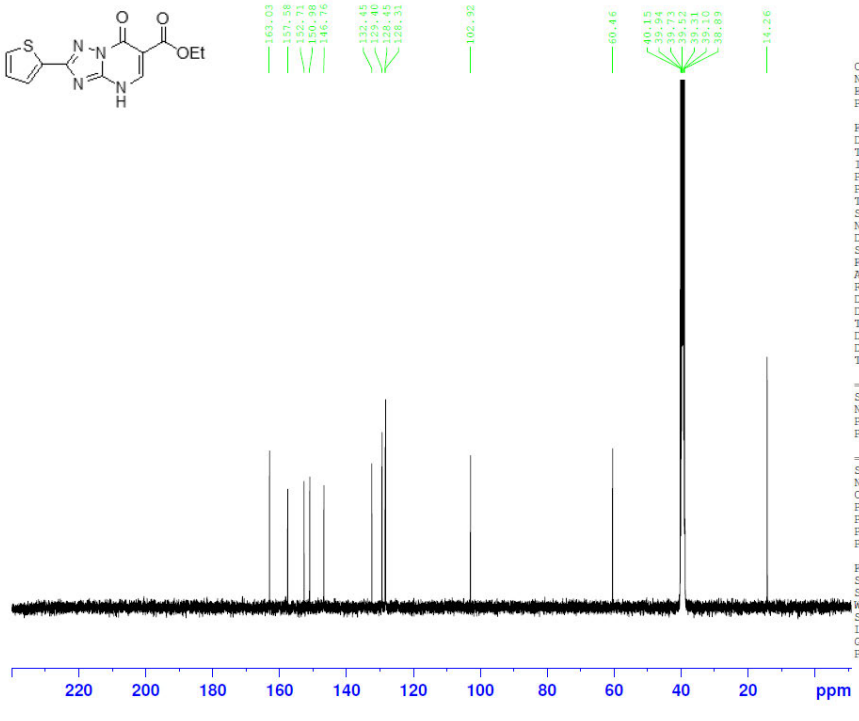
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210420
Time 15.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 80.6
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ethyl 2-(thien-2-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylate



Current Data Parameters
NAME
EXPNO 2
PROCNO 1

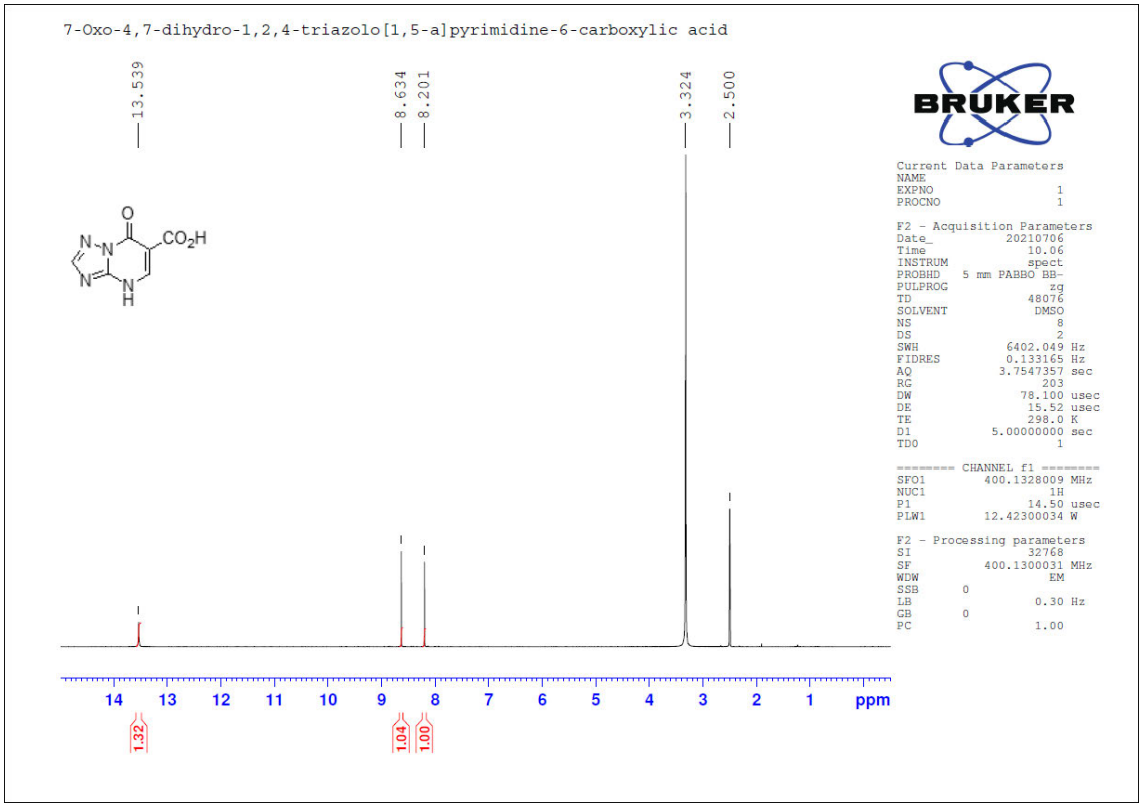
F2 - Acquisition Parameters
Date_ 20210425
Time 1.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

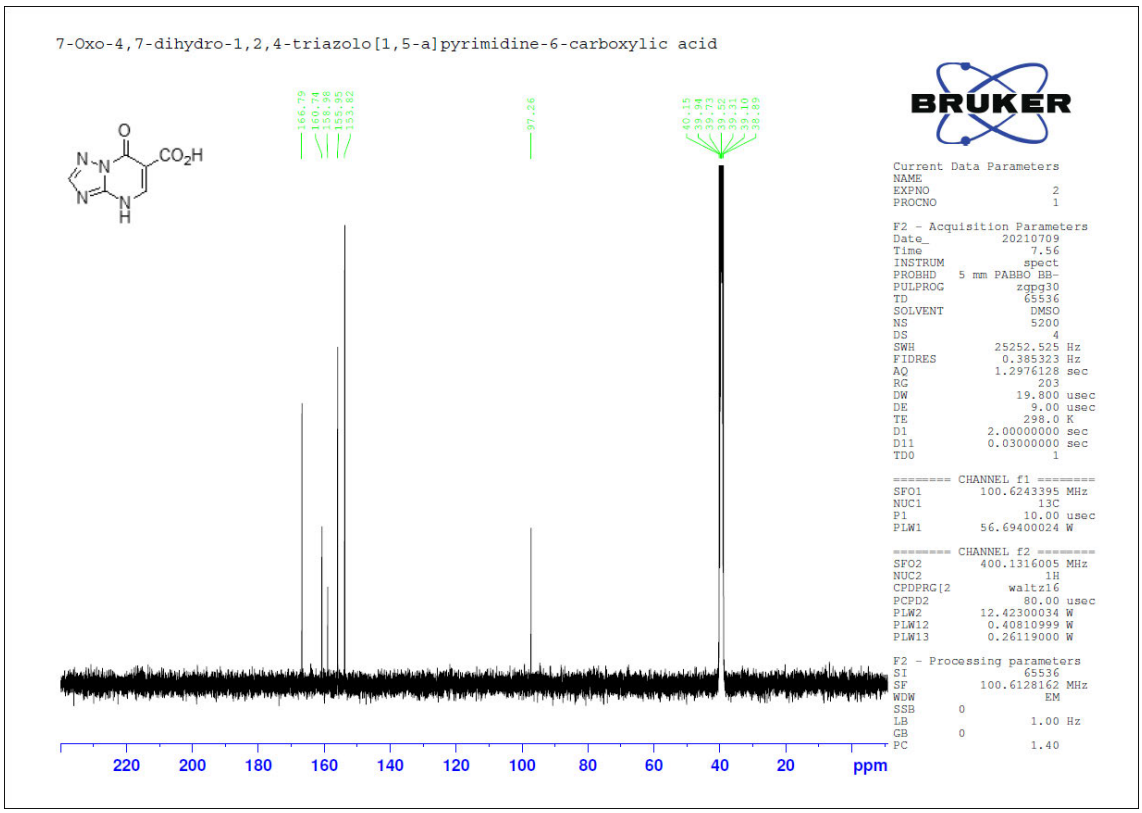
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

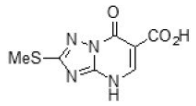
F2 - Processing parameters
SI 65536
SF 100.6128098 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Appendix 3


7-Oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
13.539
8.634
8.201
3.324
2.500
CO2H
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.32
1.04
1.00
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210706
Time 10.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


7-Oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
166.79
160.74
158.98
155.95
153.82
97.26
40.15
39.94
39.73
39.52
39.31
39.10
38.89
CO2H
220 200 180 160 140 120 100 80 60 40 20 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210709
Time 7.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 5200
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128162 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-Methylthio-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

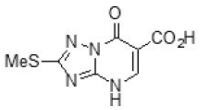
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210503
Time              10.14
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES        0.133165 Hz
AQ            3.7547357 sec
RG                  161
DW               78.100 usec
DE               15.52 usec
TE                298.0 K
D1            5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300031 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2-Methylthio-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

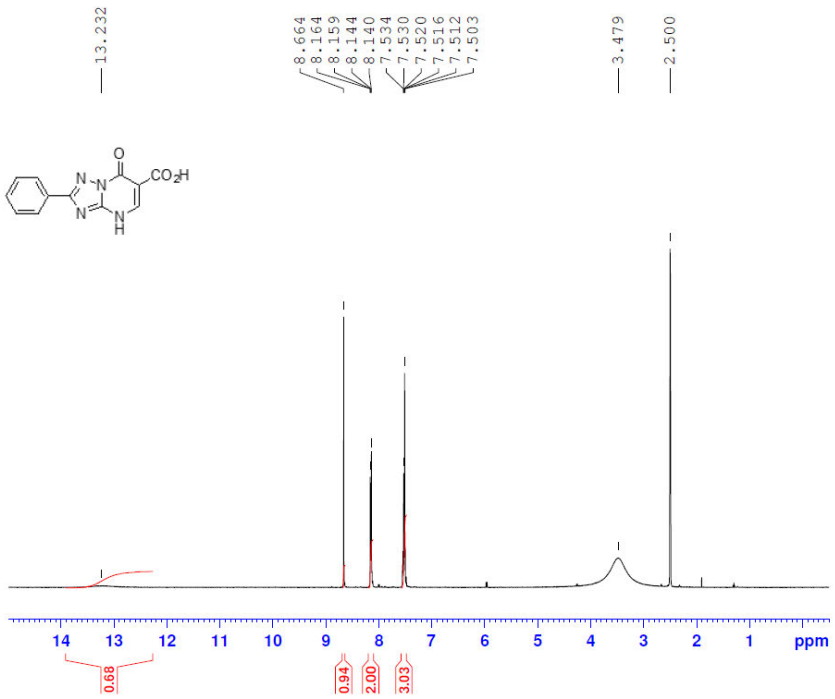
F2 - Acquisition Parameters
Date_           20210504
Time               8.06
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 6950
DS                    4
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                13C
P1                10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128154 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2-Phenyl-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

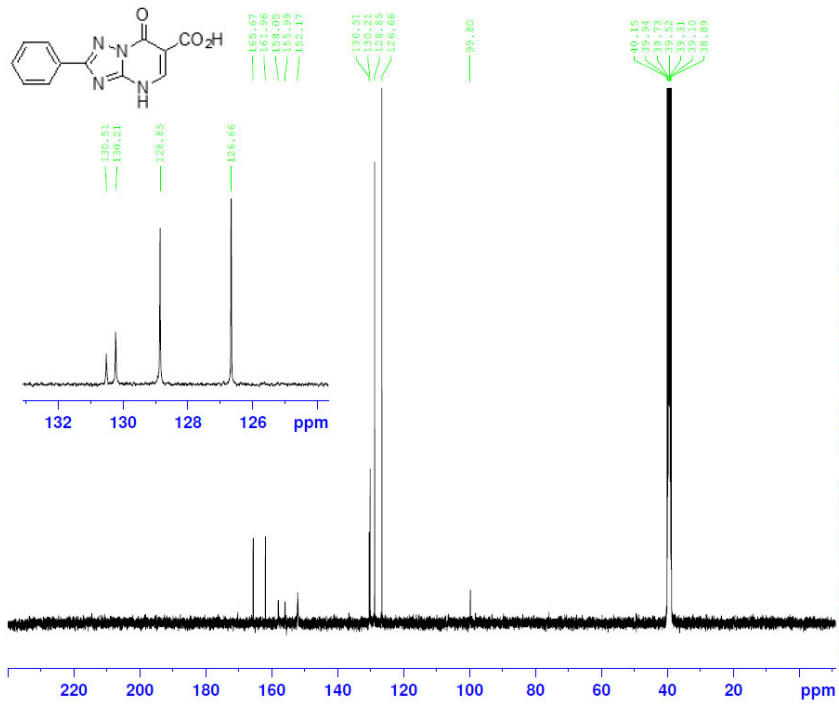
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210622
Time 15.00
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-Phenyl-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

Current Data Parameters
NAME
EXPNO 2
PROCNO 1

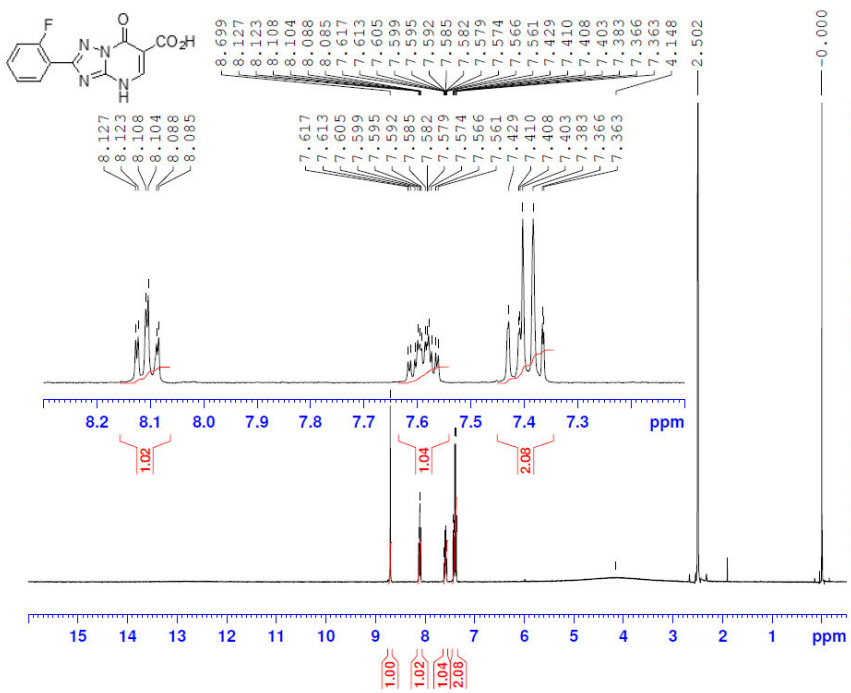
F2 - Acquisition Parameters
Date_ 20210719
Time 1.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128155 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(2-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



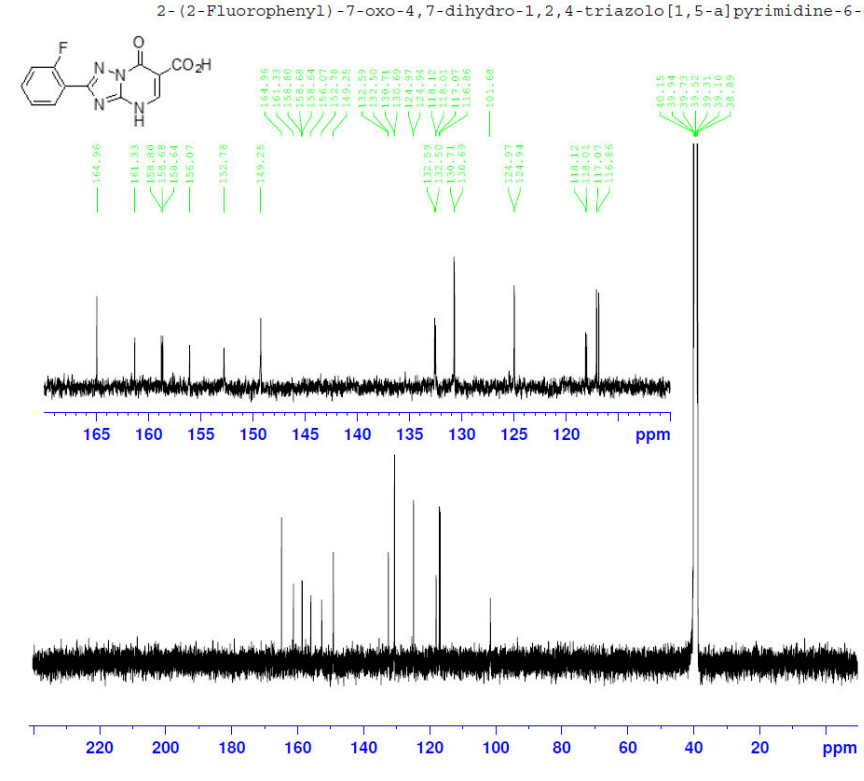
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150625
Time               9.37
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH           7194.245 Hz
FIDRES        0.149643 Hz
AQ            3.341281 sec
RG                  161
DW             69.500 usec
DE             12.64 usec
TE                298.0 K
D1           5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        400.1330010 MHz
NUC1                1H
P1             14.50 usec
PLW1       12.42300034 W

F2 - Processing parameters
SI                32768
SF          400.1300022 MHz
WDW                  EM
SSB       0
LB             0.30 Hz
GB        0
PC             1.00
```

2-(2-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



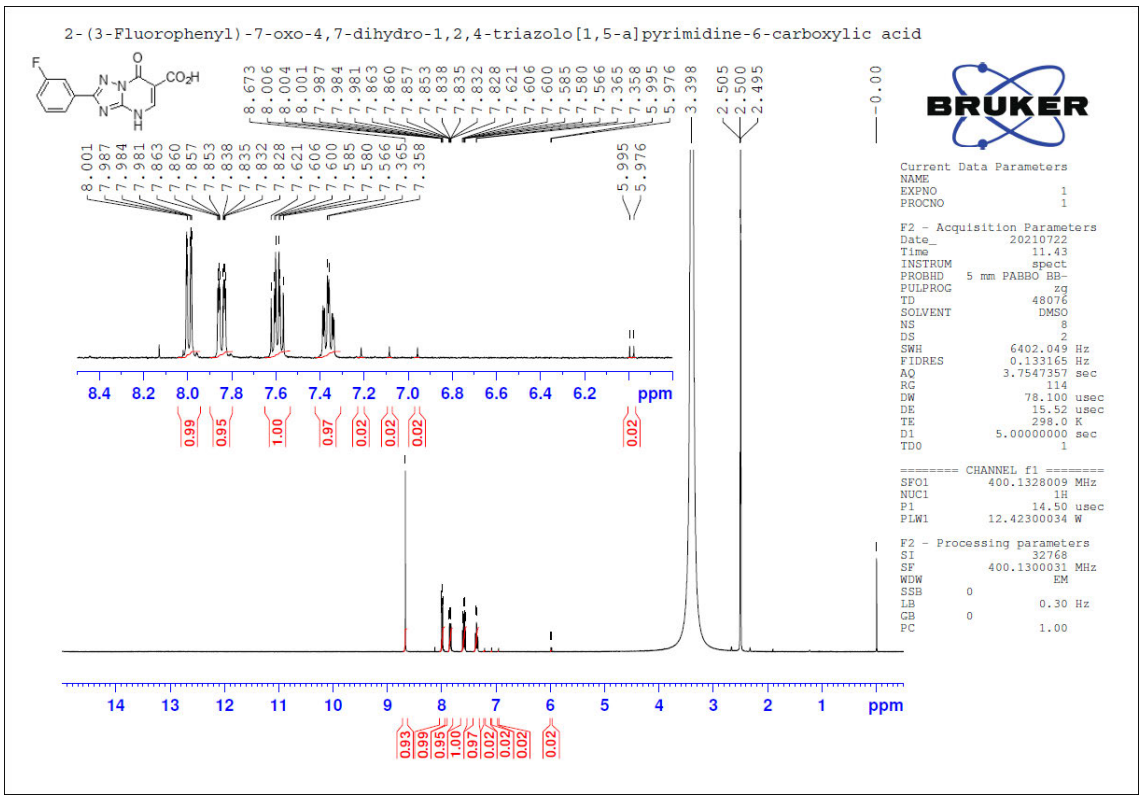
```
Current Data Parameters
NAME
EXPNO                  9
PROCNO                 1

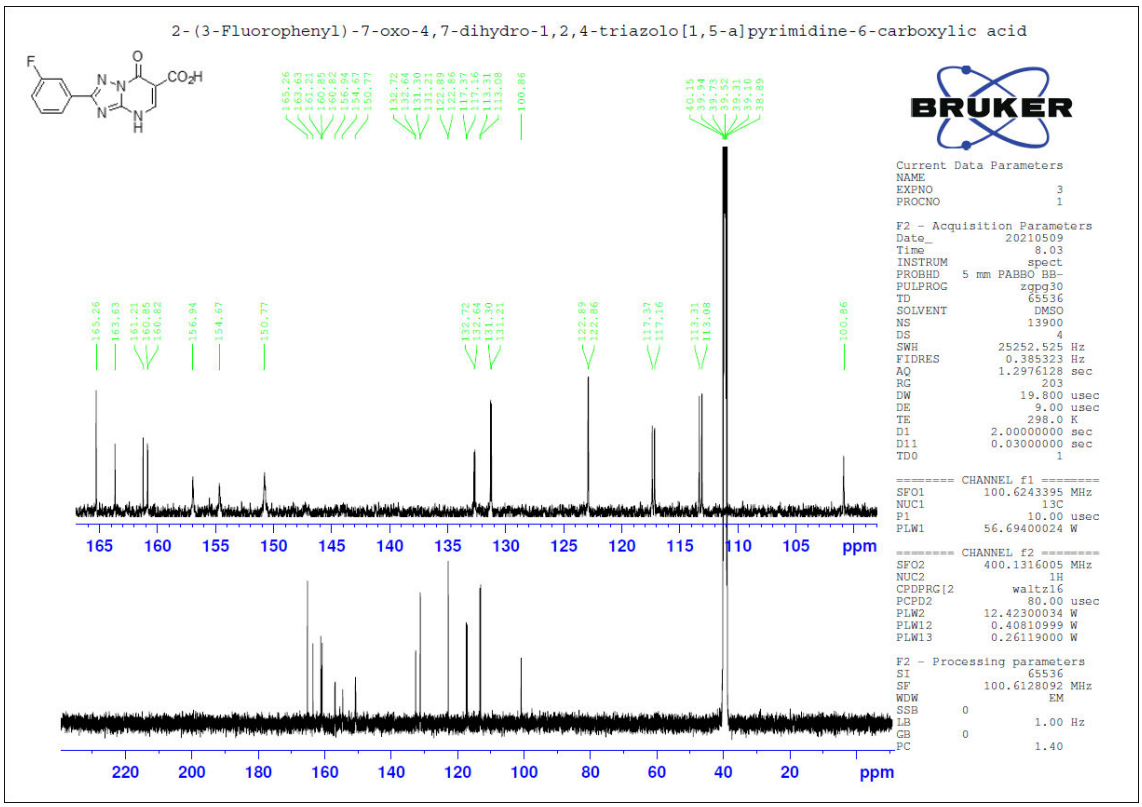
F2 - Acquisition Parameters
Date_           20160702
Time              17.58
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                24000
DS                    4
SWH          25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                  203
DW             19.800 usec
DE              9.00 usec
TE                299.8 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

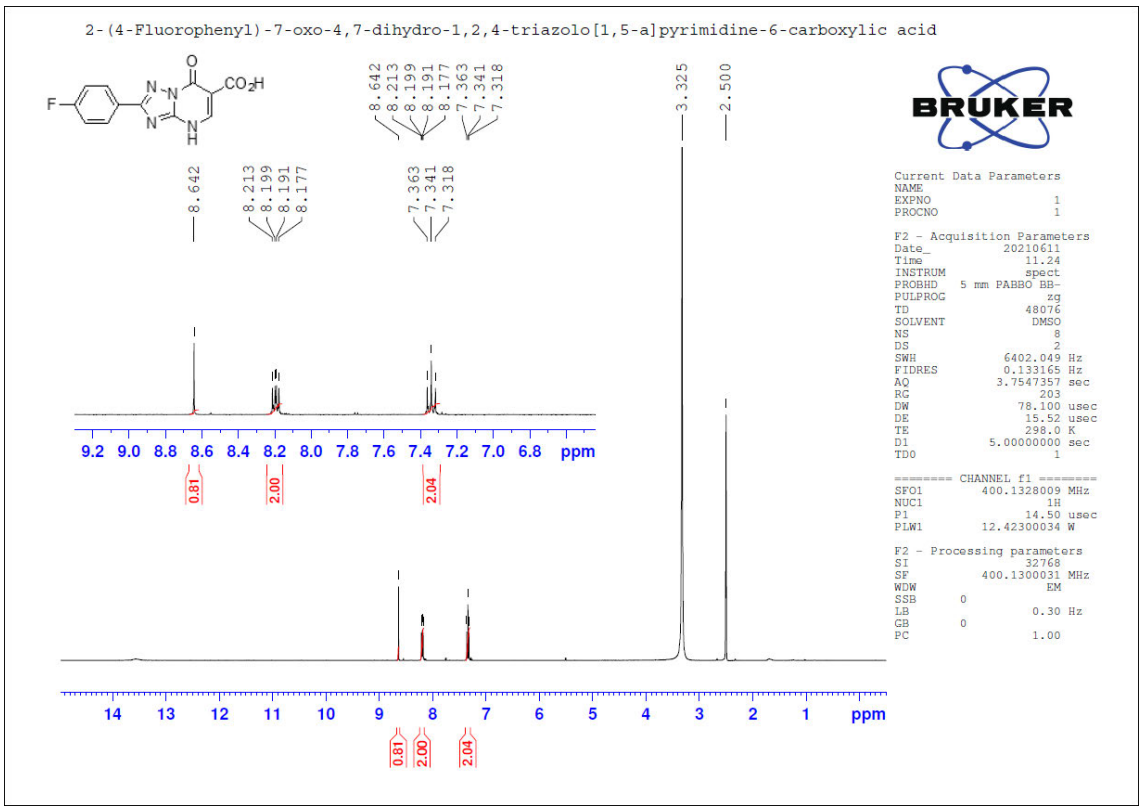
======== CHANNEL f1 ========
SFO1        100.6243395 MHz
NUC1                13C
P1             10.00 usec
PLW1       56.69400024 W

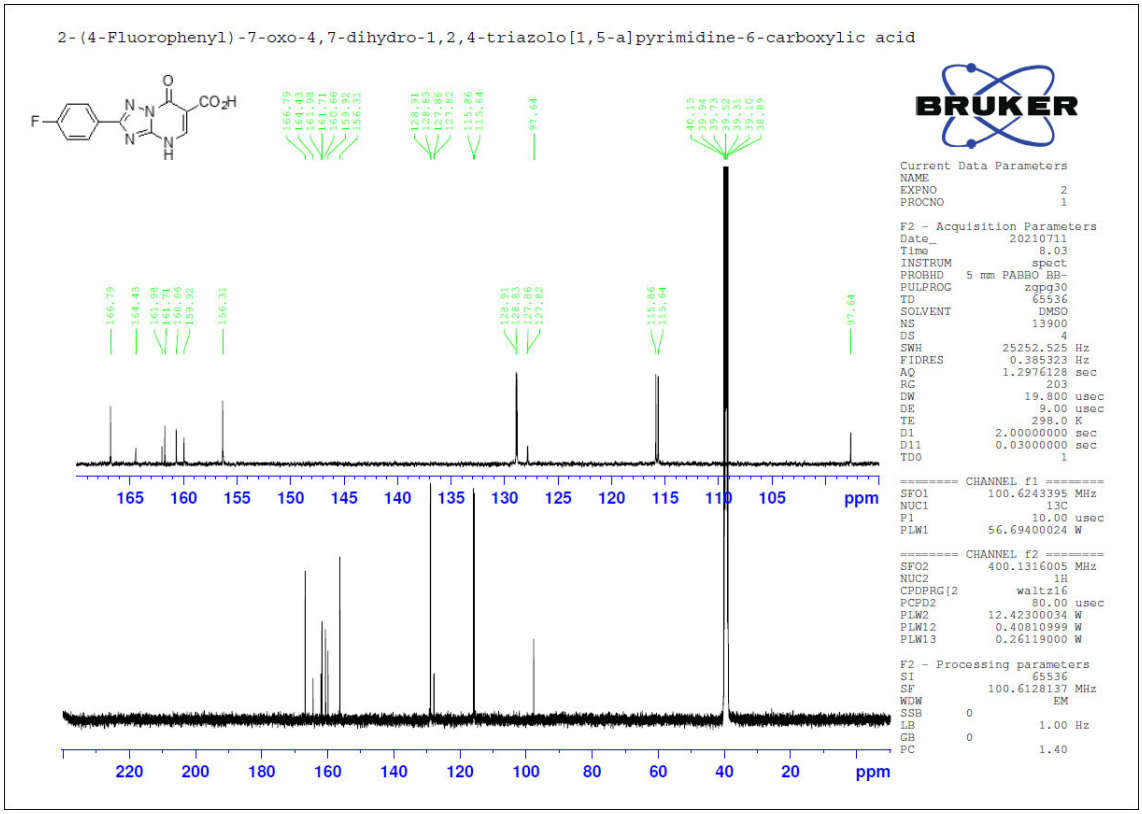
======== CHANNEL f2 ========
SFO2        400.1316005 MHz
NUC2                1H
CPDPRG[2        waltz16
PCPD2            80.00 usec
PLW2       12.42300034 W
PLW12       0.40810999 W
PLW13       0.26119000 W

F2 - Processing parameters
SI                65536
SF          100.6128008 MHz
WDW                  EM
SSB       0
LB             1.00 Hz
GB        0
PC             1.40
```

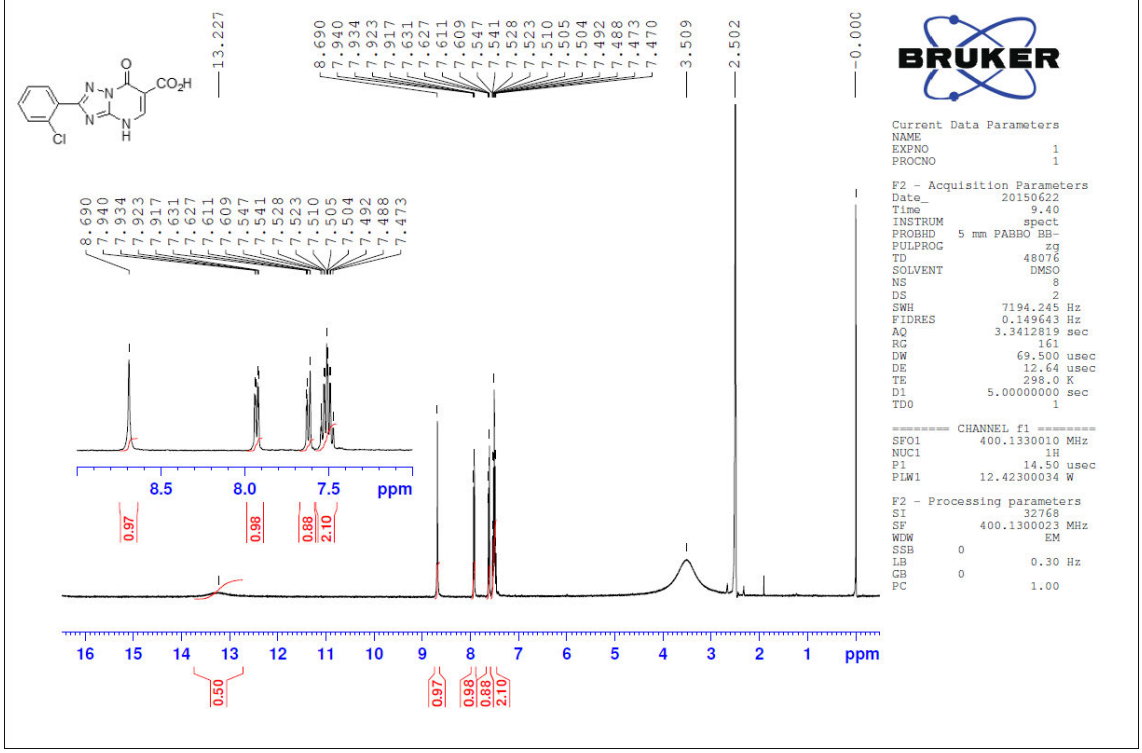

2-(3-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210722
Time 11.43
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 114
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(3-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
BRUKER
Current Data Parameters
NAME
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210509
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128092 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


2-(4-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
8.642
8.213
8.199
8.191
8.177
7.363
7.341
7.318
3.325
2.500
0.81
2.00
2.04
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210611
Time 11.24
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(4-Fluorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
166.79
164.43
161.98
161.71
160.66
159.92
156.31
128.91
128.83
127.86
127.82
115.86
115.64
97.64
40.15
39.94
39.73
39.52
39.31
39.10
38.89
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210711
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
CHANNEL f2
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128137 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(2-Chlorophenyl)7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

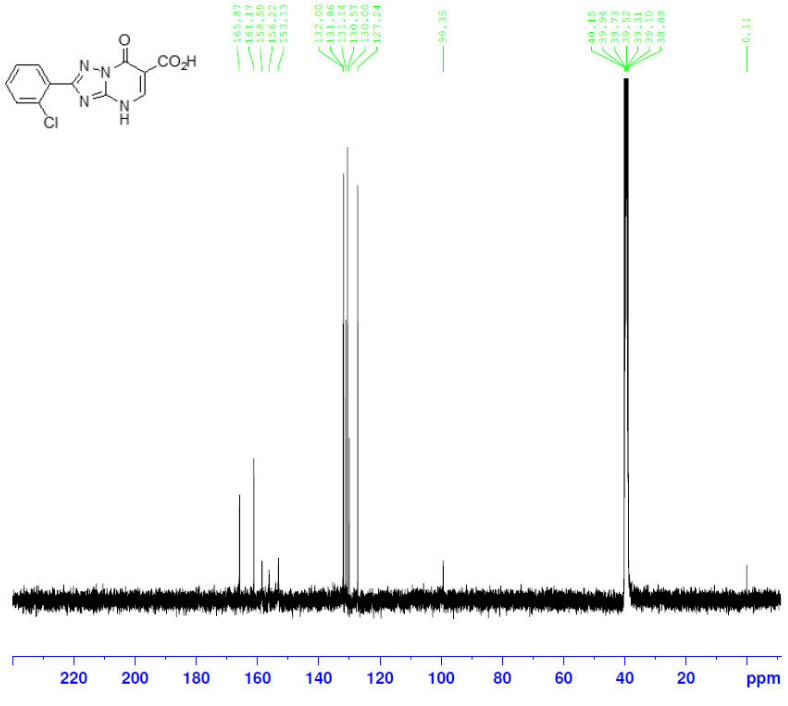
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150622
Time               9.40
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            7194.245 Hz
FIDRES        0.149643 Hz
AQ            3.3412819 sec
RG                  161
DW             69.500 usec
DE             12.64 usec
TE                298.0 K
D1           5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1330010 MHz
NUC1                 1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300023 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

2-(2-Chlorophenyl)7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

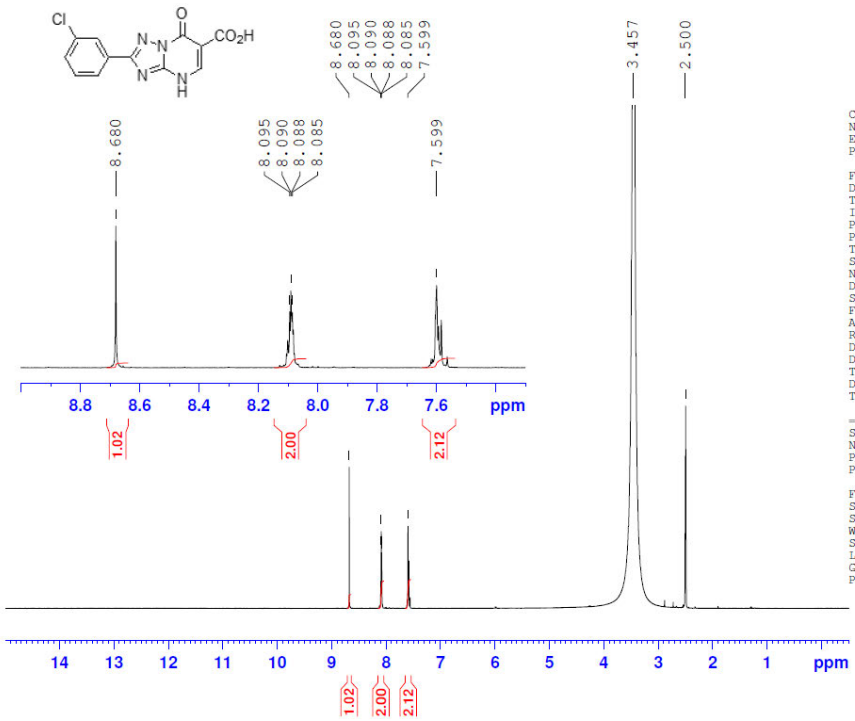
F2 - Acquisition Parameters
Date_           20150713
Time               6.00
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                65536
SOLVENT            DMSO
NS                 7000
DS                    4
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                  203
DW             19.800 usec
DE              9.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                 13C
P1               10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2            80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128155 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

2-(3-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



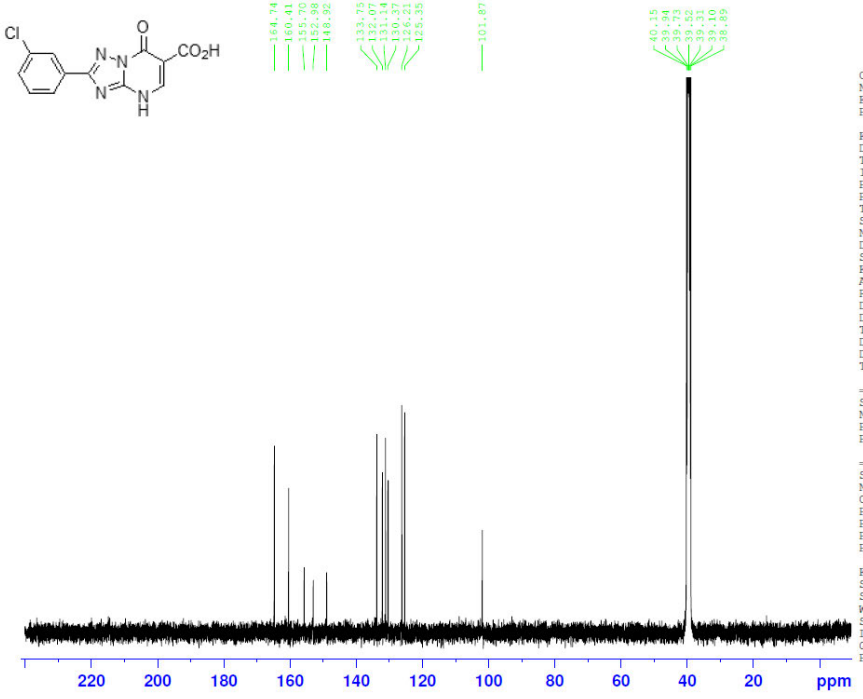

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210510
Time              10.07
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG             zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES         0.133165 Hz
AQ             3.7547357 sec
RG                 80.6
DW               78.100 usec
DE               15.52 usec
TE                298.0 K
D1             5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                 1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300031 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2-(3-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid




```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

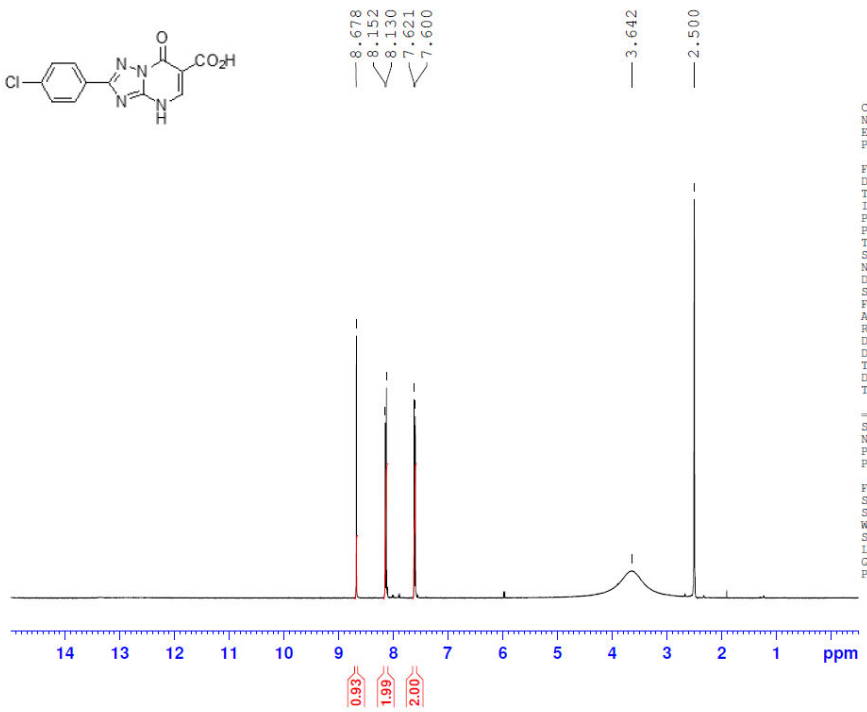
F2 - Acquisition Parameters
Date_           20210513
Time               8.03
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                13900
DS                    4
SWH           25252.525 Hz
FIDRES         0.385323 Hz
AQ             1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                 13C
P1                10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128101 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2-(4-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

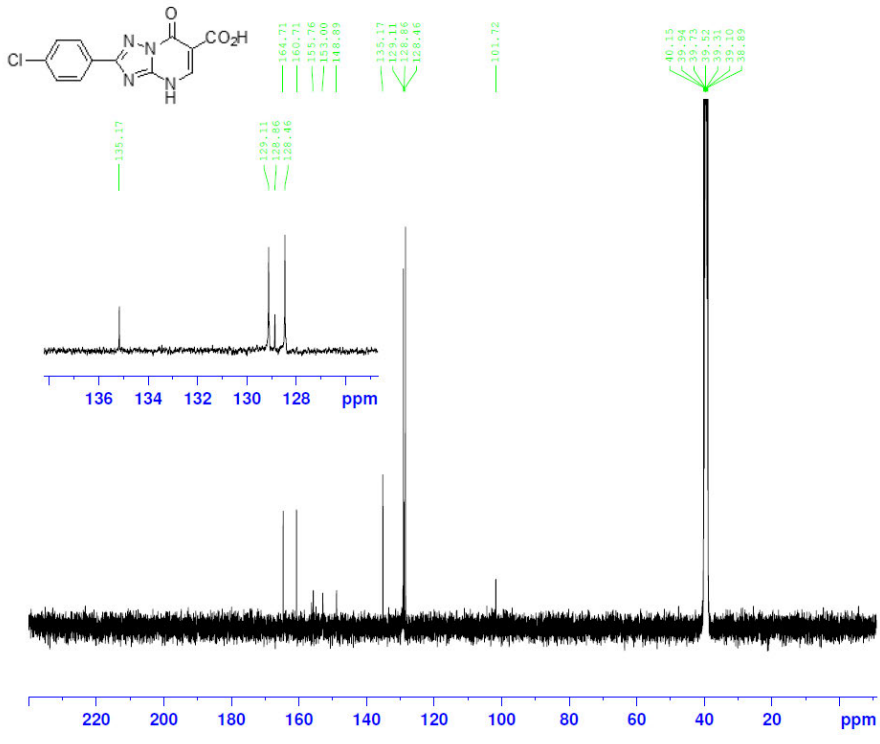
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210706
Time 10.16
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(4-Chlorophenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

Current Data Parameters
NAME
EXPNO 2
PROCNO 1

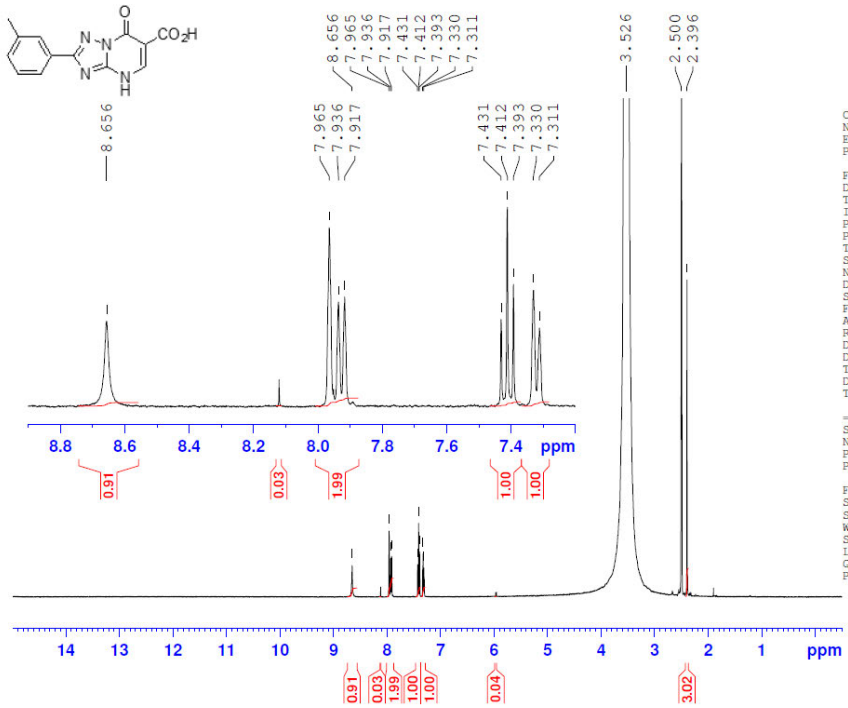
F2 - Acquisition Parameters
Date_ 20210724
Time 1.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128156 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(3-Methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



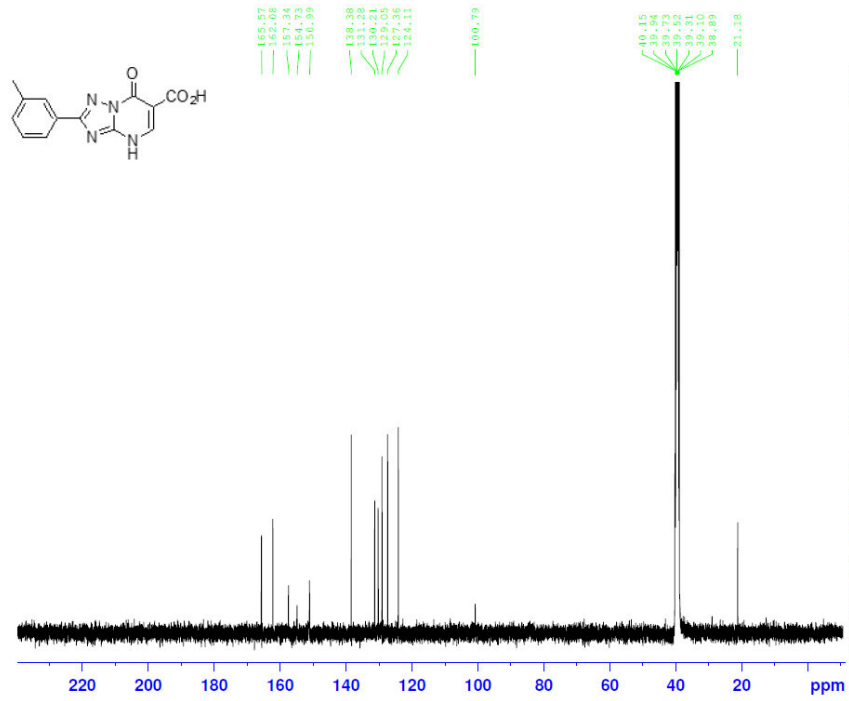

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_               20210331
Time                   15.57
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG                zg
TD                     48076
SOLVENT                DMSO
NS                     8
DS                     2
SWH                 6402.049 Hz
FIDRES              0.133165 Hz
AQ                  3.7547357 sec
RG                     32
DW                     78.100 usec
DE                     15.52 usec
TE                     298.3 K
D1                  5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1                400.1328009 MHz
NUC1                   1H
P1                     14.50 usec
PLW1                12.42300034 W

F2 - Processing parameters
SI                     32768
SF                  400.1300031 MHz
WDW                    EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.00
```

2-(3-Methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid




```
Cu
NAME
EXPNO                  2
PROCNO                 1

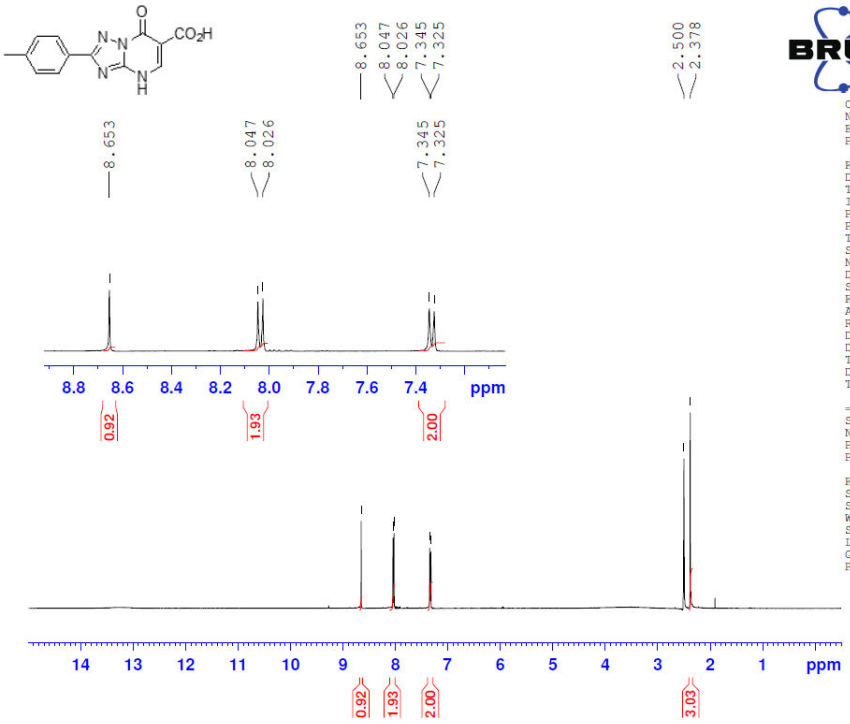
F2 - Acquisition Parameters
Date_               20210504
Time                   1.33
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                     65536
SOLVENT                DMSO
NS                     6950
DS                     4
SWH                 25252.525 Hz
FIDRES              0.385323 Hz
AQ                  1.2976128 sec
RG                     203
DW                     19.800 usec
DE                     9.00 usec
TE                     298.0 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1                100.6243395 MHz
NUC1                   13C
P1                     10.00 usec
PLW1                56.69400024 W

======== CHANNEL f2 ========
SFO2                400.1316005 MHz
NUC2                   1H
CPDPRG[2               waltz16
PCPD2                  80.00 usec
PLW2                12.42300034 W
PLW12               0.40810999 W
PLW13               0.26119000 W

F2 - Processing parameters
SI                     65536
SF                  100.6127943 MHz
WDW                    EM
SSB          0
LB                     1.00 Hz
GB           0
PC                     1.40
```

2-(4-Methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



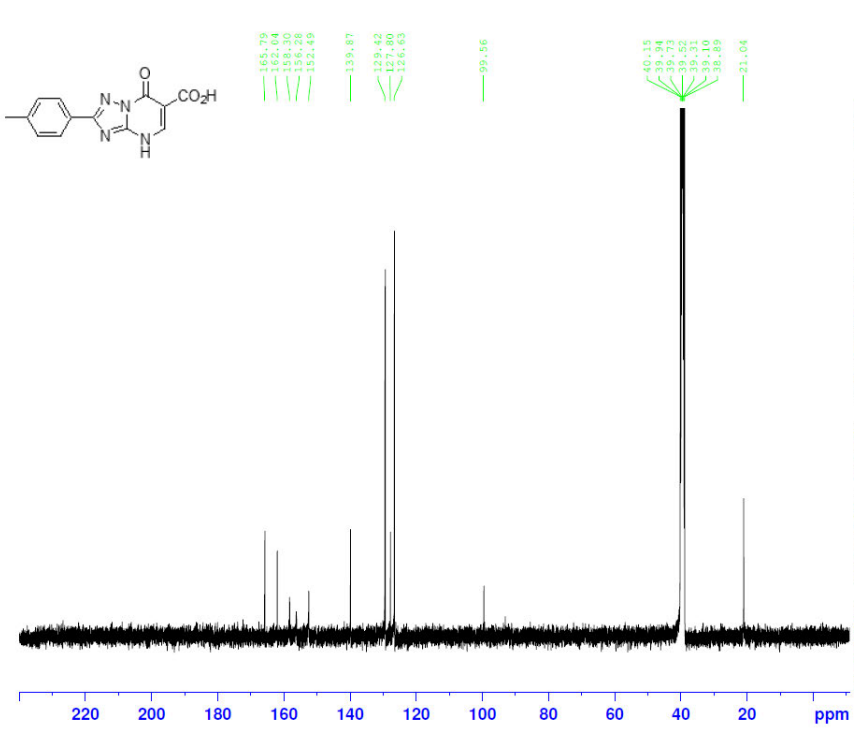

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210526
Time               9.56
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES         0.133165 Hz
AQ             3.7547357 sec
RG                  203
DW                78.100 usec
DE                15.52 usec
TE                298.0 K
D1             5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300031 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2-(4-Methylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid




```
Current Data Parameters
NAME
EXPNO                  3
PROCNO                 1

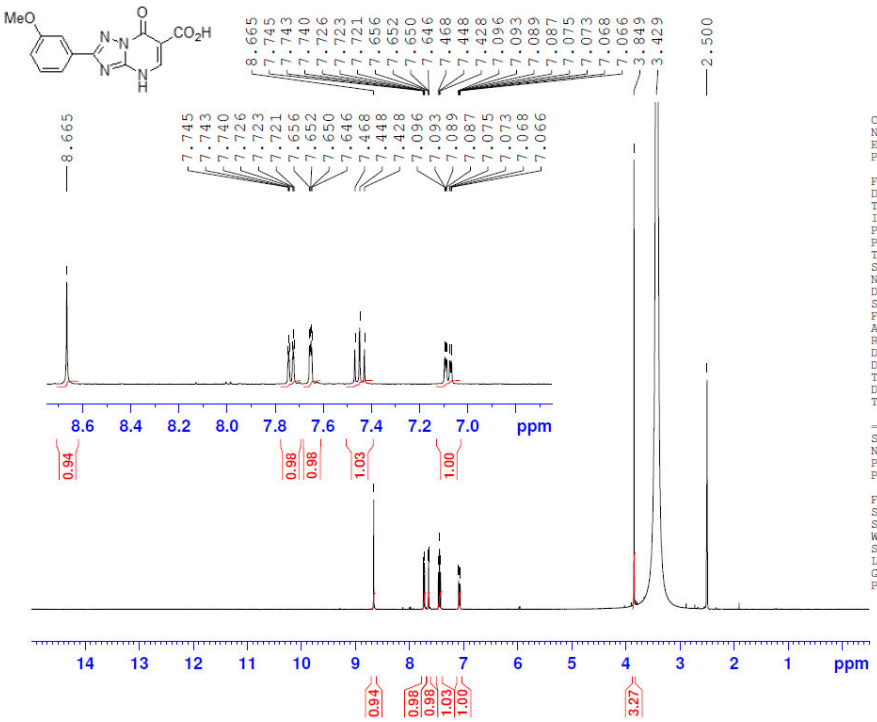
F2 - Acquisition Parameters
Date_           20210709
Time               3.01
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 8500
DS                    4
SWH           25252.525 Hz
FIDRES         0.385323 Hz
AQ             1.2976128 sec
RG                  203
DW                19.800 usec
DE                 9.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                13C
P1                10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128152 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2-(3-Methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

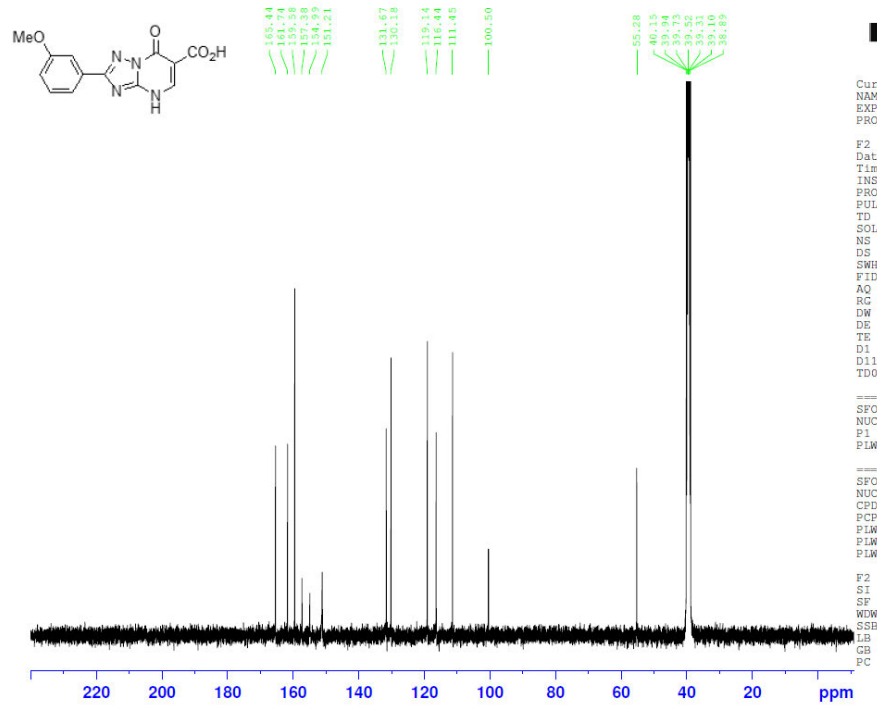
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210419
Time 9.54
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 80.6
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300029 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(3-Methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

Current Data Parameters
NAME
EXPNO 2
PROCNO 1

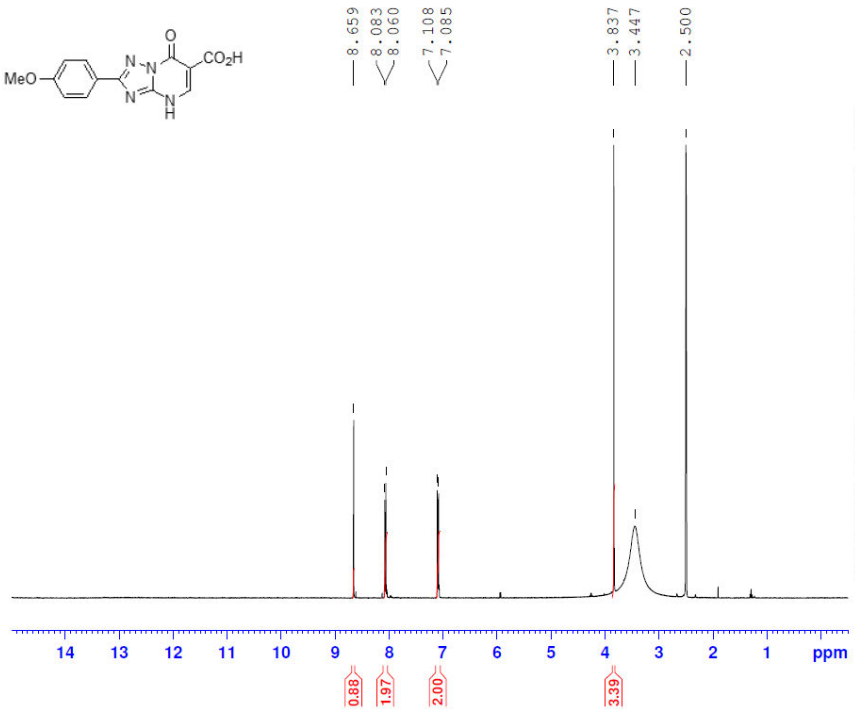
F2 - Acquisition Parameters
Date_ 20210516
Time 8.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(4-Methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



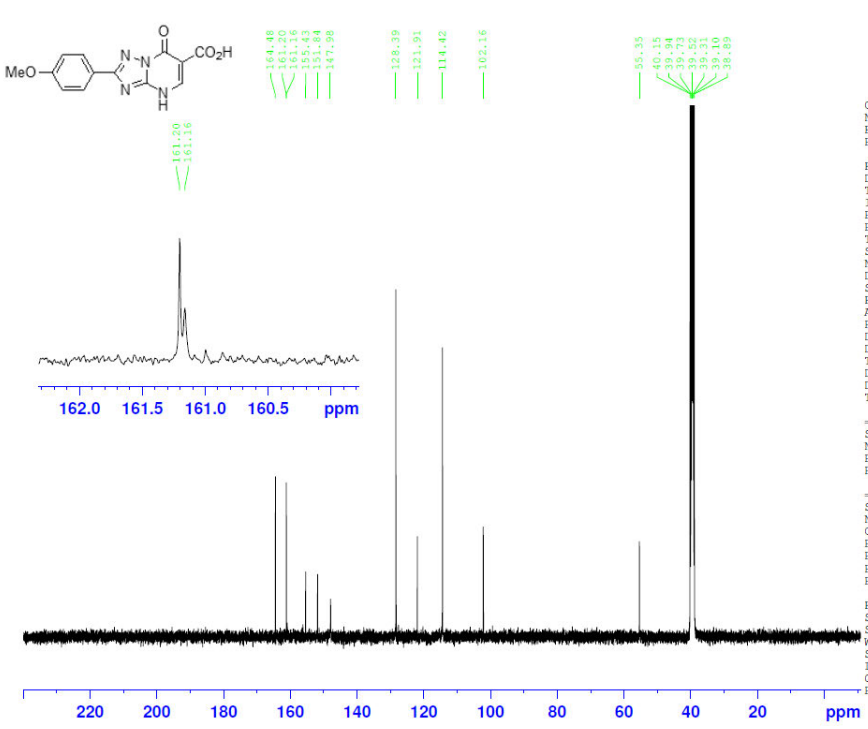

```
Current Data Parameters
NAME
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_               20210709
Time                   10.37
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG                 zg
TD                     48076
SOLVENT                DMSO
NS                         8
DS                         2
SWH                 6402.049 Hz
FIDRES              0.133165 Hz
AQ                  3.7547357 sec
RG                       203
DW                    78.100 usec
DE                    15.52 usec
TE                    298.0 K
D1                  5.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1              400.1328009 MHz
NUC1                      1H
P1                     14.50 usec
PLW1              12.42300034 W

F2 - Processing parameters
SI                         32768
SF                400.1300031 MHz
WDW                           EM
SSB          0
LB                      0.30 Hz
GB           0
PC                      1.00
```

2-(4-Methoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



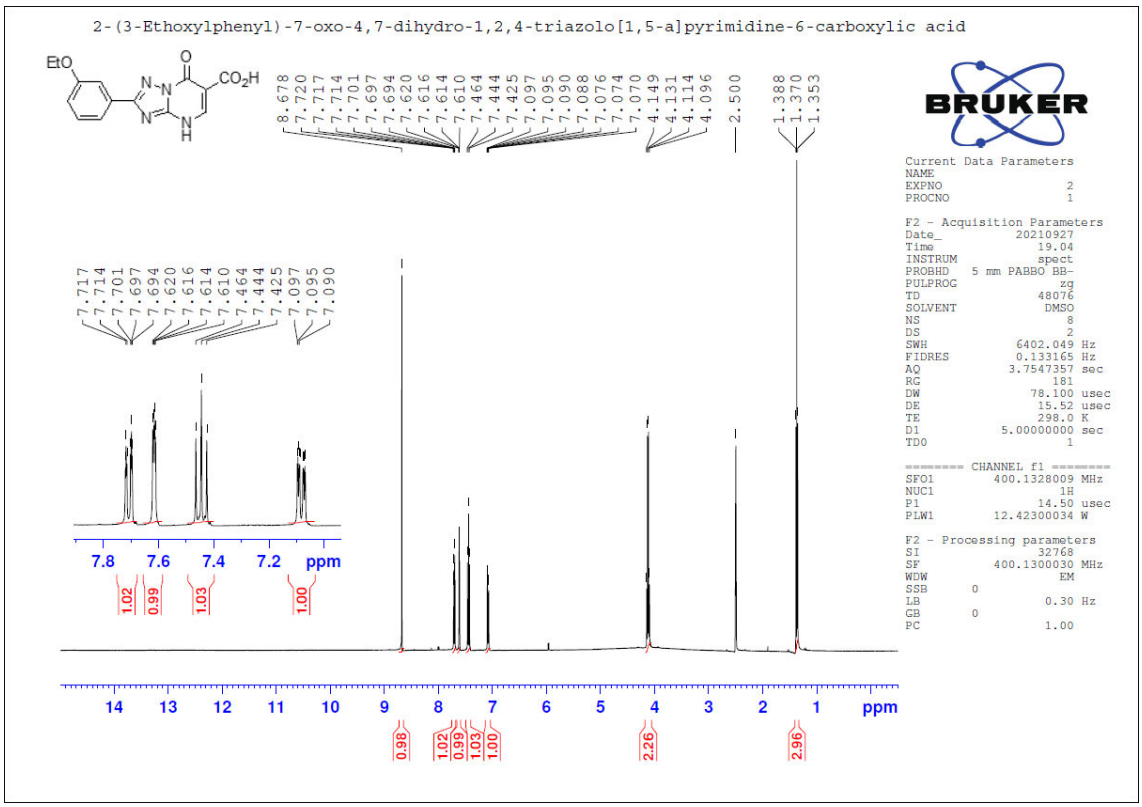

```
Current Data Parameters
NAME
EXPNO                   2
PROCNO                  1

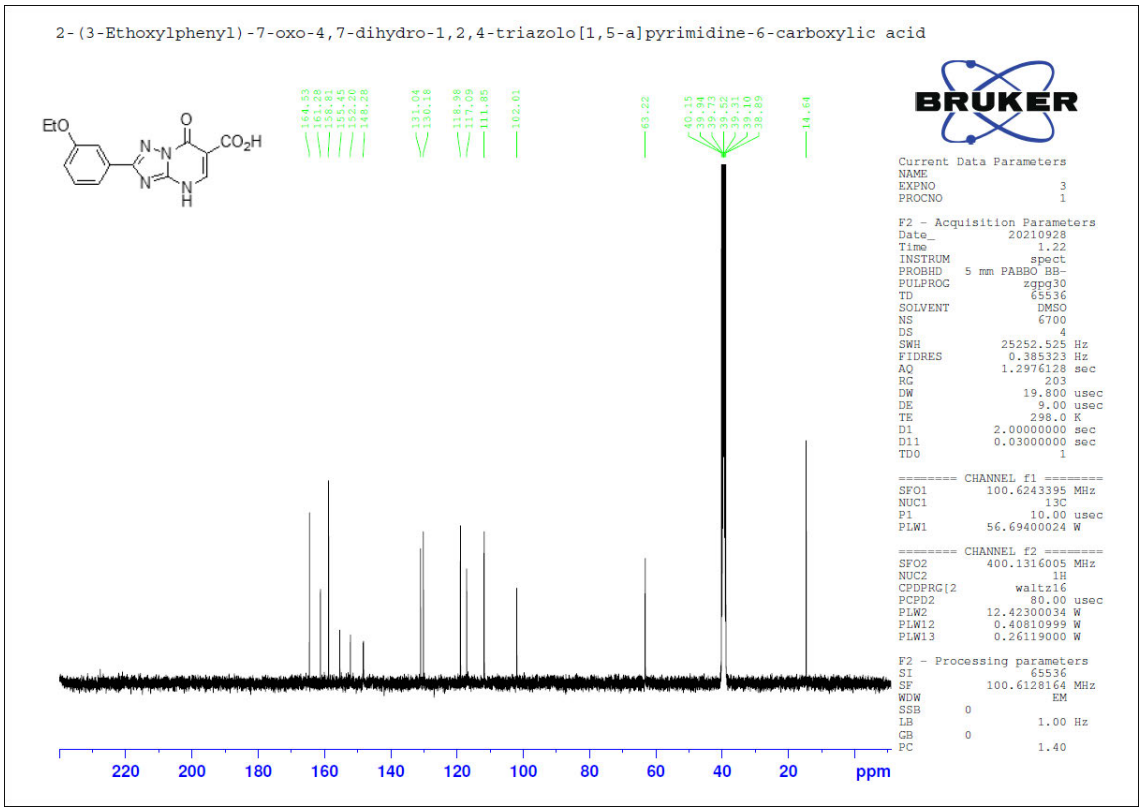
F2 - Acquisition Parameters
Date_               20210902
Time                    8.06
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                     65536
SOLVENT                DMSO
NS                      6950
DS                         4
SWH                25252.525 Hz
FIDRES              0.385323 Hz
AQ                  1.2976128 sec
RG                       203
DW                    19.800 usec
DE                     9.00 usec
TE                    298.0 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1              100.6243395 MHz
NUC1                      13C
P1                     10.00 usec
PLW1              56.69400024 W

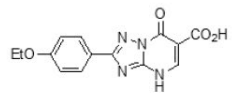
======== CHANNEL f2 ========
SFO2              400.1316005 MHz
NUC2                      1H
CPDPRG[2             waltz16
PCPD2                  80.00 usec
PLW2              12.42300034 W
PLW12              0.40810999 W
PLW13              0.26119000 W

F2 - Processing parameters
SI                         65536
SF                100.6128141 MHz
WDW                           EM
SSB          0
LB                      1.00 Hz
GB           0
PC                      1.40
```


2-(3-Ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
EtO
CO2H
8.678
7.720
7.717
7.714
7.701
7.697
7.694
7.620
7.616
7.614
7.610
7.464
7.444
7.425
7.097
7.095
7.090
7.088
7.076
7.074
7.070
4.149
4.131
4.114
4.096
2.500
1.388
1.370
1.353
7.8
7.6
7.4
7.2
ppm
1.02
0.99
1.03
1.00
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.98
1.02
0.99
1.03
1.00
2.26
2.96
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210927
Time 19.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(3-Ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
EtO
CO2H
164.53
161.28
158.83
155.45
152.20
148.28
131.04
130.18
118.88
117.09
111.85
102.01
63.22
40.15
39.94
39.73
39.52
39.31
39.10
38.89
14.64
220
200
180
160
140
120
100
80
60
40
20
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210928
Time 1.22
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6700
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128164 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(4-Ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

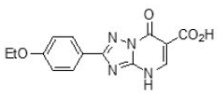
```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210928
Time                1.26
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH             6402.049 Hz
FIDRES          0.133165 Hz
AQ              3.7547357 sec
RG                   181
DW                78.100 usec
DE                15.52 usec
TE                298.0 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1328009 MHz
NUC1                  1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                 32768
SF            400.1300030 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

2-(4-Ethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

```
Current Data Parameters
NAME
EXPNO                  3
PROCNO                 1

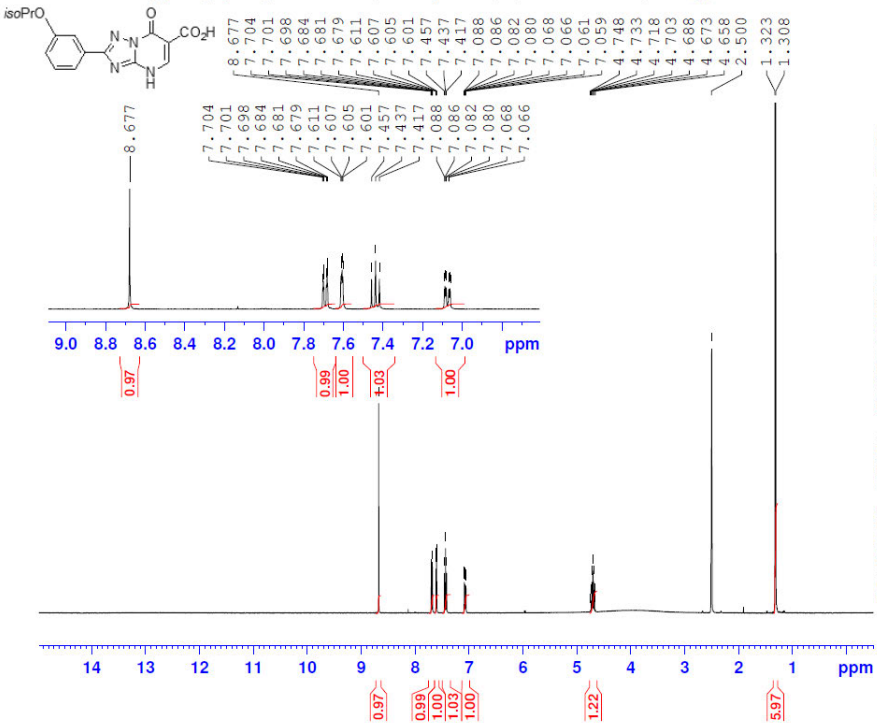
F2 - Acquisition Parameters
Date_           20210928
Time                7.44
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG            zgpg30
TD                 65536
SOLVENT             DMSO
NS                  6700
DS                     4
SWH            25252.525 Hz
FIDRES          0.385323 Hz
AQ              1.2976128 sec
RG                   203
DW                19.800 usec
DE                 9.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

F2 - Processing parameters
SI                 65536
SF            100.6128164 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

2-(3-isoPropyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

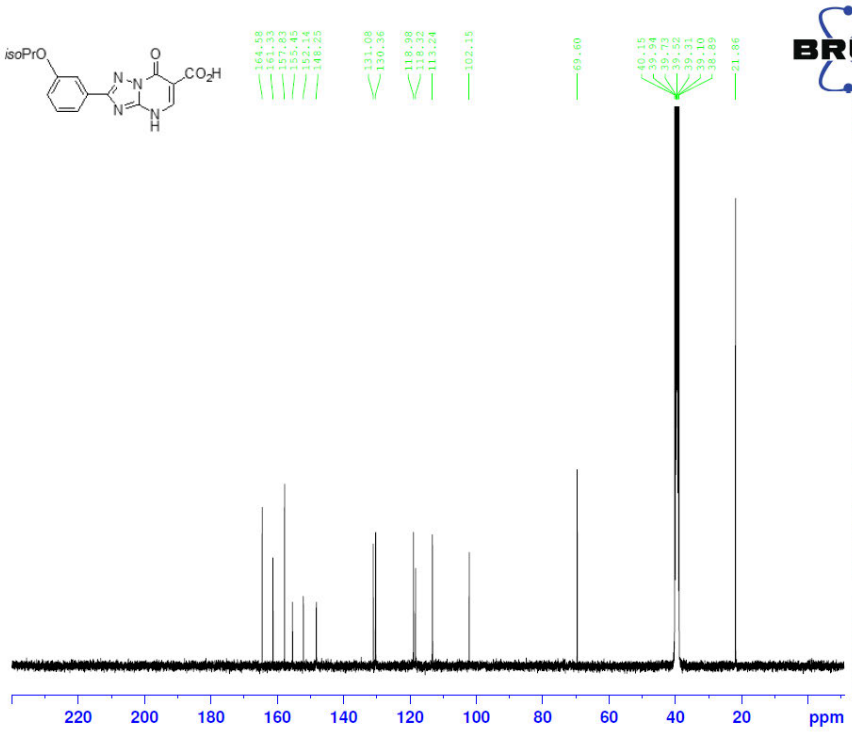
```
Current Data Parameters
NAME
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210602
Time              10.38
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES         0.133165 Hz
AQ             3.7547357 sec
RG                  203
DW               78.100 usec
DE               15.52 usec
TE               298.0 K
D1            5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1               1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300031 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
```

2-(3-isoPropyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

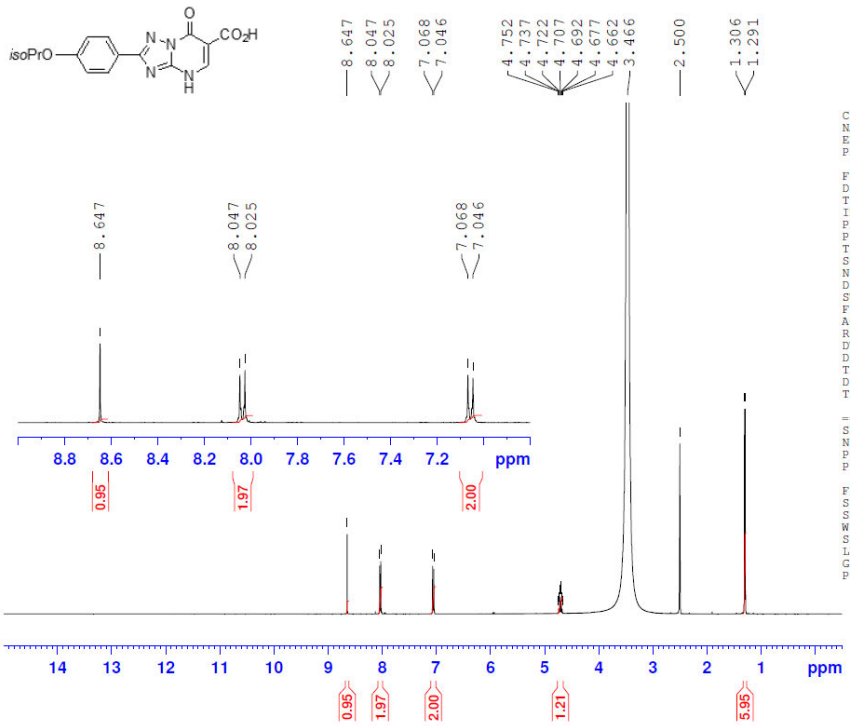
F2 - Acquisition Parameters
Date_           20210515
Time               8.03
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                13900
DS                    4
SWH           25252.525 Hz
FIDRES         0.385323 Hz
AQ             1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1               13C
P1                10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128091 MHz
WDW                EM
SSB       0
LB              1.00 Hz
GB        0
PC              1.40
```

2-(4-isoPropyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



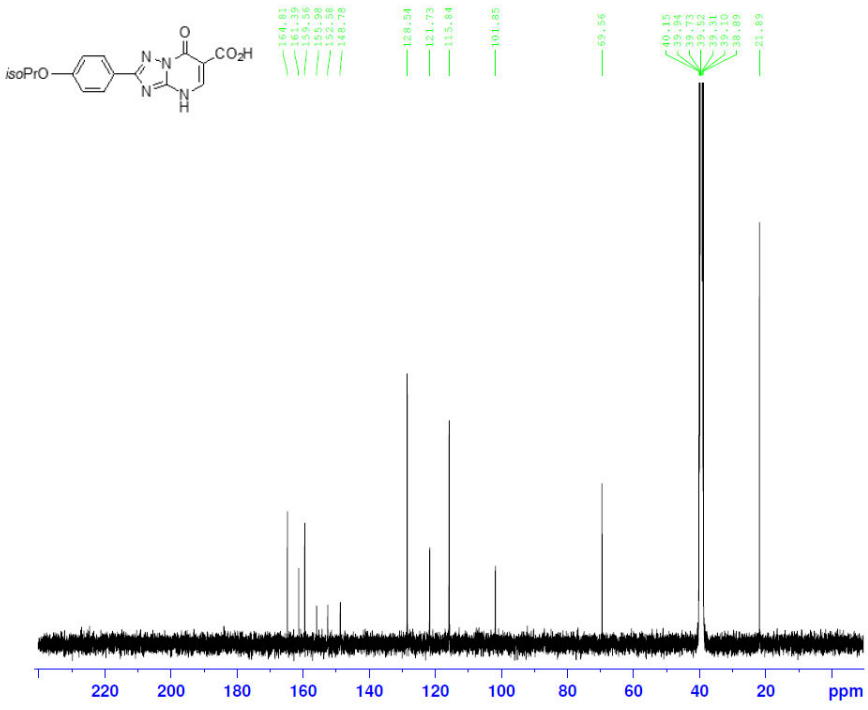
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210401
Time               14.51
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH             6402.049 Hz
FIDRES          0.133165 Hz
AQ              3.7547357 sec
RG                  45.2
DW                78.100 usec
DE                15.52 usec
TE                298.1 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1328009 MHz
NUC1                    1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                 32768
SF            400.1300032 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

2-(4-isoPropyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

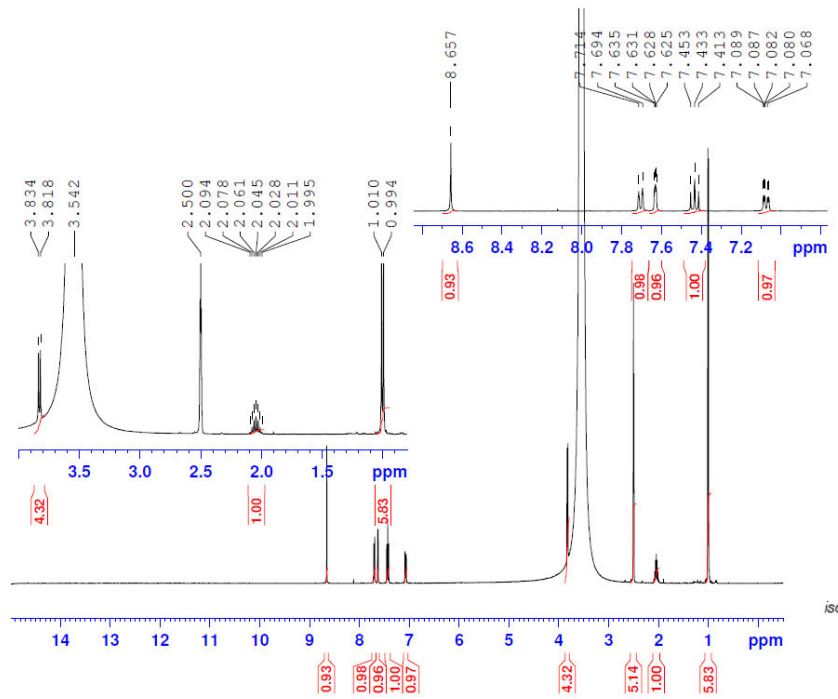
F2 - Acquisition Parameters
Date_           20210411
Time               8.28
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                 13900
DS                     4
SWH            25252.525 Hz
FIDRES          0.385323 Hz
AQ              1.2976128 sec
RG                   203
DW                19.800 usec
DE                 9.00 usec
TE                 296.7 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                    13C
P1                10.00 usec
PLW1          56.69400024 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                    1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

F2 - Processing parameters
SI                 65536
SF            100.6128041 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

2-(3-isoButyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid

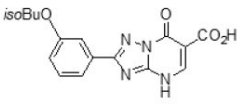


Current Data Parameters
NAME
EXPNO 1
PROCNO 1

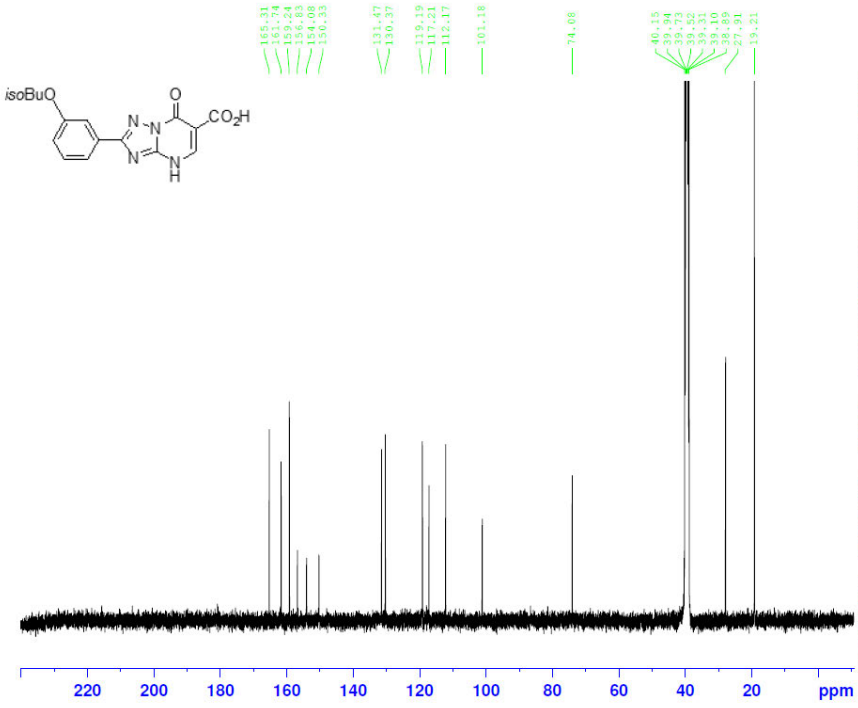
F2 - Acquisition Parameters
Date_ 20210324
Time 13.49
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 32
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



2-(3-isoButyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



Current Data Parameters
NAME
EXPNO 2
PROCNO 1

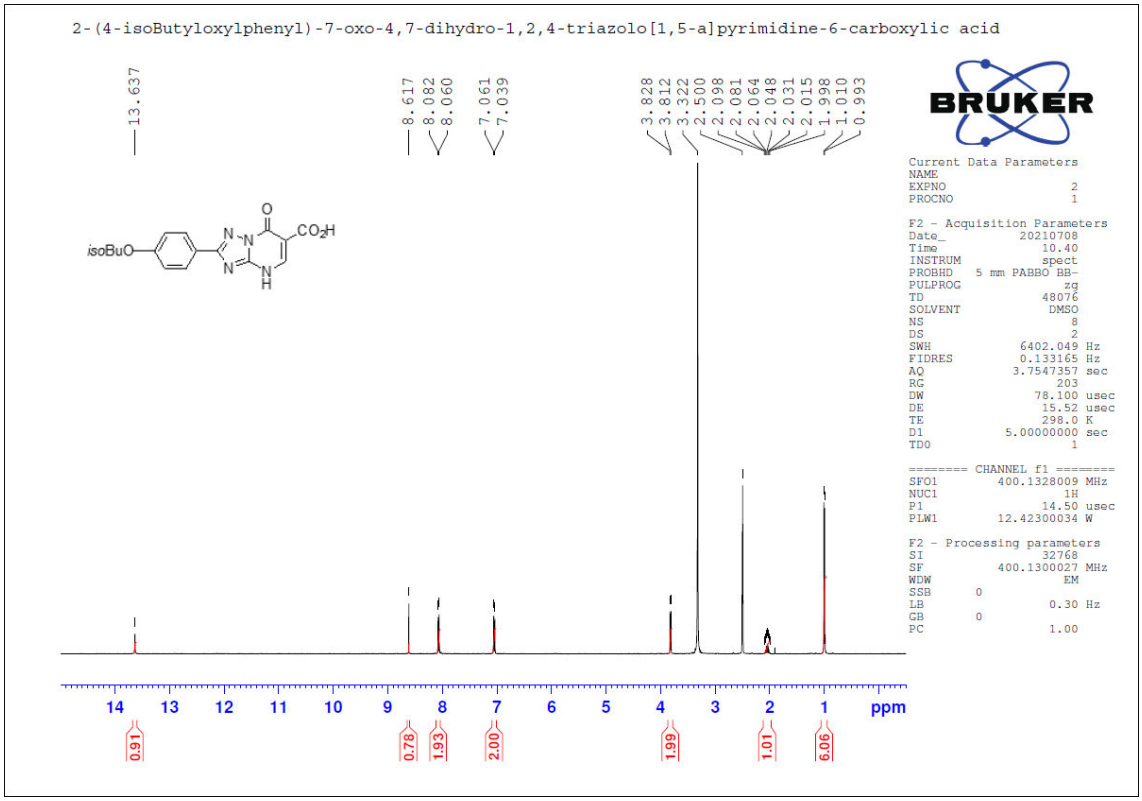
F2 - Acquisition Parameters
Date_ 20210328
Time 16.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 25700
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6127962 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(4-isoButyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



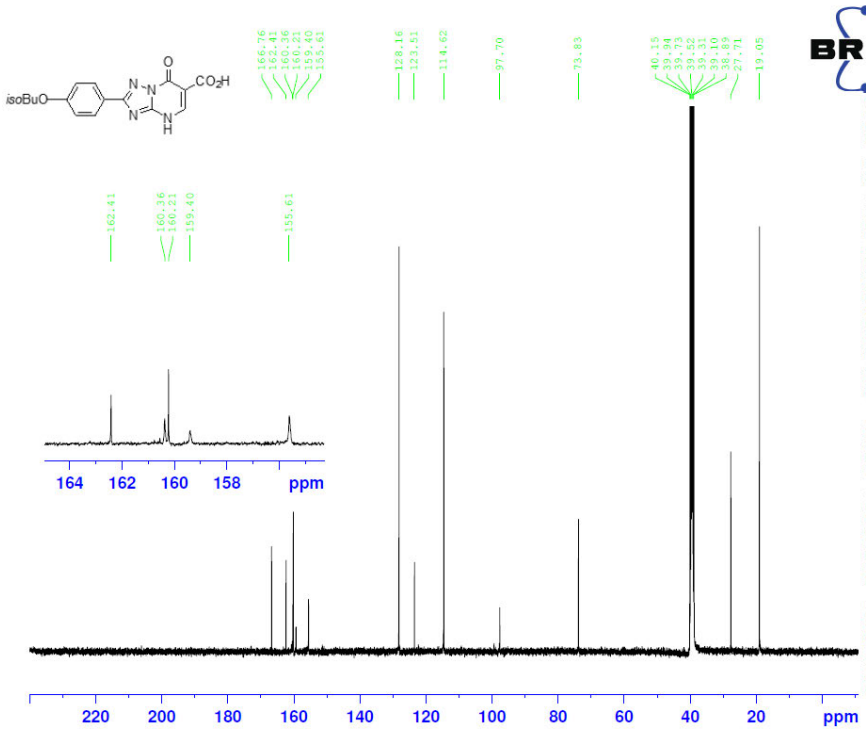
Current Data Parameters
NAME
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210708
Time 10.40
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300027 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(4-isoButyloxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



Current Data Parameters
NAME
EXPNO 4
PROCNO 1

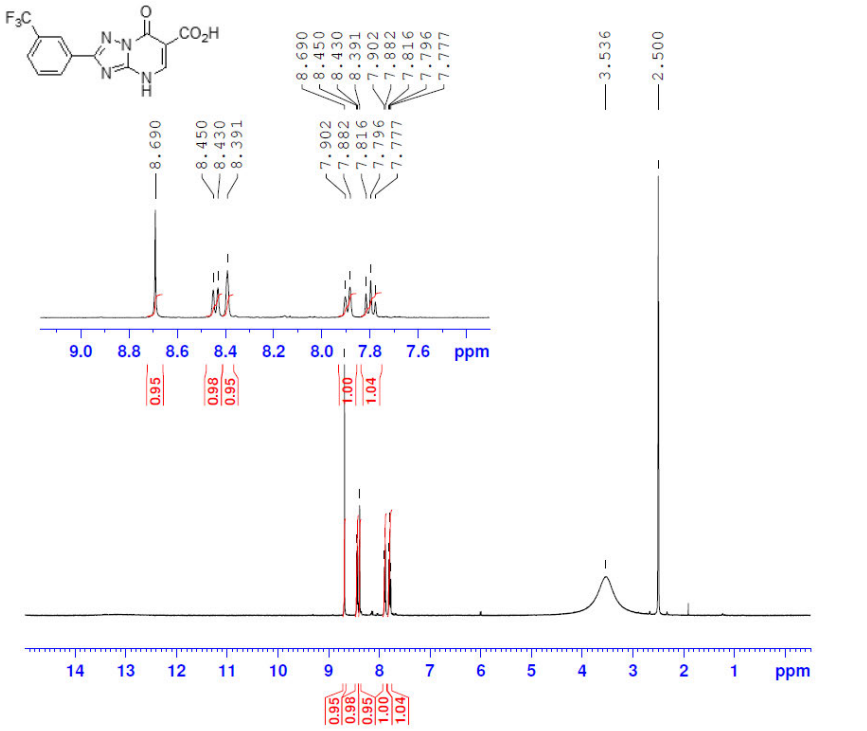
F2 - Acquisition Parameters
Date_ 20210821
Time 8.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128143 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(3-Trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



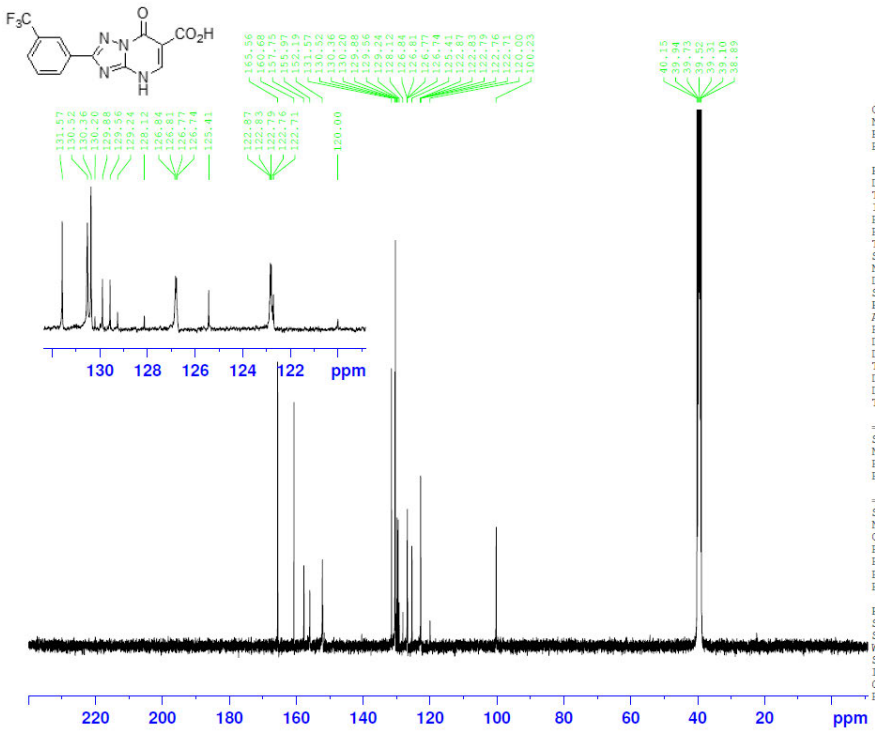
```
Current Data Parameters
NAME
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210621
Time               8.55
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES        0.133165 Hz
AQ            3.7547357 sec
RG                  203
DW               78.100 usec
DE               15.52 usec
TE               298.0 K
D1            5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                1H
P1               14.50 usec
PLW1        12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300030 MHz
WDW                  EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```

2-(3-Trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



```
Current Data Parameters
NAME
EXPNO                  4
PROCNO                 1

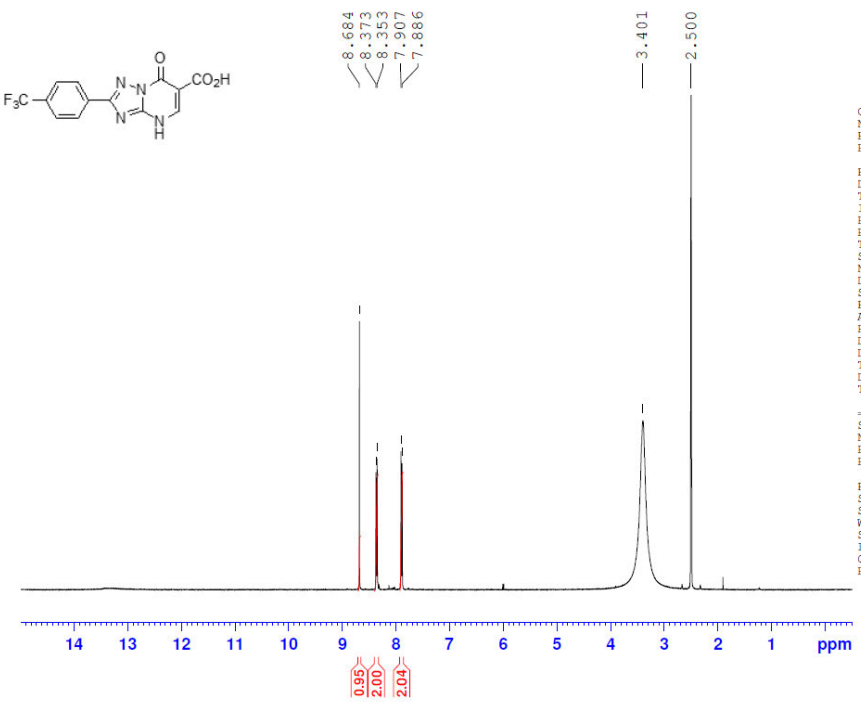
F2 - Acquisition Parameters
Date_           20211007
Time               8.02
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                13900
DS                    4
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                13C
P1               10.00 usec
PLW1        56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2            80.00 usec
PLW2        12.42300034 W
PLW12        0.40810999 W
PLW13        0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128115 MHz
WDW                  EM
SSB       0
LB               1.00 Hz
GB        0
PC               1.40
```

2-(4-Trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



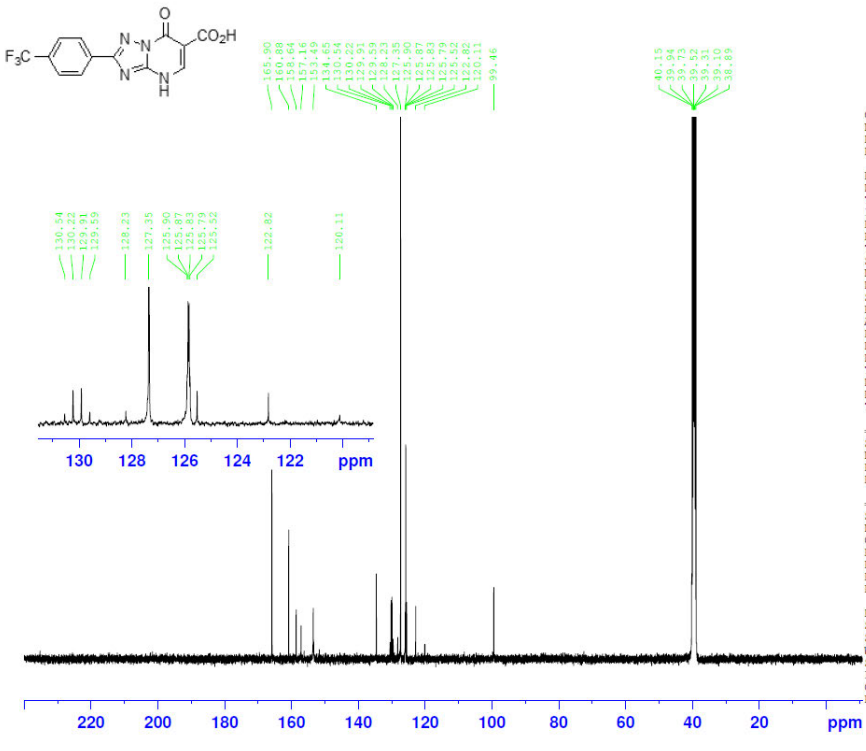
Current Data Parameters
NAME
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210624
Time 10.49
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(4-Trifluoromethylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



Current Data Parameters
NAME
EXPNO 5
PROCNO 1

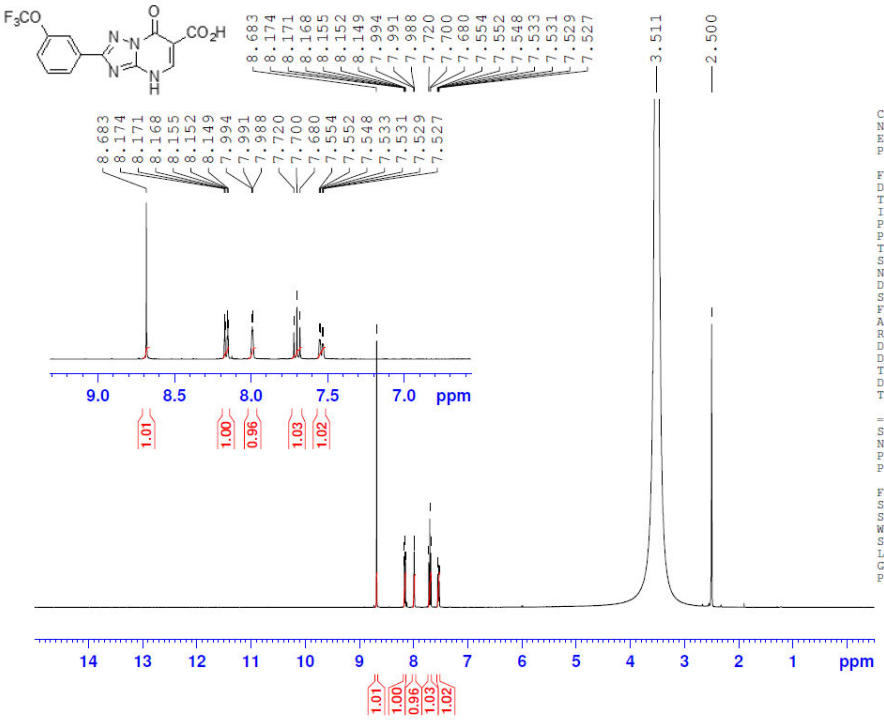
F2 - Acquisition Parameters
Date_ 20211003
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

F2 - Processing parameters
SI 65536
SF 100.6128132 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(3-Trifluoromethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

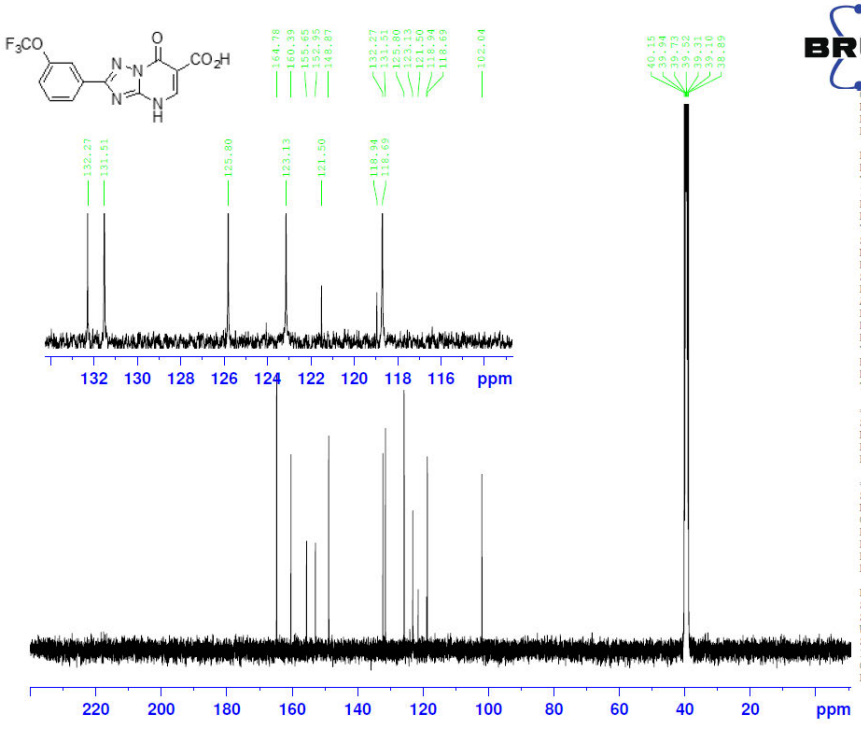
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210413
Time              10.01
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES        0.133165 Hz
AQ            3.7547357 sec
RG                 45.2
DW               78.100 usec
DE               15.52 usec
TE                295.2 K
D1            5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                 1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300030 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2-(3-Trifluoromethoxylphenyl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

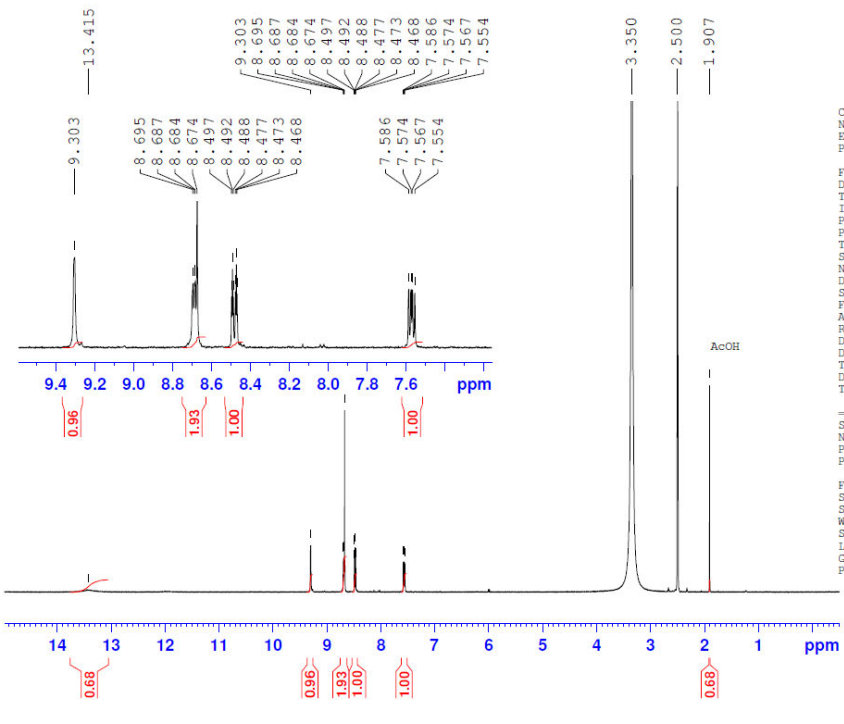
F2 - Acquisition Parameters
Date_           20210523
Time              1.30
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                65536
SOLVENT            DMSO
NS                 6900
DS                    4
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

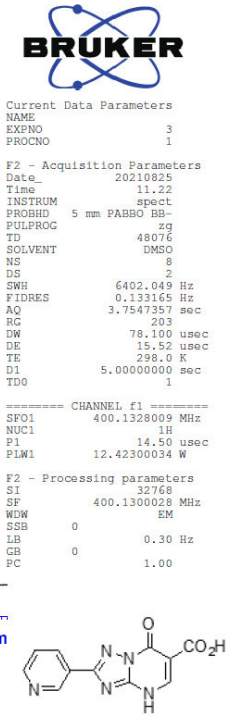
======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                 13C
P1                10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128029 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2-(Pyridin-3-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid





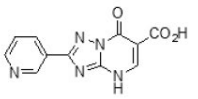
Current Data Parameters
NAME
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210825
Time 11.22
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300028 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(Pyridin-3-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid



Current Data Parameters
NAME
EXPNO 4
PROCNO 1

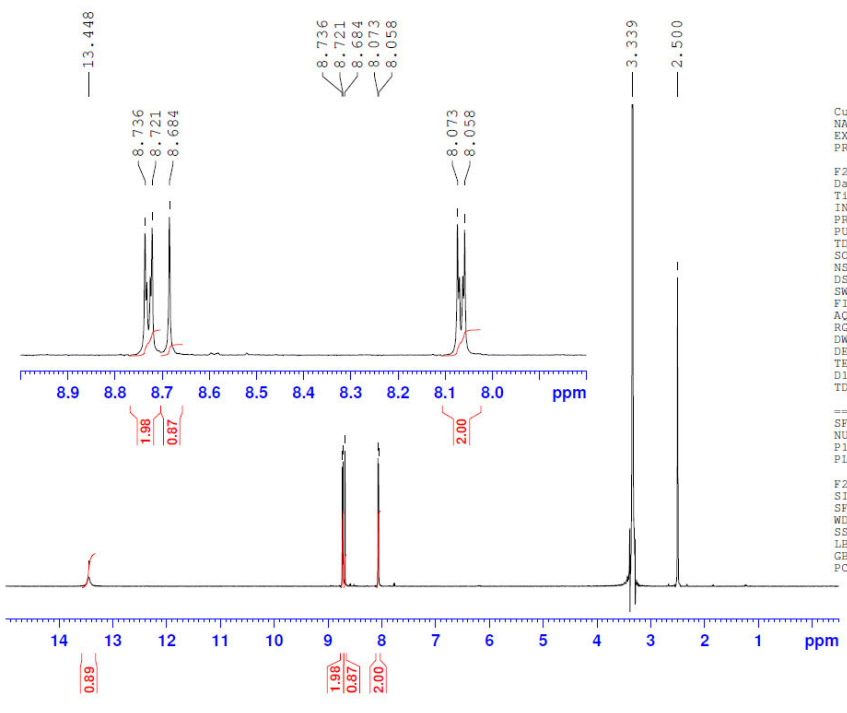
F2 - Acquisition Parameters
Date_ 20211114
Time 19.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 14336
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 14

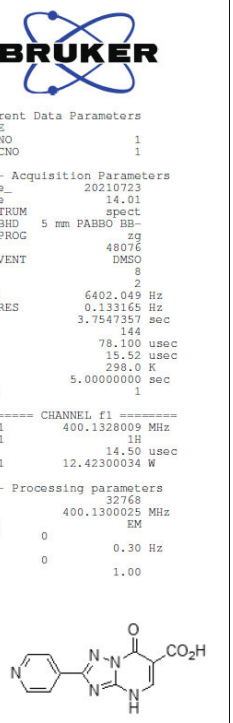
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

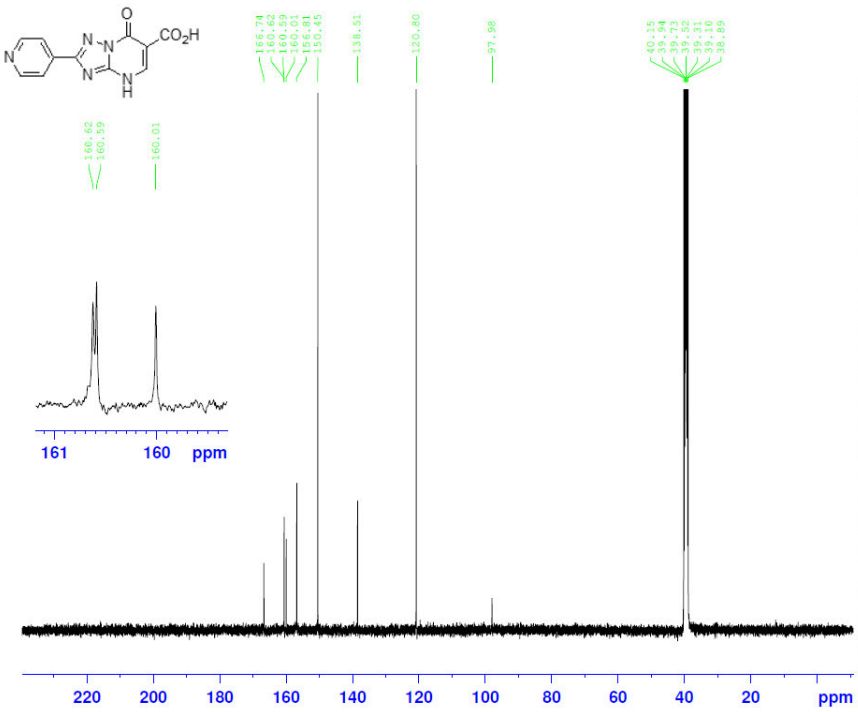
F2 - Processing parameters
SI 65536
SF 100.6132911 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

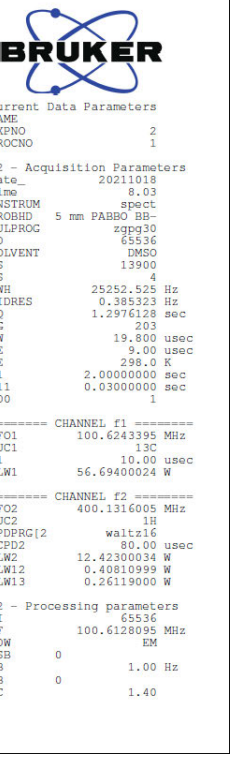
2-(Pyridin-4-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid

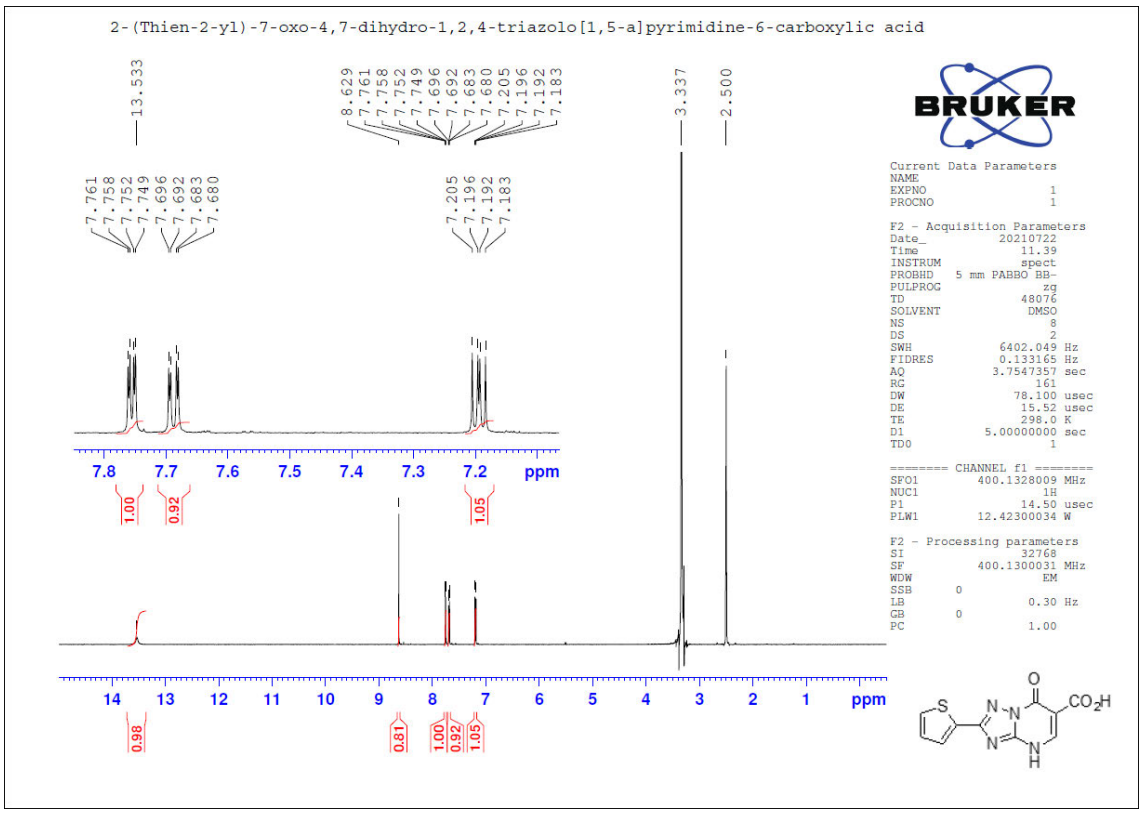


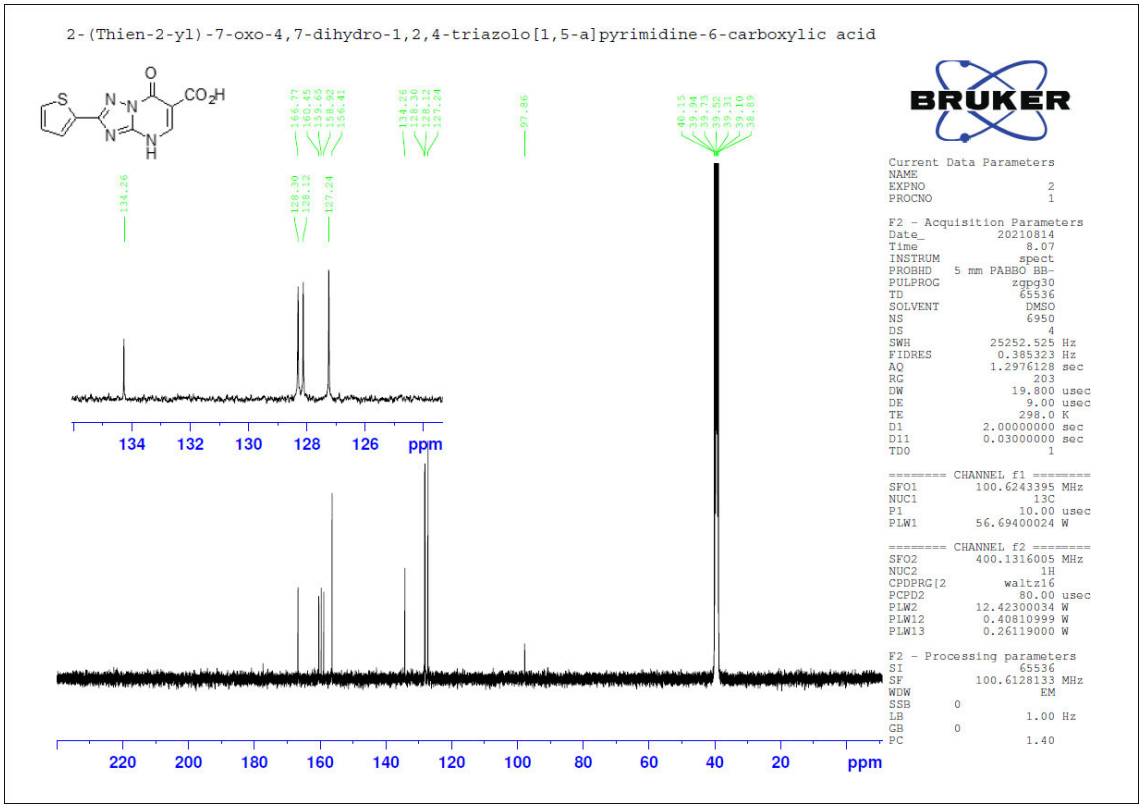


2-(Pyridin-4-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid

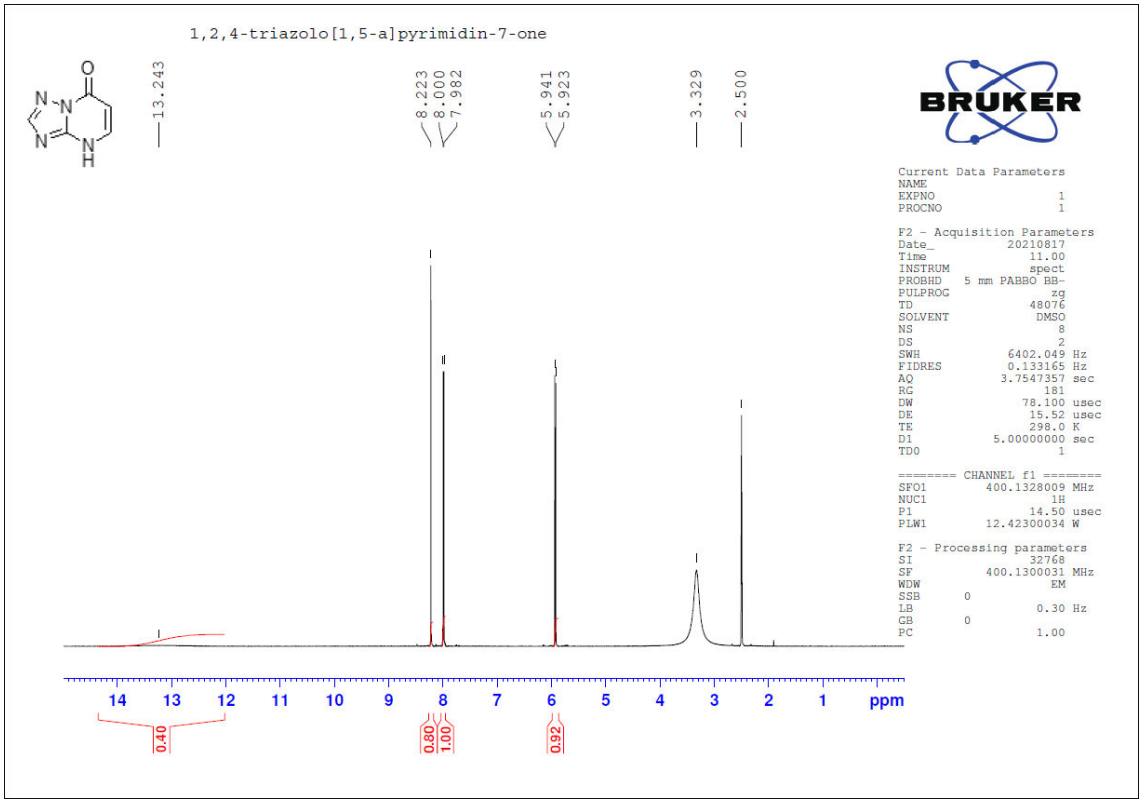


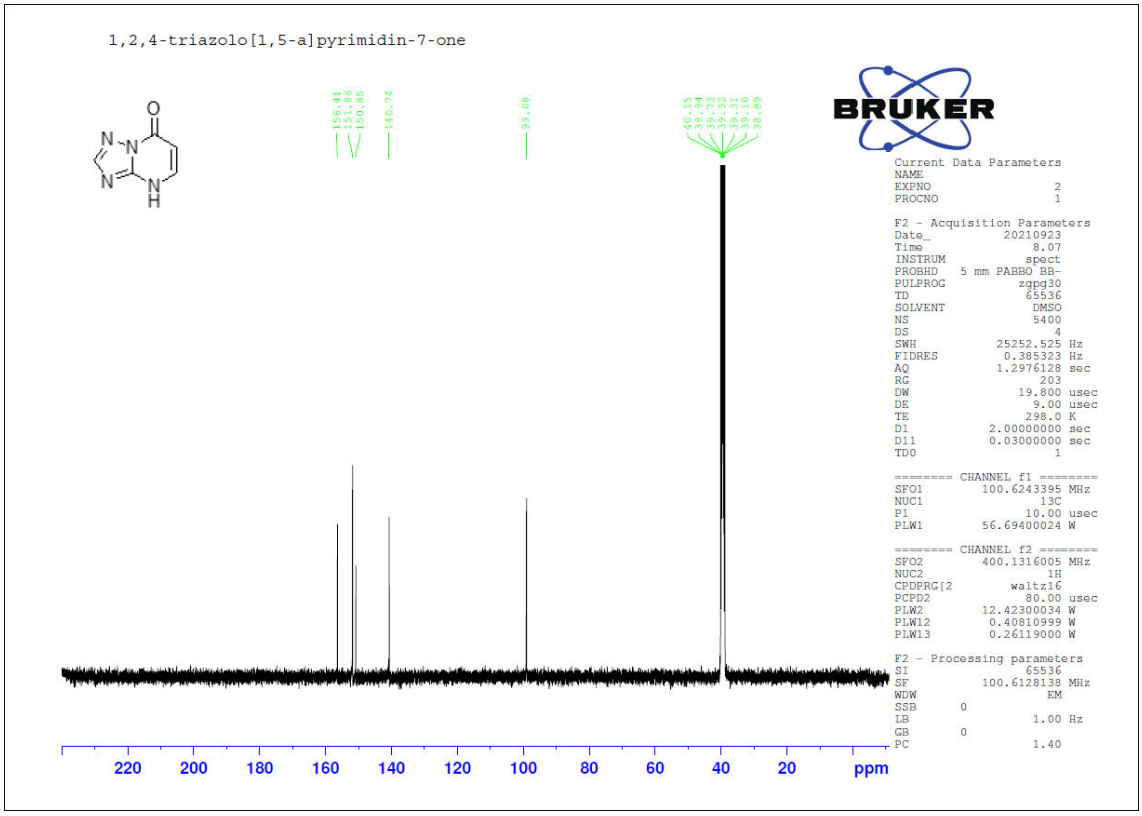



2-(Thien-2-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
13.533
8.629
7.761
7.758
7.752
7.749
7.696
7.692
7.683
7.680
7.205
7.196
7.192
7.183
3.337
2.500
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210722
Time 11.39
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
CO2H

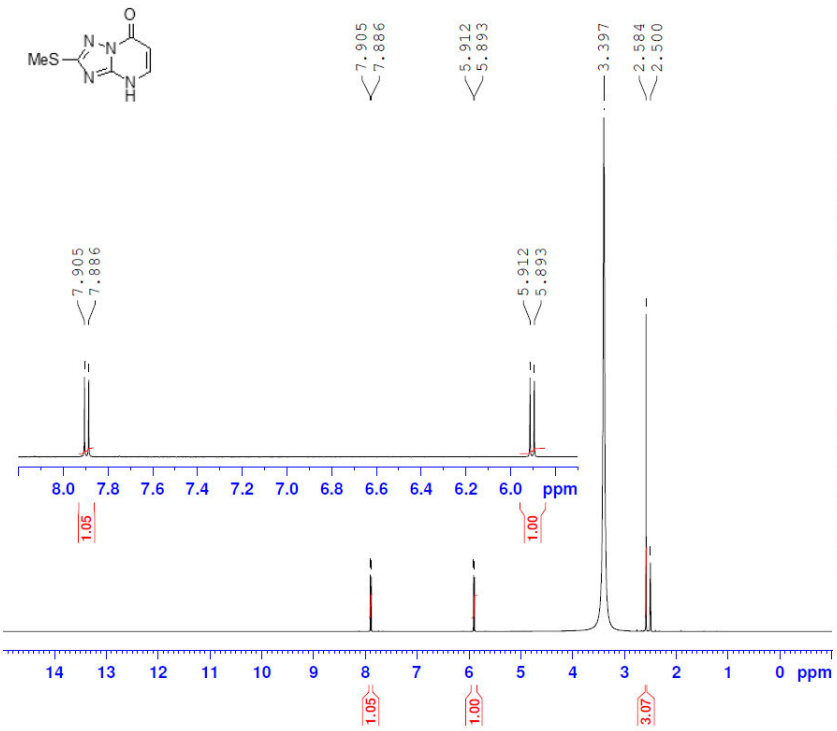

2-(Thien-2-yl)-7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic acid
166.77
160.45
148.62
138.92
134.26
128.30
128.12
127.24
97.86
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210814
Time 8.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128133 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm
CO2H

Appendix 4


1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.243
8.223
8.000
7.982
5.941
5.923
3.329
2.500
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210817
Time 11.00
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.40
0.80
1.00
0.92


1,2,4-triazolo[1,5-a]pyrimidin-7-one
156.41
151.86
150.85
140.74
99.08
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210923
Time 8.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 5400
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128138 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

2-Methylthio-1,2,4-triazolo[1,5-a]pyrimidin-7-one



BRUKER

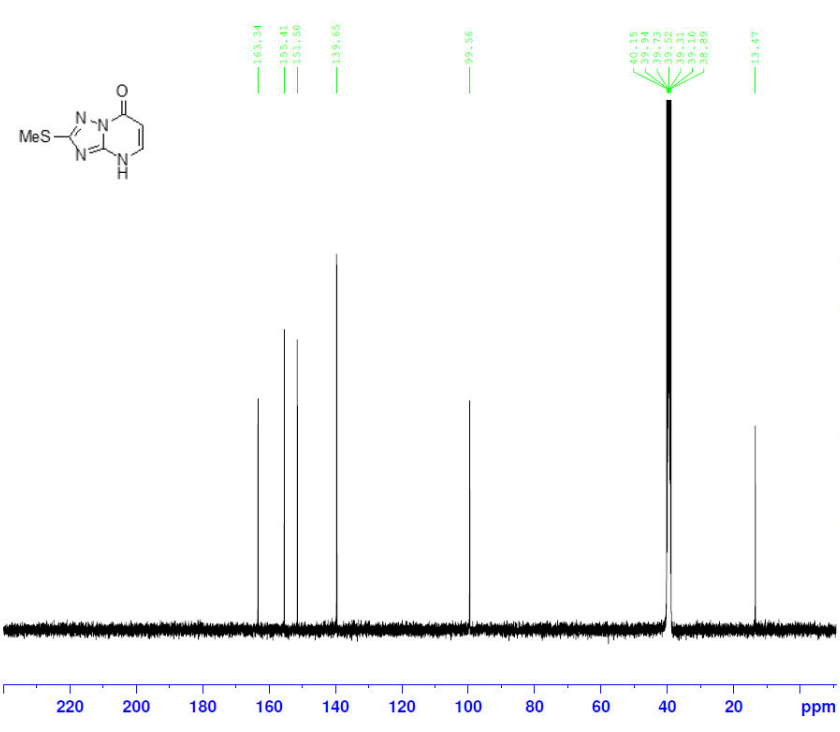
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210517
Time               9.56
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES         0.133165 Hz
AQ             3.7547357 sec
RG                 71.8
DW               78.100 usec
DE               15.52 usec
TE                298.0 K
D1             5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                 1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300031 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2-Methylthio-1,2,4-triazolo[1,5-a]pyrimidin-7-one



BRUKER

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

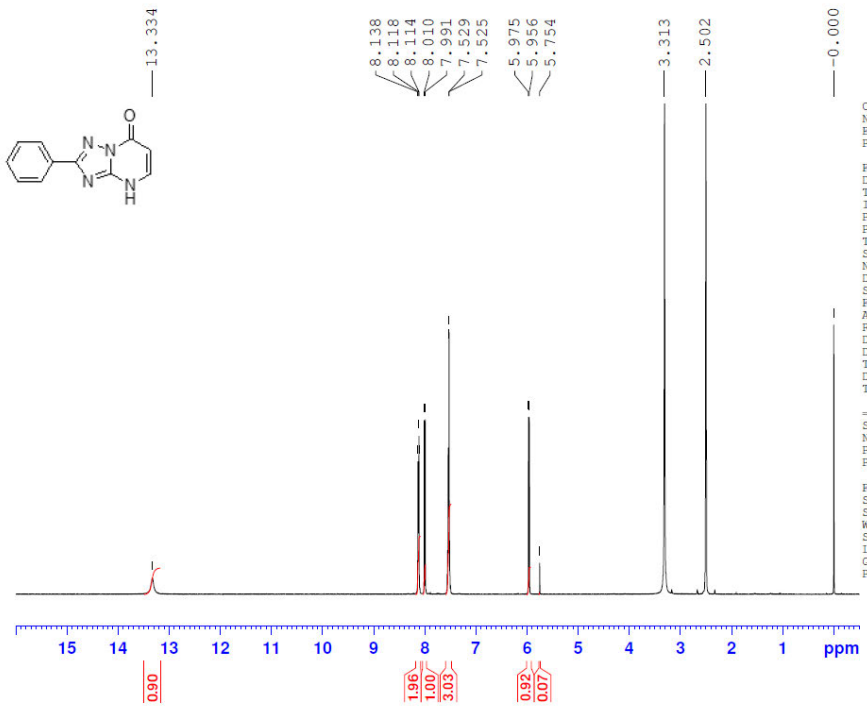
F2 - Acquisition Parameters
Date_           20210522
Time               8.01
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                 6900
DS                    4
SWH           25252.525 Hz
FIDRES         0.385323 Hz
AQ             1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                 13C
P1                10.00 usec
PLW1         56.69400024 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

F2 - Processing parameters
SI                65536
SF           100.6128088 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2-Phenyl-1,2,4-triazolo[1,5-a]pyrimidin-7-one



BRUKER

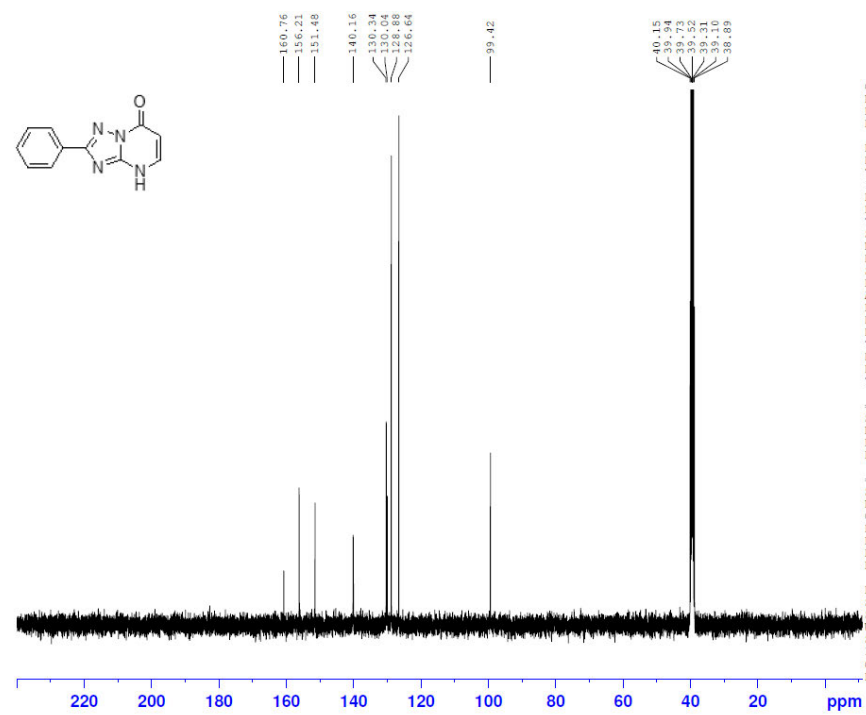
```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150512
Time               11.28
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG               zg
TD                 48076
SOLVENT             DMSO
NS                     8
DS                     2
SWH             7194.245 Hz
FIDRES          0.149643 Hz
AQ              3.3412819 sec
RG                   161
DW                69.500 usec
DE                12.64 usec
TE                299.9 K
D1             5.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1330010 MHz
NUC1                  1H
P1                 14.50 usec
PLW1          12.42300034 W

F2 - Processing parameters
SI                 32768
SF            400.1300019 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

2-Phenyl-1,2,4-triazolo[1,5-a]pyrimidin-7-one



BRUKER

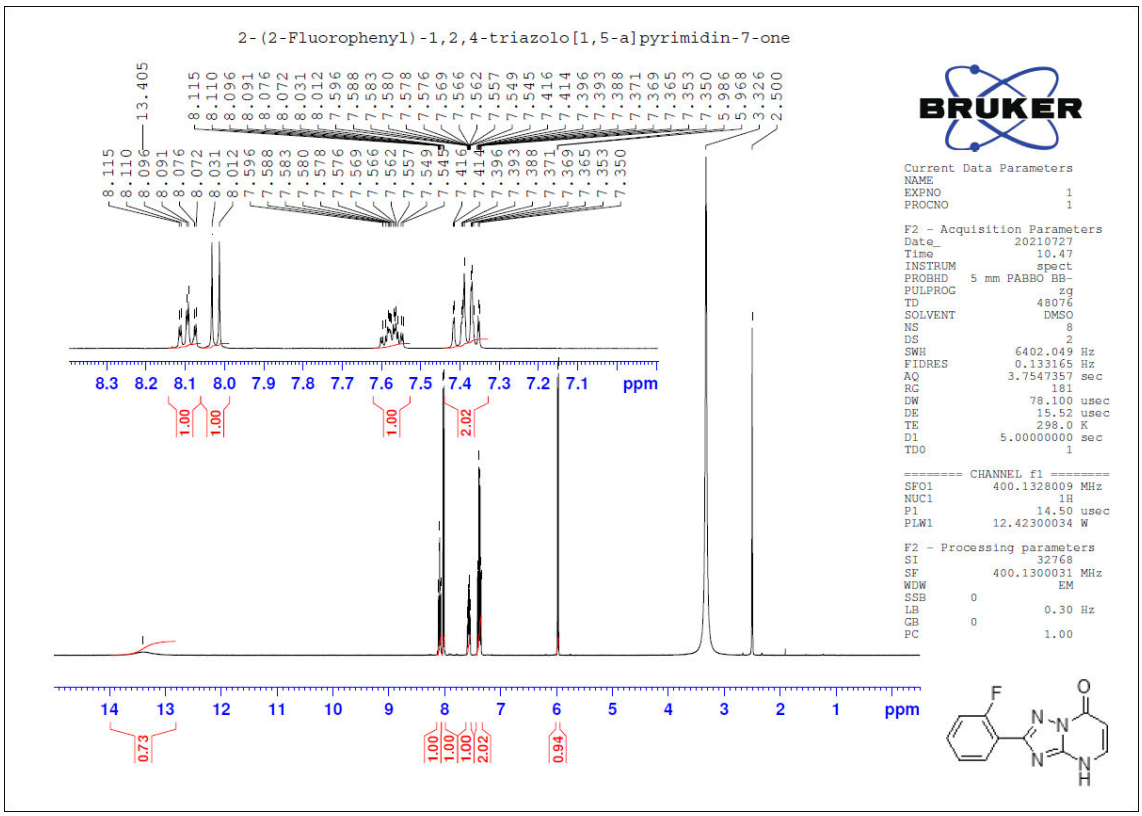
```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

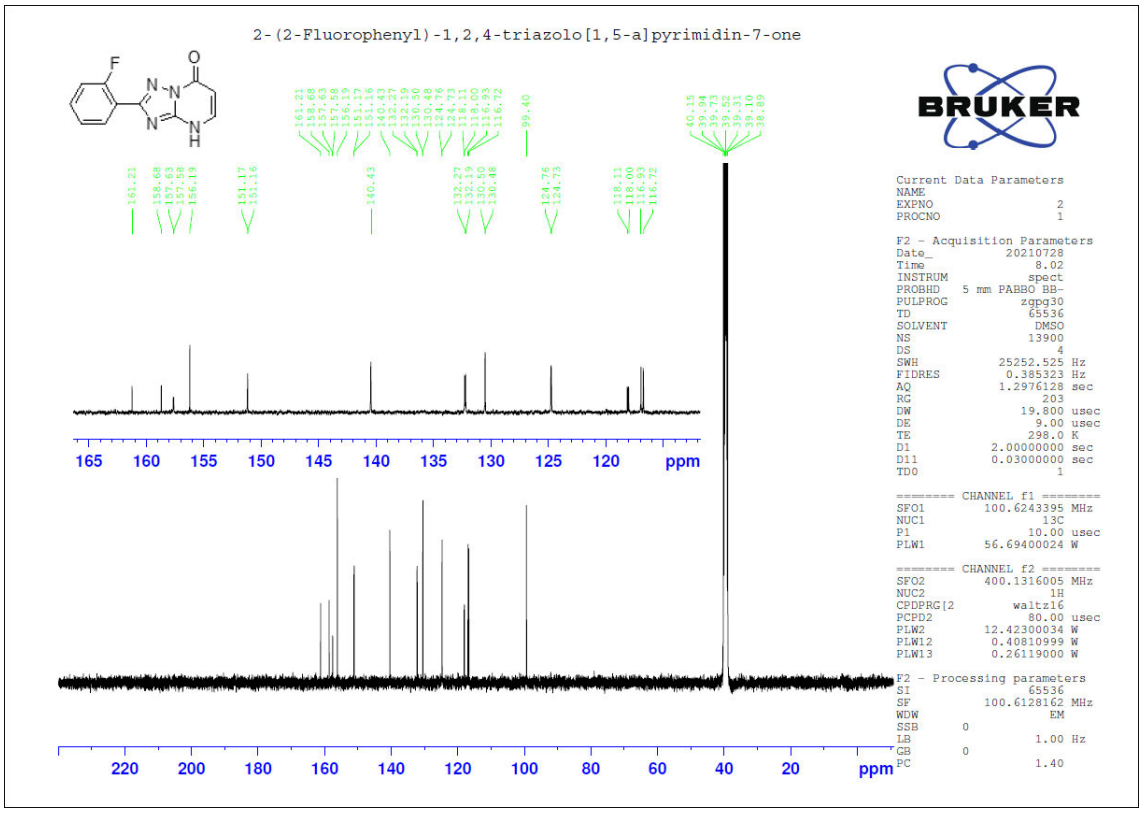
F2 - Acquisition Parameters
Date_           20150514
Time               11.03
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                   256
DS                     4
SWH            25252.525 Hz
FIDRES          0.385323 Hz
AQ              1.2976128 sec
RG                   203
DW                19.800 usec
DE                 9.00 usec
TE                299.9 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

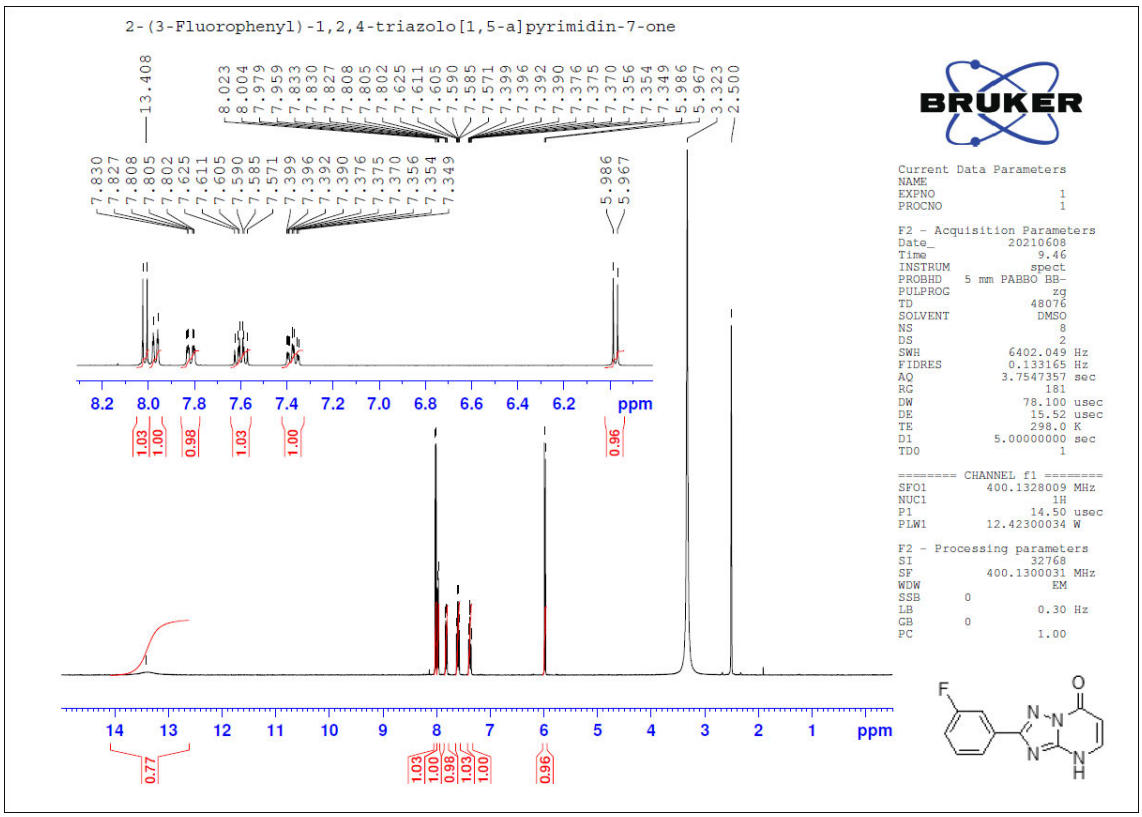
======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                10.00 usec
PLW1          56.69400024 W

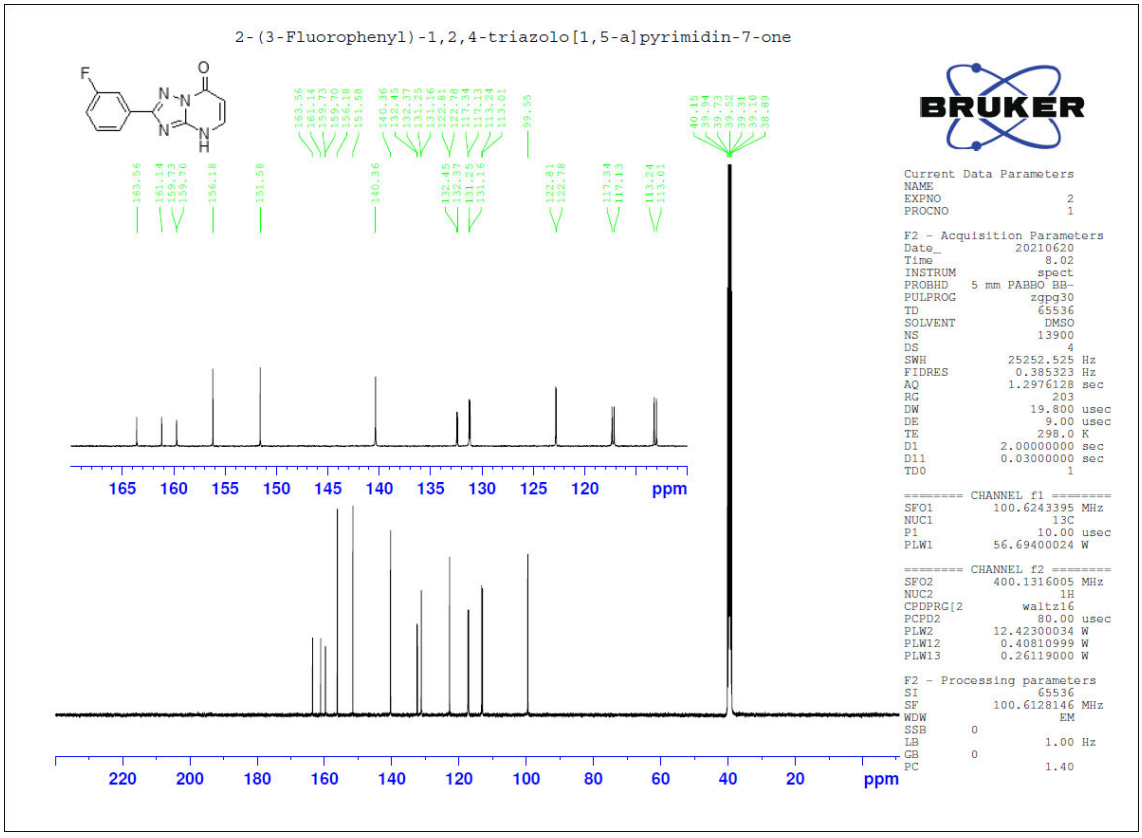
======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          12.42300034 W
PLW12          0.40810999 W
PLW13          0.26119000 W

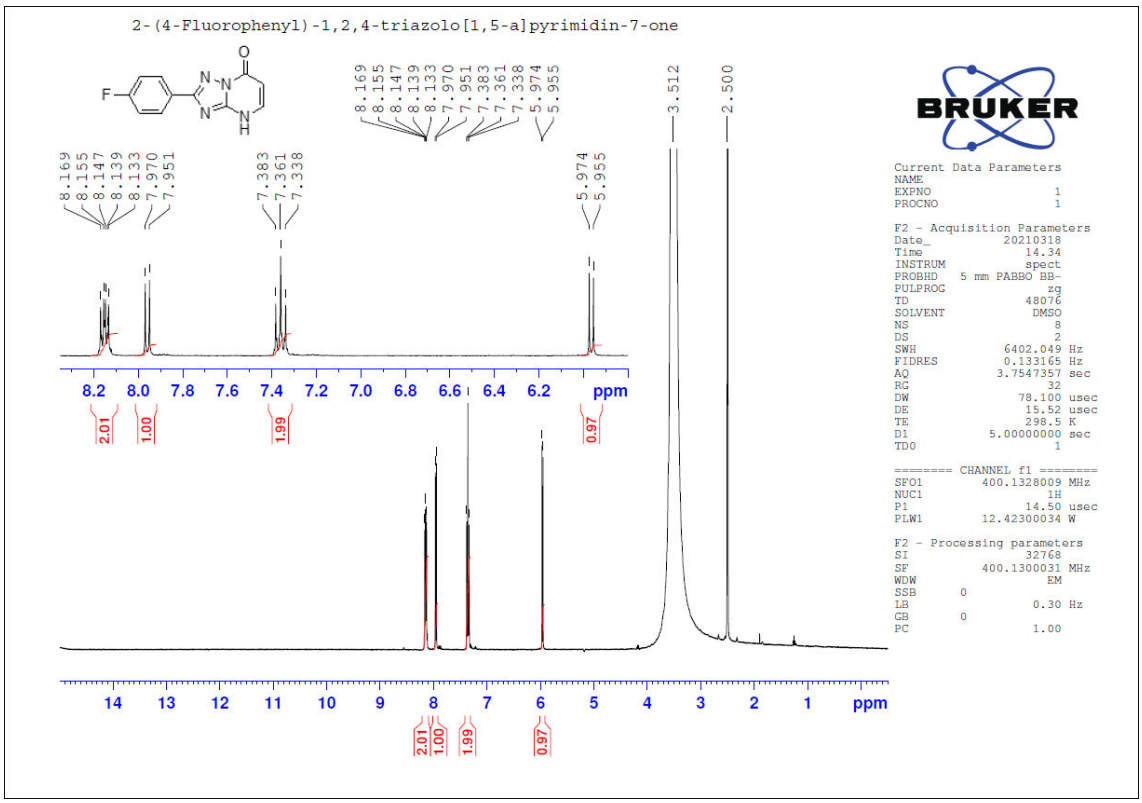
F2 - Processing parameters
SI                 65536
SF            100.6128175 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

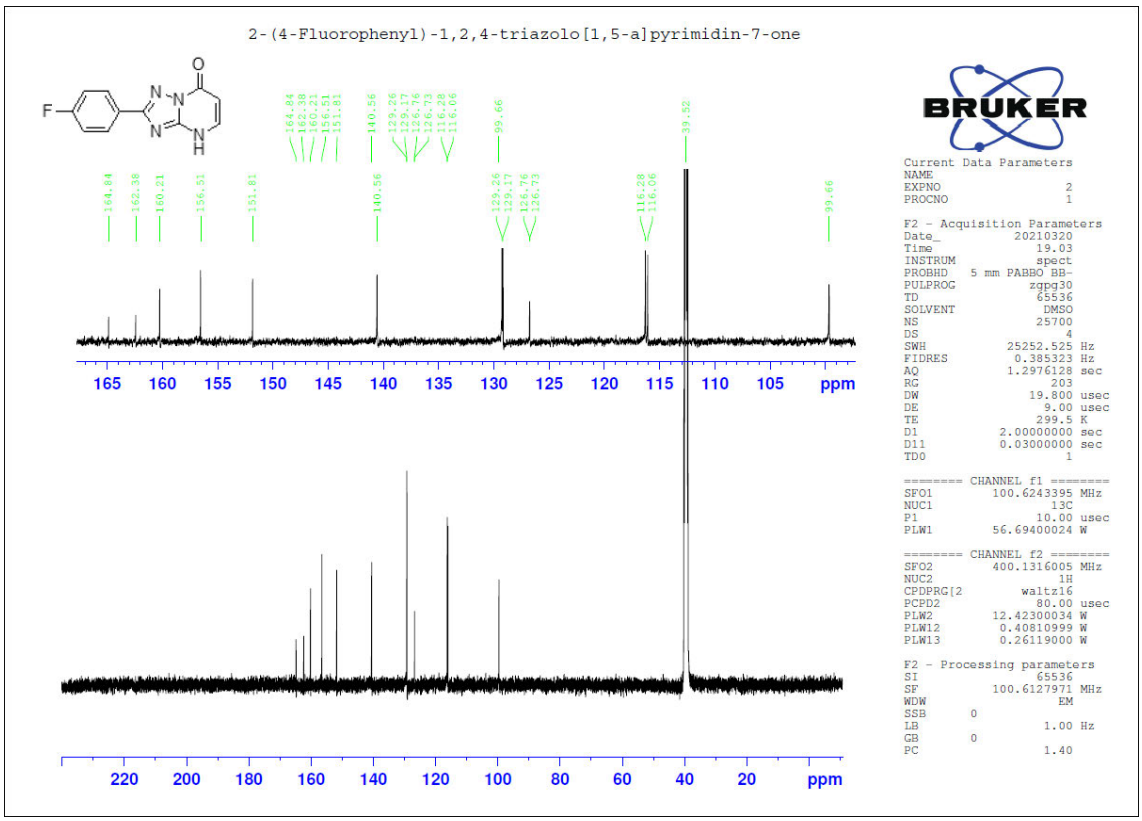

2-(2-Fluorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210727
Time 10.47
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

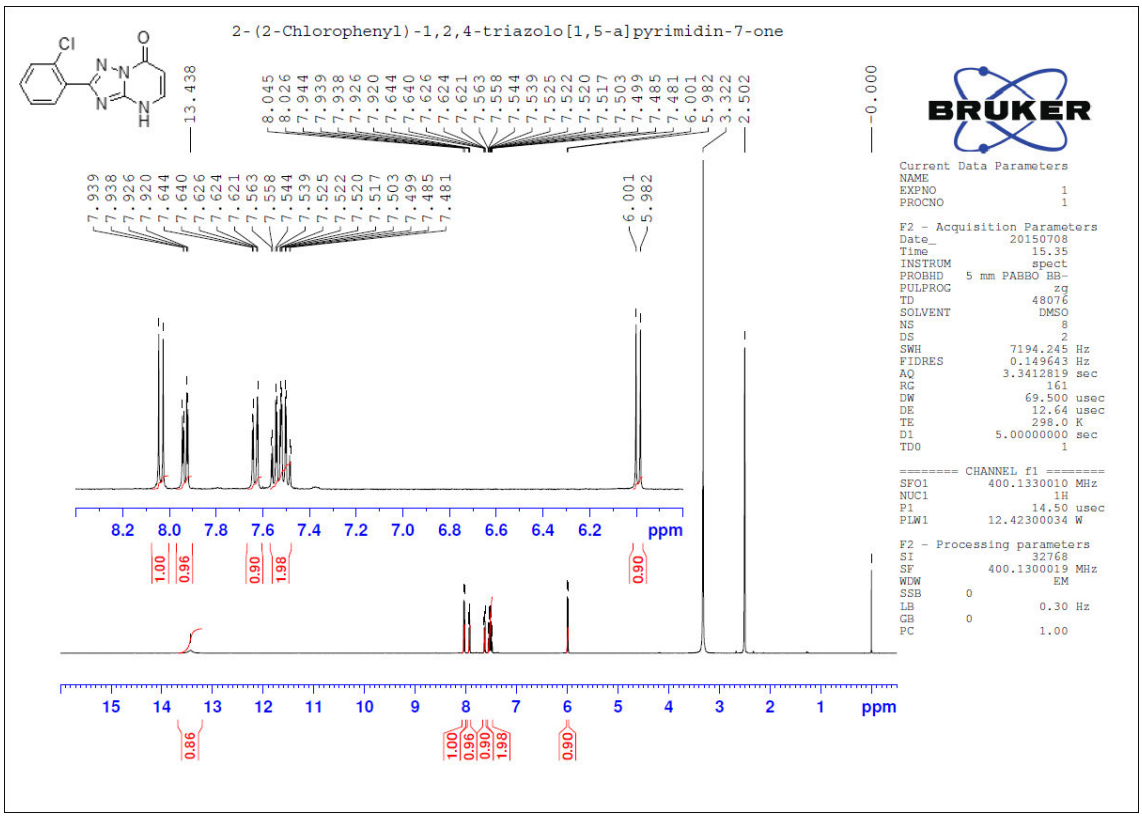

2-(2-Fluorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210728
Time 8.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128162 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm

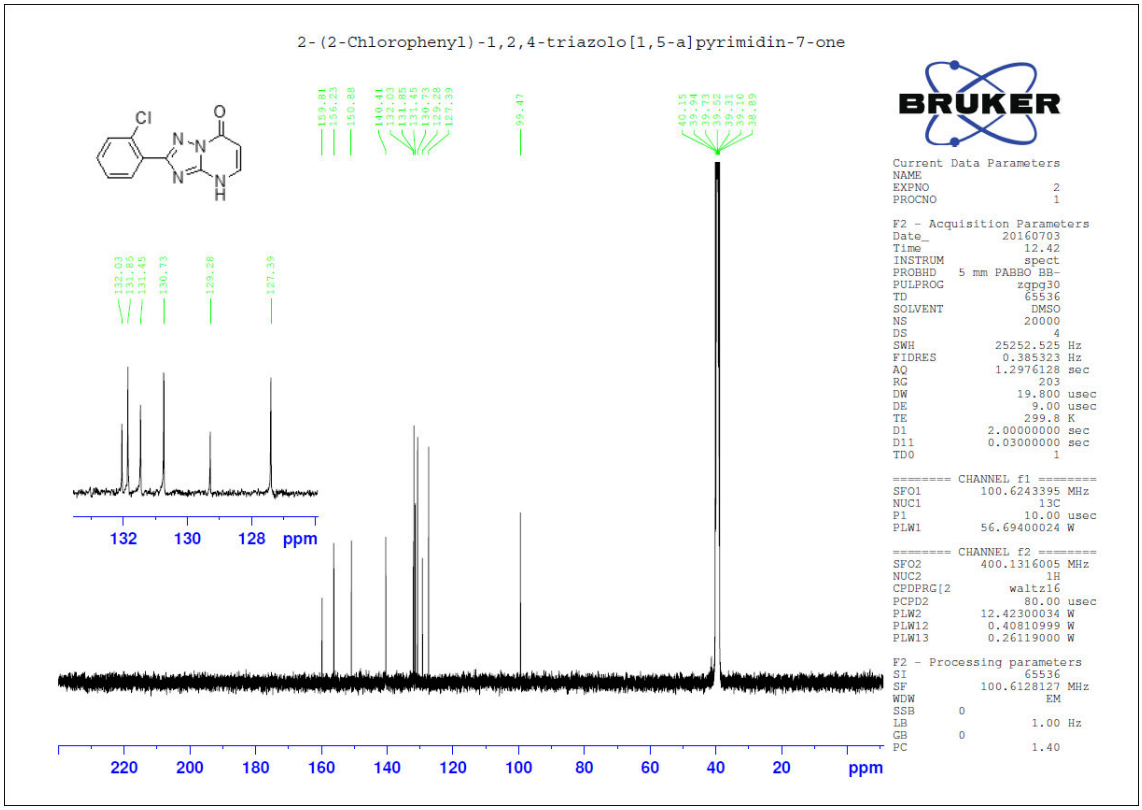

2-(3-Fluorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210608
Time 9.46
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

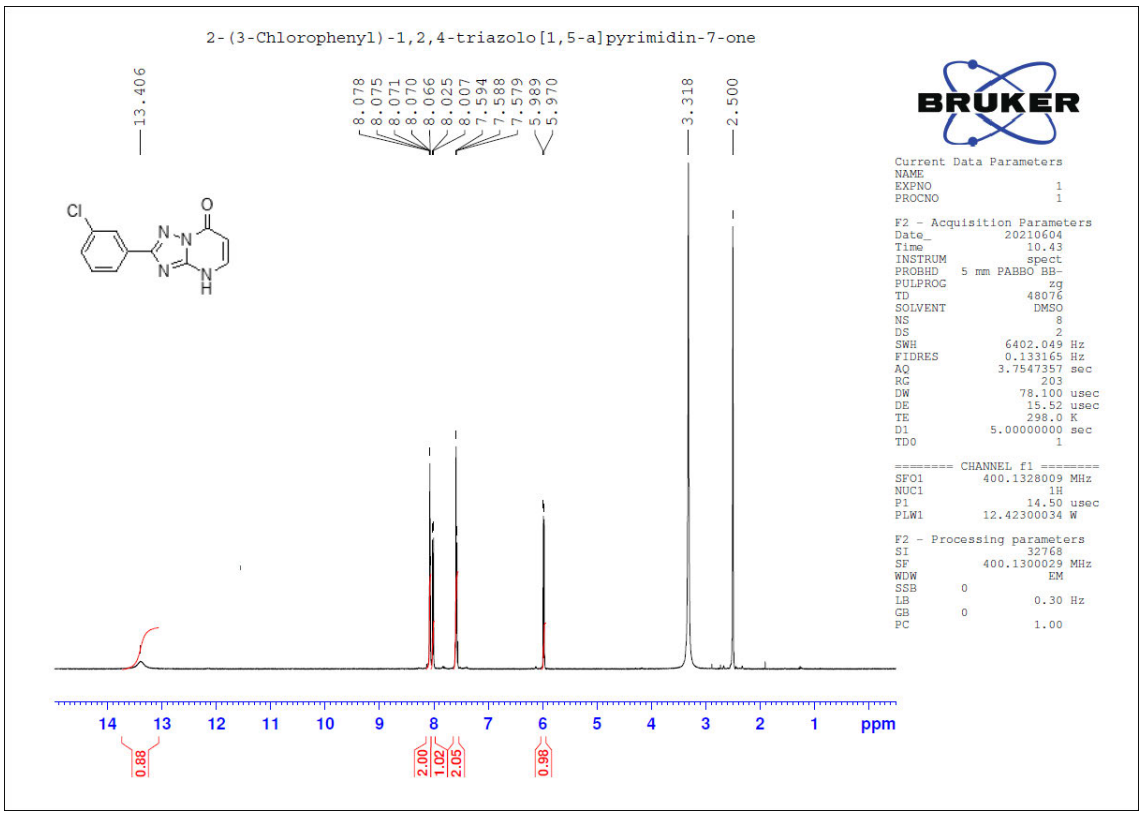

2-(3-Fluorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210620
Time 8.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128146 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm

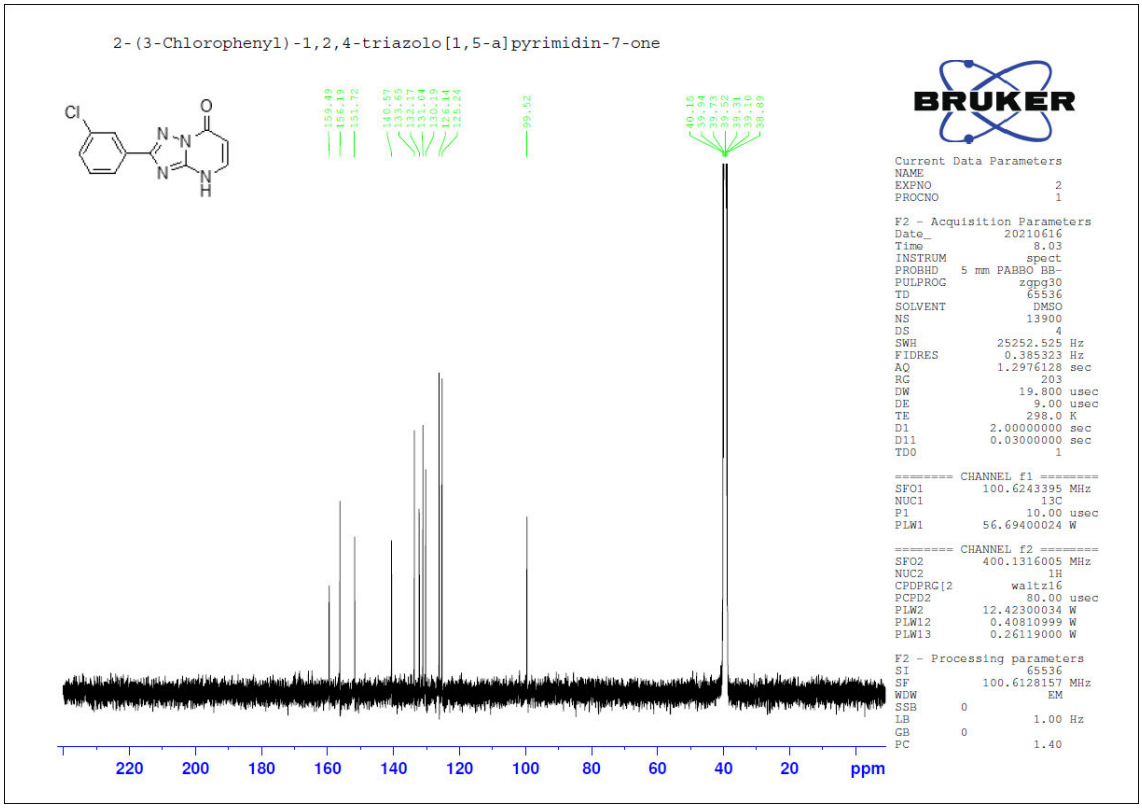

2-(4-Fluorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
8.169
8.155
8.147
8.139
8.133
7.970
7.951
7.383
7.361
7.338
5.974
5.955
3.512
2.500
ppm
2.01
1.00
1.99
0.97
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210318
Time 14.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 32
DW 78.100 usec
DE 15.52 usec
TE 298.5 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(4-Fluorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
164.84
162.38
160.21
156.51
151.81
140.56
129.26
129.17
126.76
126.73
116.28
116.06
99.66
39.52
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210320
Time 19.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 25700
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.5 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6127971 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

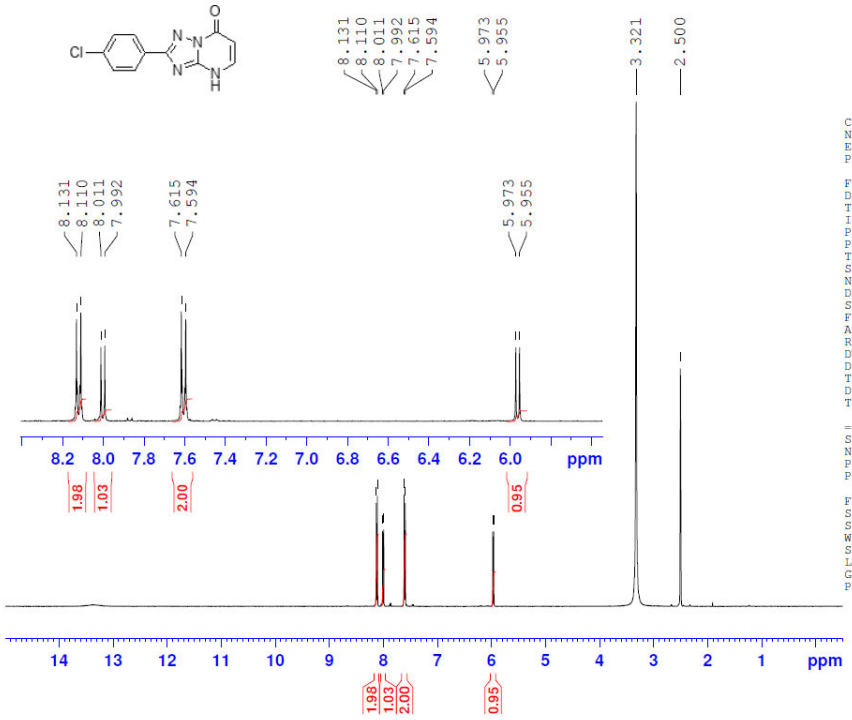

2-(2-Chlorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
Cl
O
N
N
N
H
13.438
8.045
8.026
7.944
7.939
7.938
7.926
7.920
7.644
7.640
7.626
7.624
7.621
7.563
7.558
7.544
7.539
7.525
7.522
7.520
7.517
7.503
7.499
7.485
7.481
6.001
5.982
3.322
2.502
-0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150708
Time 15.35
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 7194.245 Hz
FIDRES 0.149643 Hz
AQ 3.3412819 sec
RG 161
DW 69.500 usec
DE 12.64 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300019 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 ppm
1.00 0.96 0.90 1.98 0.90
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.86 1.00 0.96 0.90 1.98 0.90


2-(2-Chlorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
Cl
O
N
N
N
H
159.81
156.23
150.88
140.41
132.03
131.85
131.45
130.73
129.28
127.39
99.47
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160703
Time 12.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 20000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.8 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128127 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
132 130 128 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm


2-(3-Chlorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.406
8.078
8.075
8.071
8.070
8.066
8.025
8.007
7.594
7.588
7.579
5.989
5.970
3.318
2.500
Cl
O
N
N
N
H
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.88
2.00
1.02
2.05
0.90
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210604
Time 10.43
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300029 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(3-Chlorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
159.49
156.19
151.72
140.57
133.65
132.17
131.04
130.19
126.14
125.24
99.52
40.15
39.94
39.73
39.52
39.31
39.10
38.89
Cl
O
N
N
N
H
220
200
180
160
140
120
100
80
60
40
20
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210616
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128157 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(4-Chlorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one



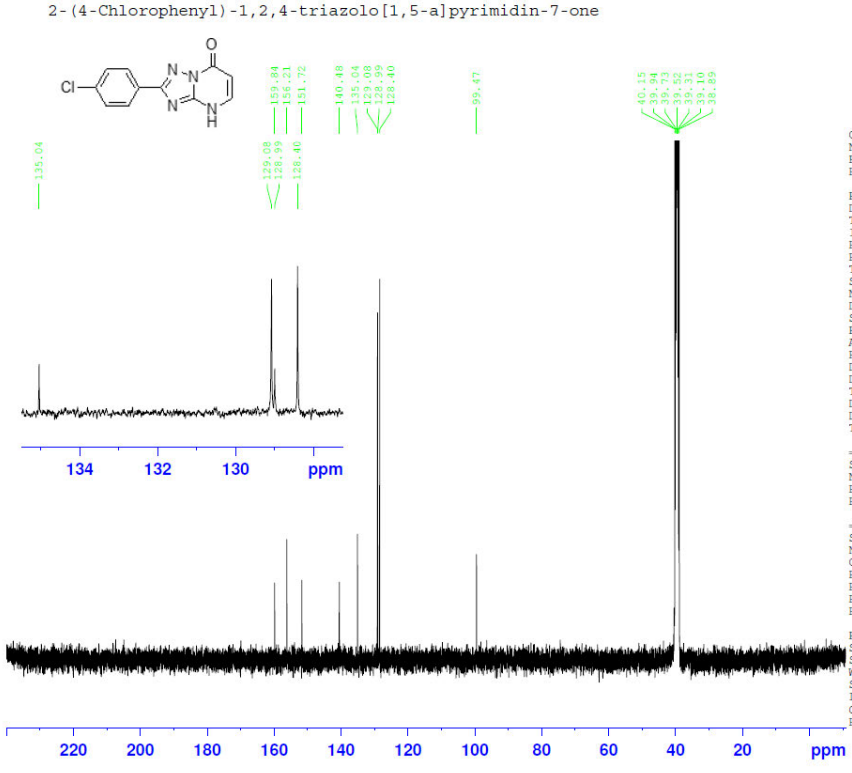
Current Data Parameters
NAME
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210706
Time 10.20
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W

F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(4-Chlorophenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one



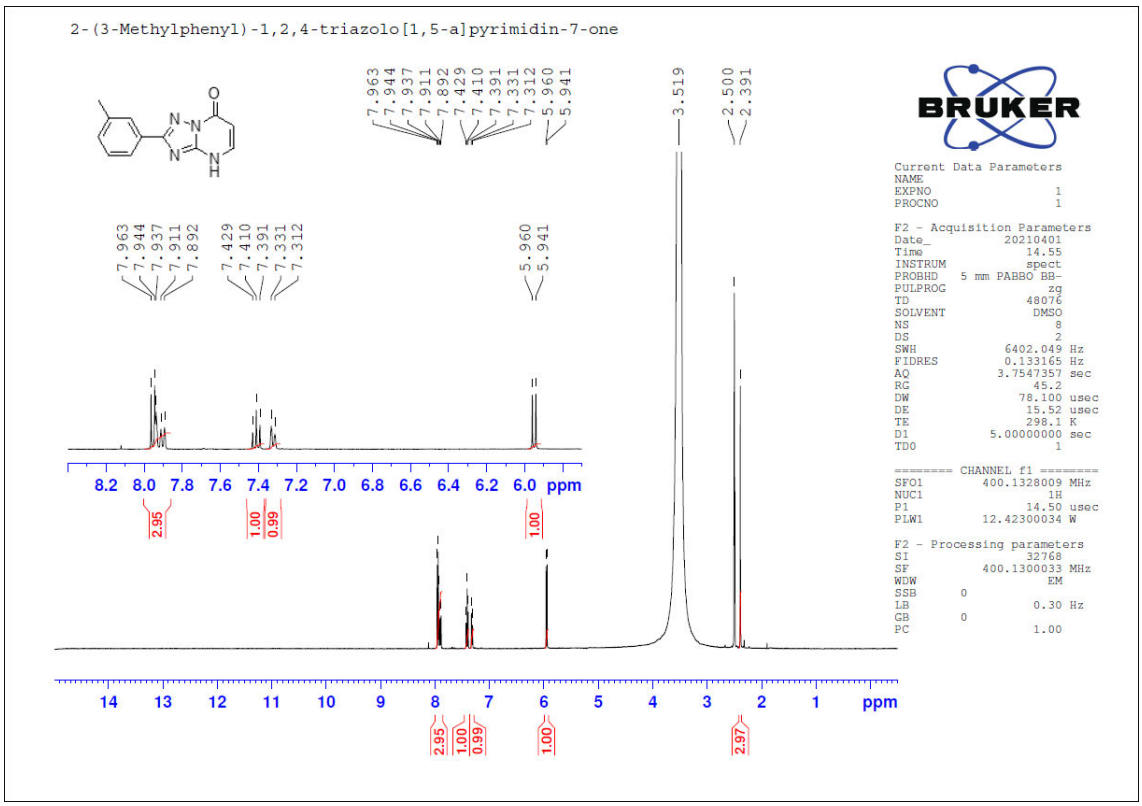
Current Data Parameters
NAME
EXPNO 2
PROCNO 1

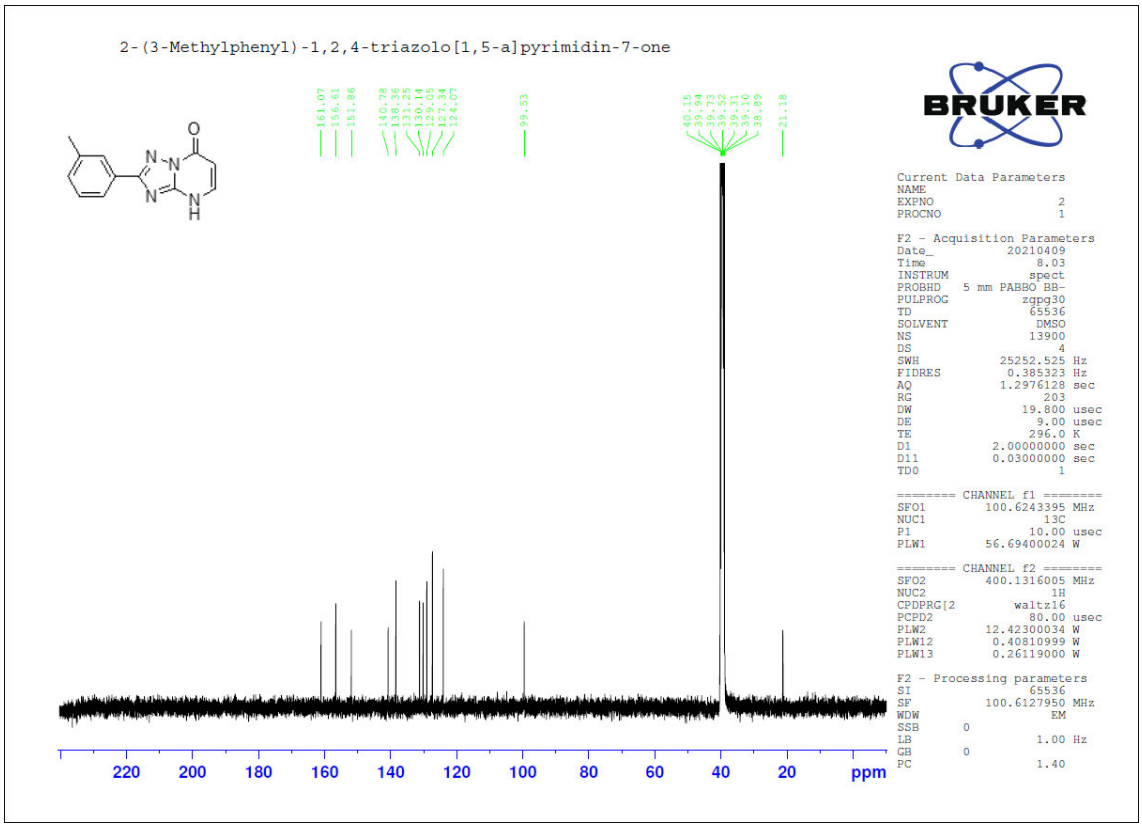
F2 - Acquisition Parameters
Date_ 20210724
Time 8.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

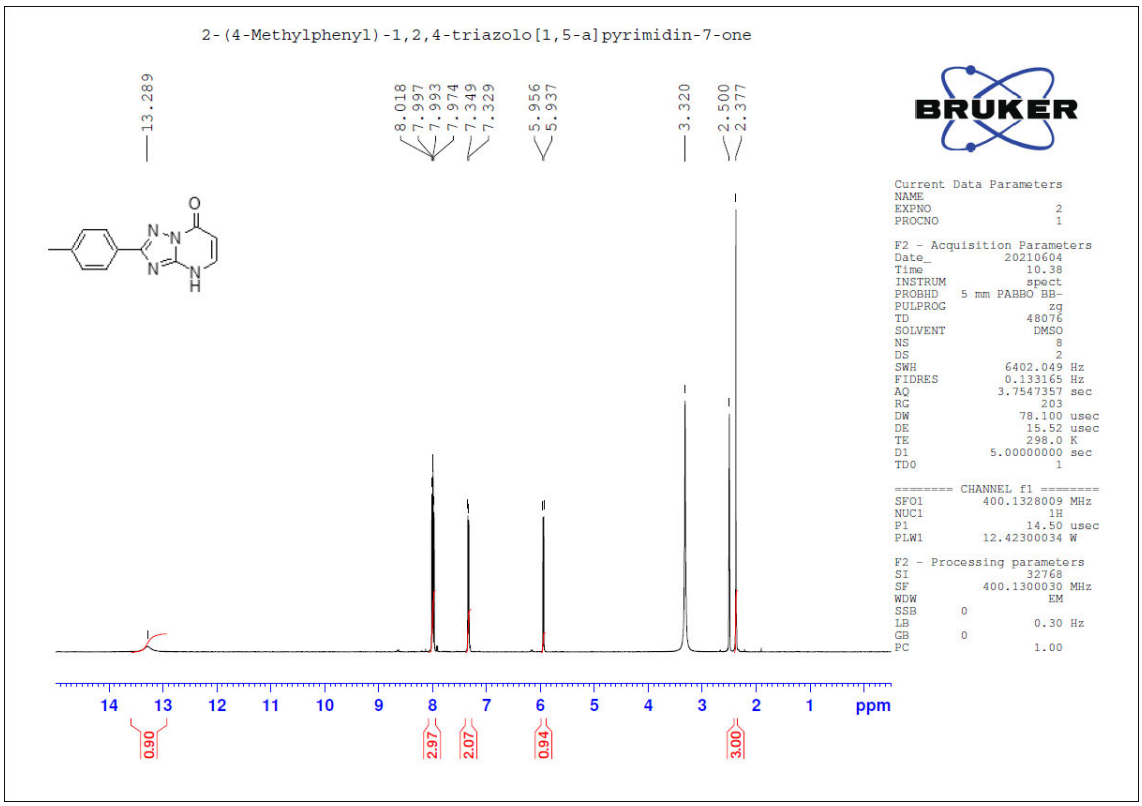
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W

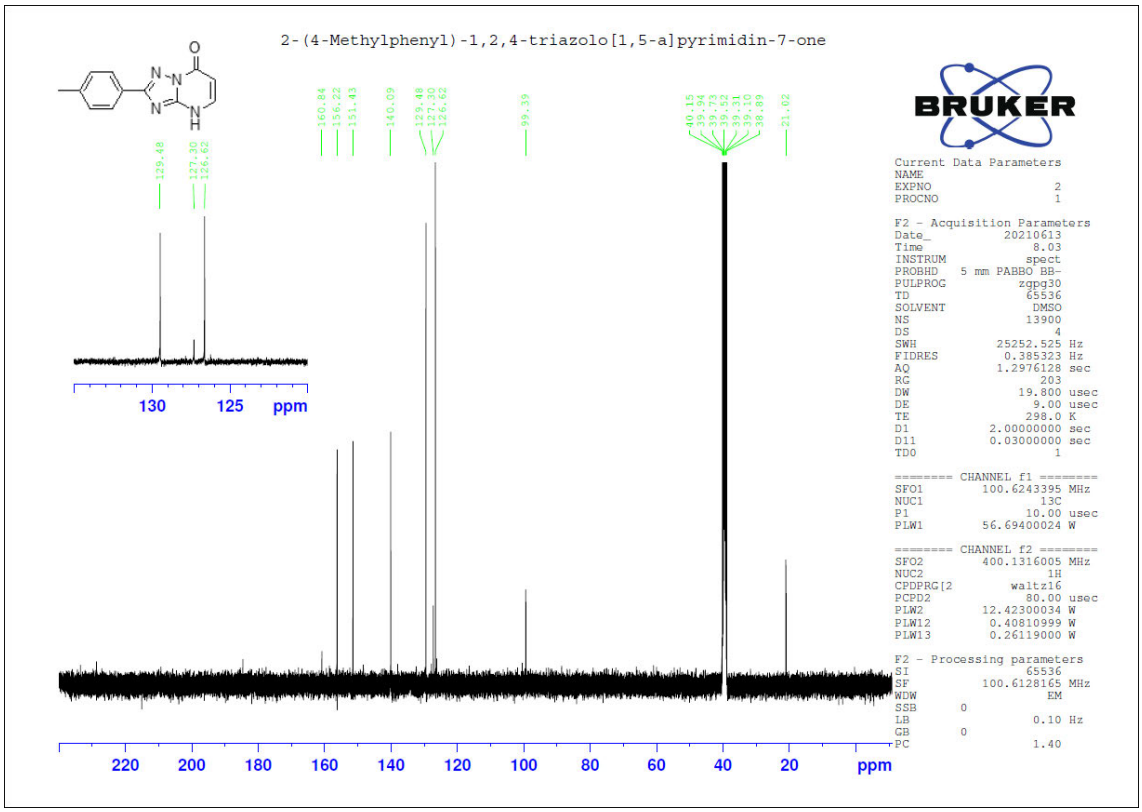
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W

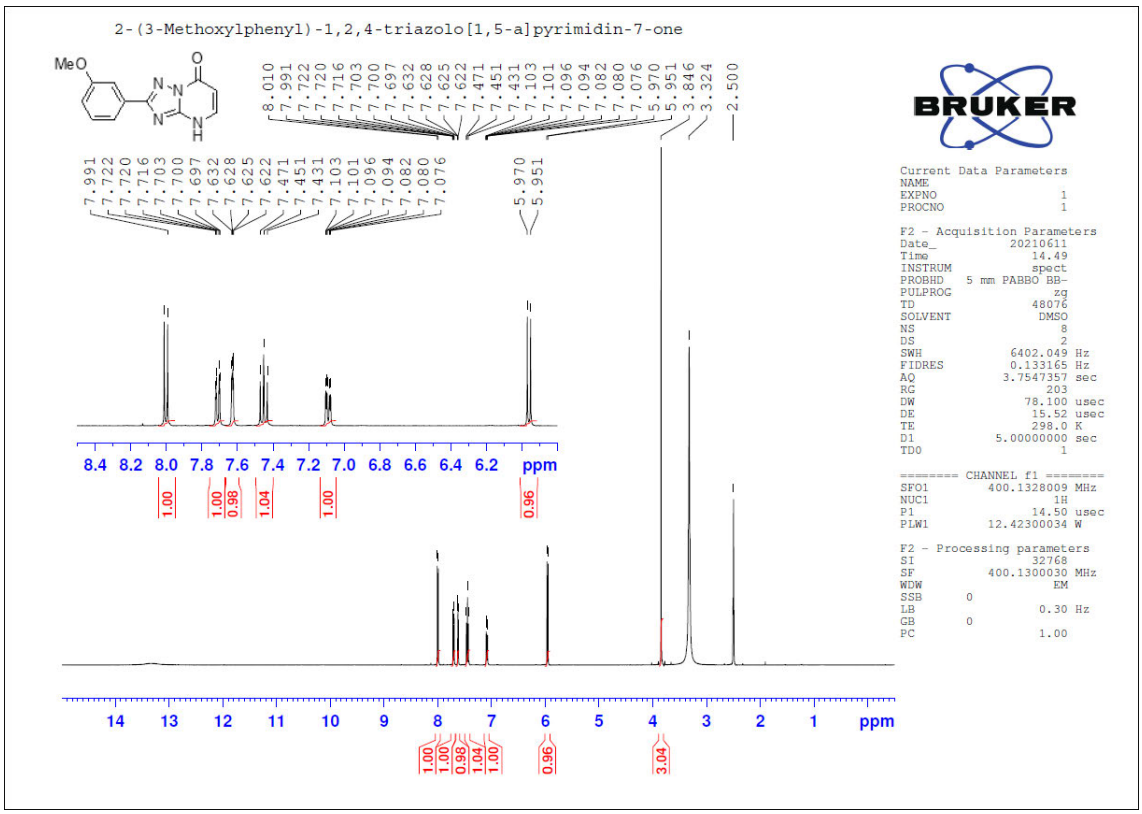
F2 - Processing parameters
SI 65536
SF 100.6128158 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

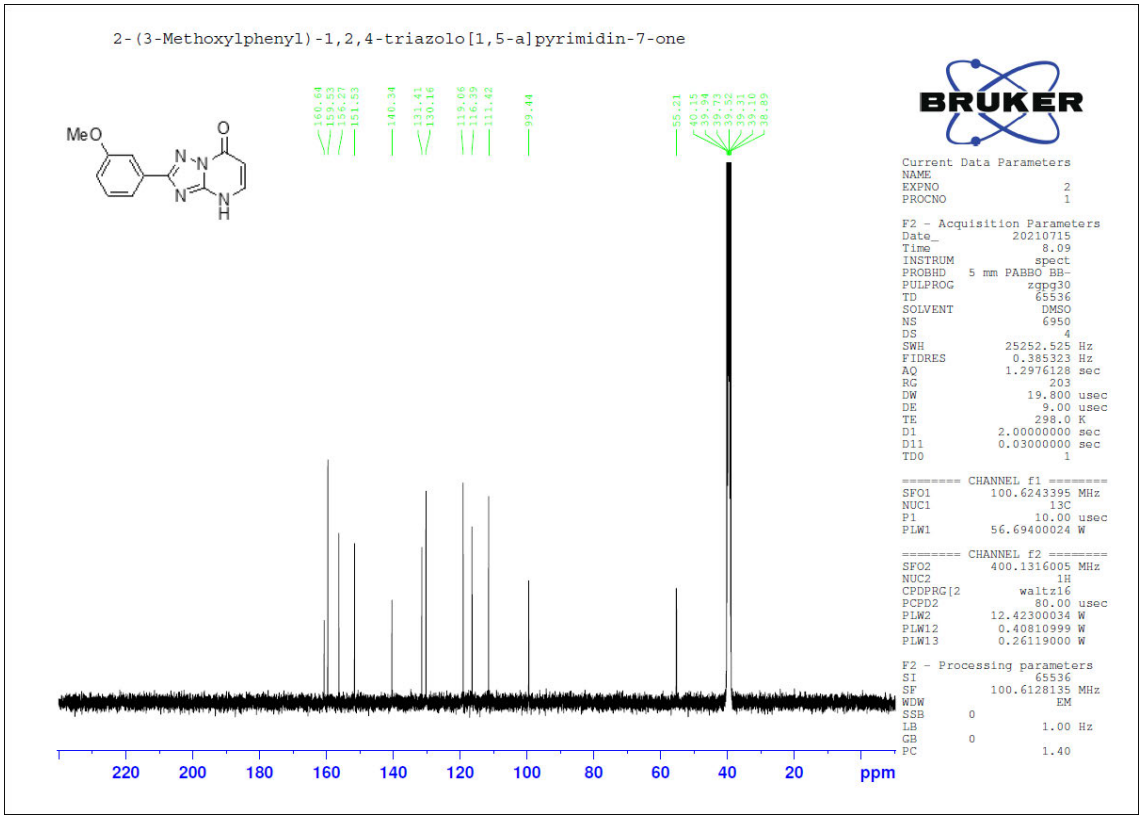

2-(3-Methylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
7.963
7.944
7.937
7.911
7.892
7.429
7.410
7.391
7.331
7.312
5.960
5.941
3.519
2.500
2.391
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 ppm
2.95
1.00
0.99
1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
2.95
1.00
0.99
1.00
2.97
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210401
Time 14.55
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 45.2
DW 78.100 usec
DE 15.52 usec
TE 298.1 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

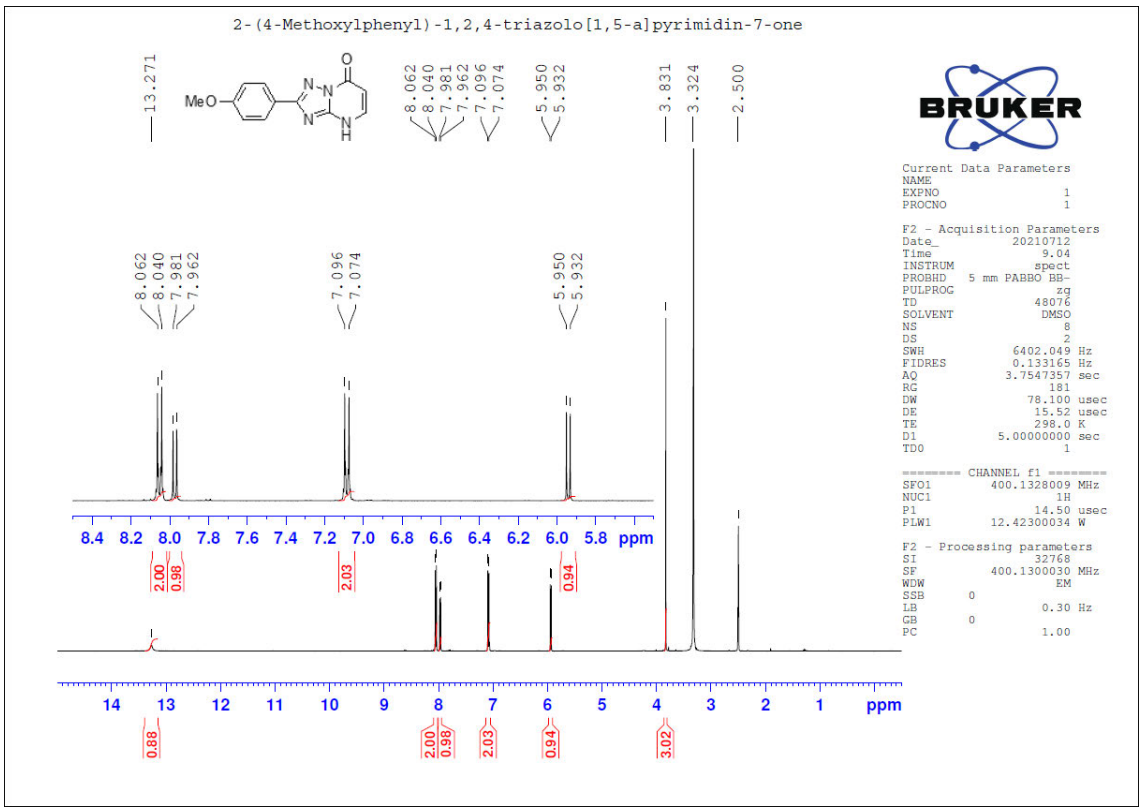

2-(3-Methylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
161.07
156.61
151.86
140.78
138.36
131.25
130.14
129.05
127.34
124.07
99.53
40.15
39.94
39.73
39.52
39.31
39.10
38.89
21.18
220 200 180 160 140 120 100 80 60 40 20 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210409
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 296.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6127950 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

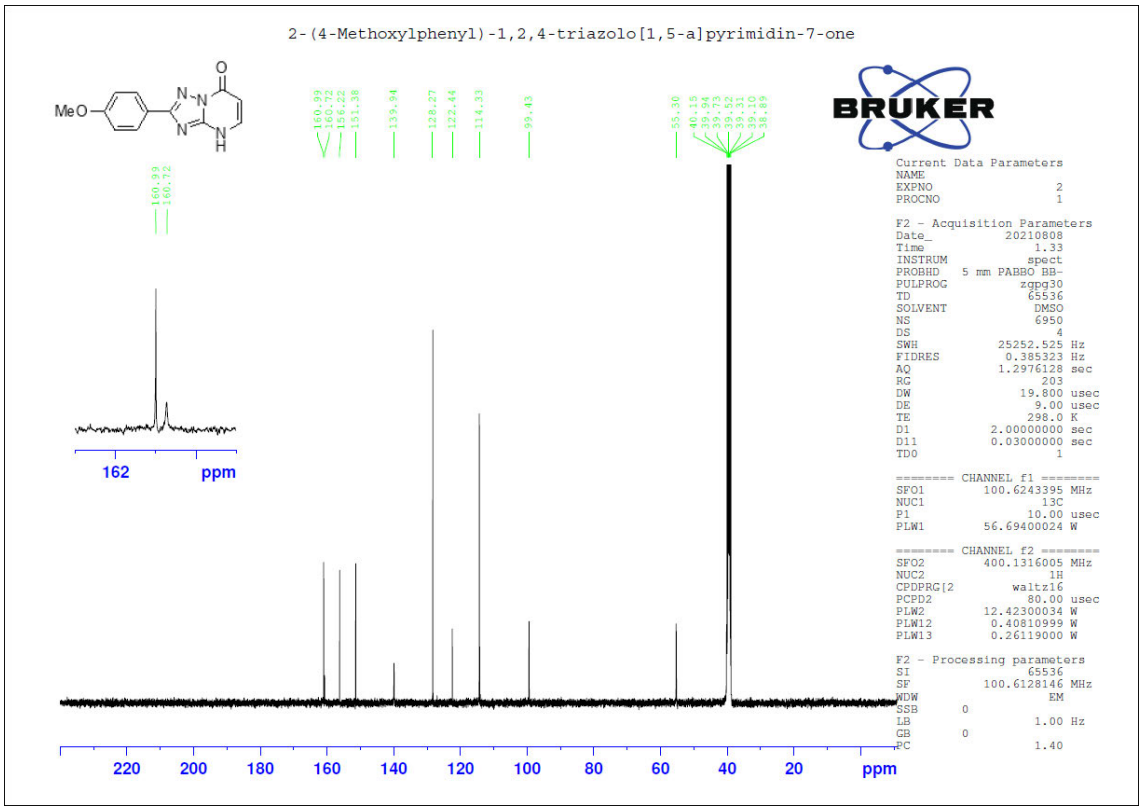

2-(4-Methylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.289
8.018
7.997
7.993
7.974
7.349
7.329
5.956
5.937
3.320
2.500
2.377
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210604
Time 10.38
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.90 2.97 2.07 0.94 3.00

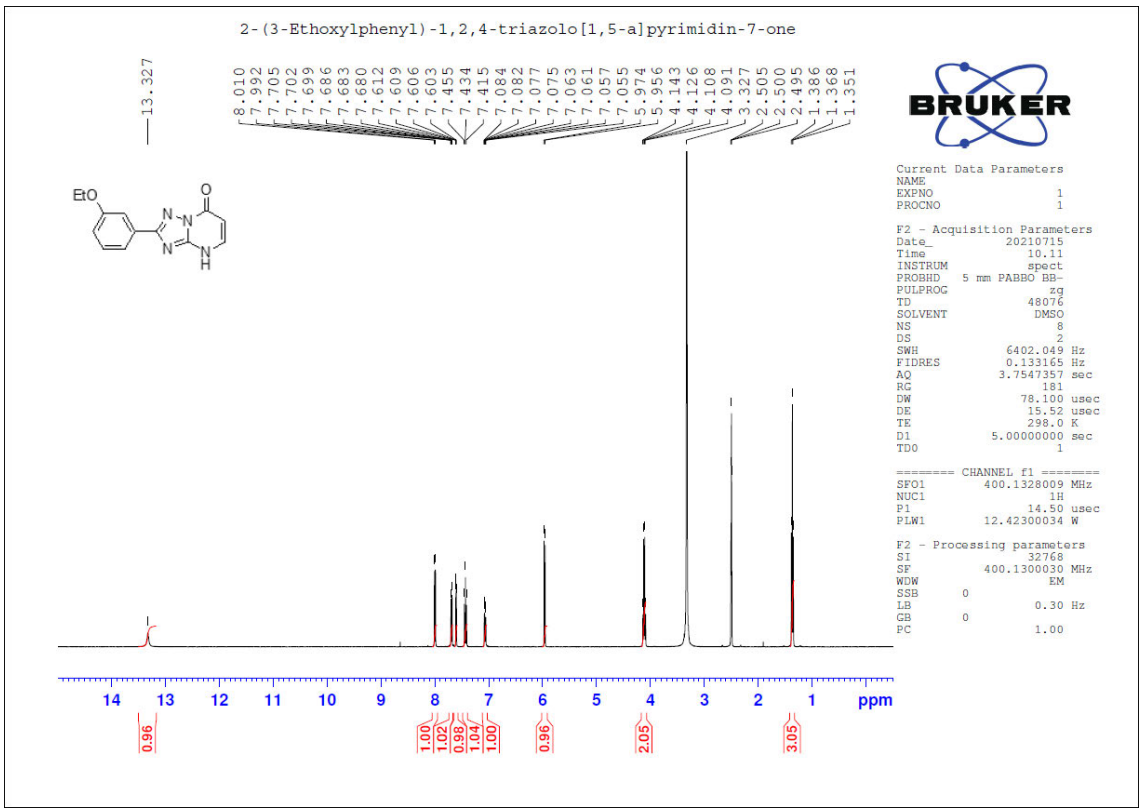

2-(4-Methylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
160.84
156.22
151.43
140.09
129.48
127.20
126.62
99.39
40.15
39.94
39.73
39.52
39.31
39.10
38.89
21.02
129.48
127.20
126.62
130 125 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210613
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128165 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

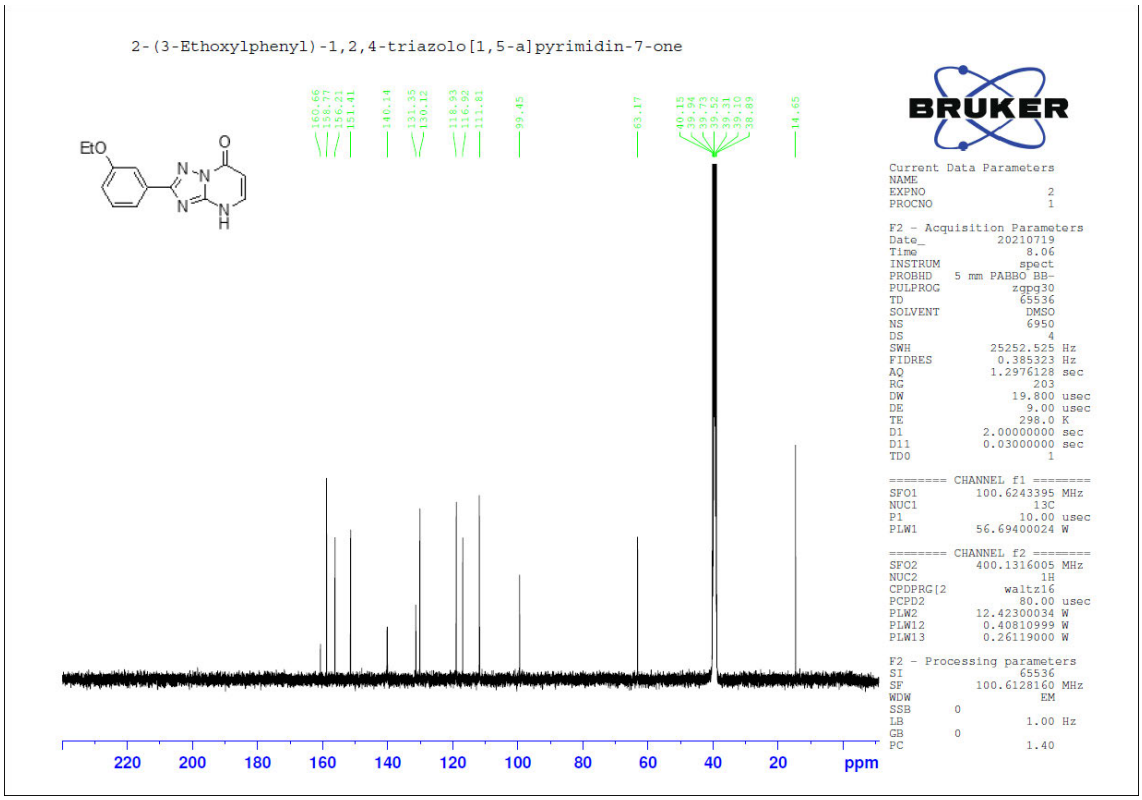

2-(3-Methoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
MeO
8.010
7.991
7.722
7.720
7.716
7.703
7.700
7.697
7.632
7.628
7.625
7.622
7.471
7.451
7.431
7.103
7.101
7.096
7.094
7.082
7.080
7.076
5.970
5.951
3.846
3.324
2.500
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 ppm
1.00 1.00 0.98 1.04 1.00 0.96
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 1.00 0.98 1.04 1.00 0.96 3.04
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210611
Time 14.49
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

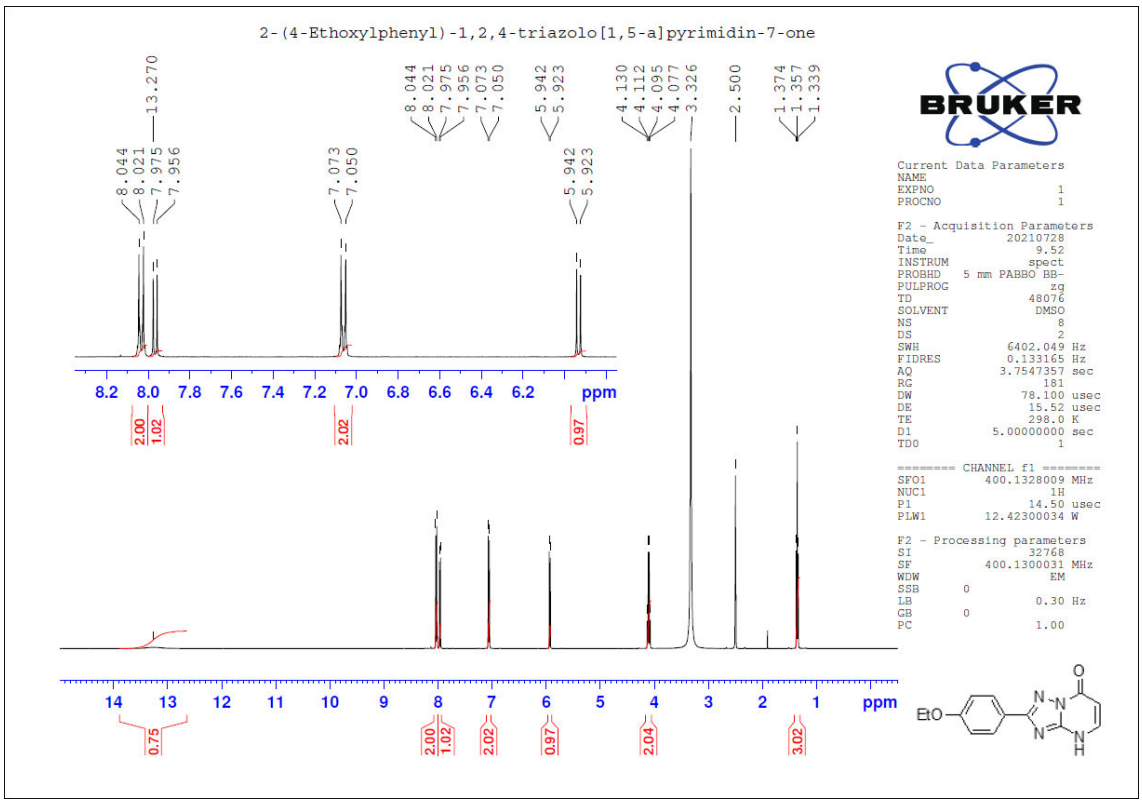

2-(3-Methoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
MeO
160.64
159.53
156.27
151.53
140.34
131.41
130.16
119.06
116.39
111.42
99.44
55.21
40.15
39.94
39.73
39.52
39.31
39.10
38.89
220 200 180 160 140 120 100 80 60 40 20 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210715
Time 8.09
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128135 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

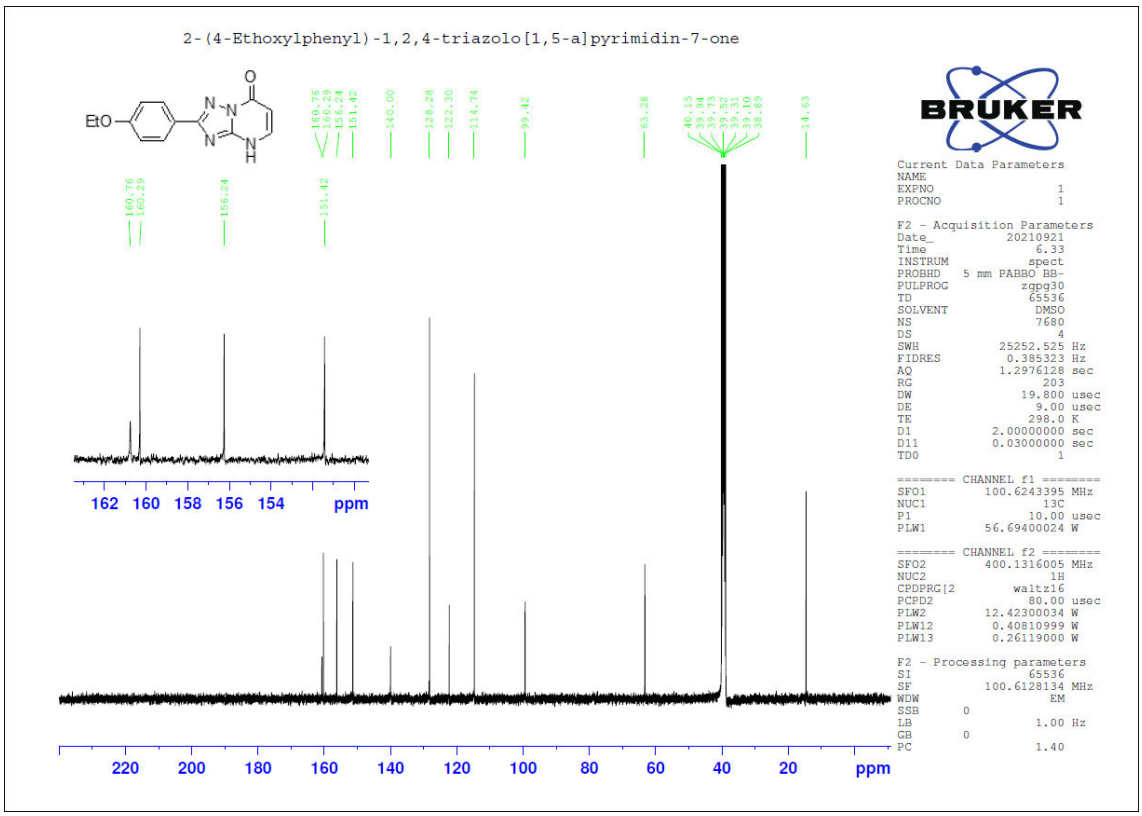

2-(4-Methoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.271
8.062
8.040
7.981
7.962
7.096
7.074
5.950
5.932
3.831
3.324
2.500
MeO
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210712
Time 9.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 ppm
2.00 0.98 2.03 0.94
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.88 2.00 0.98 2.03 0.94 3.02

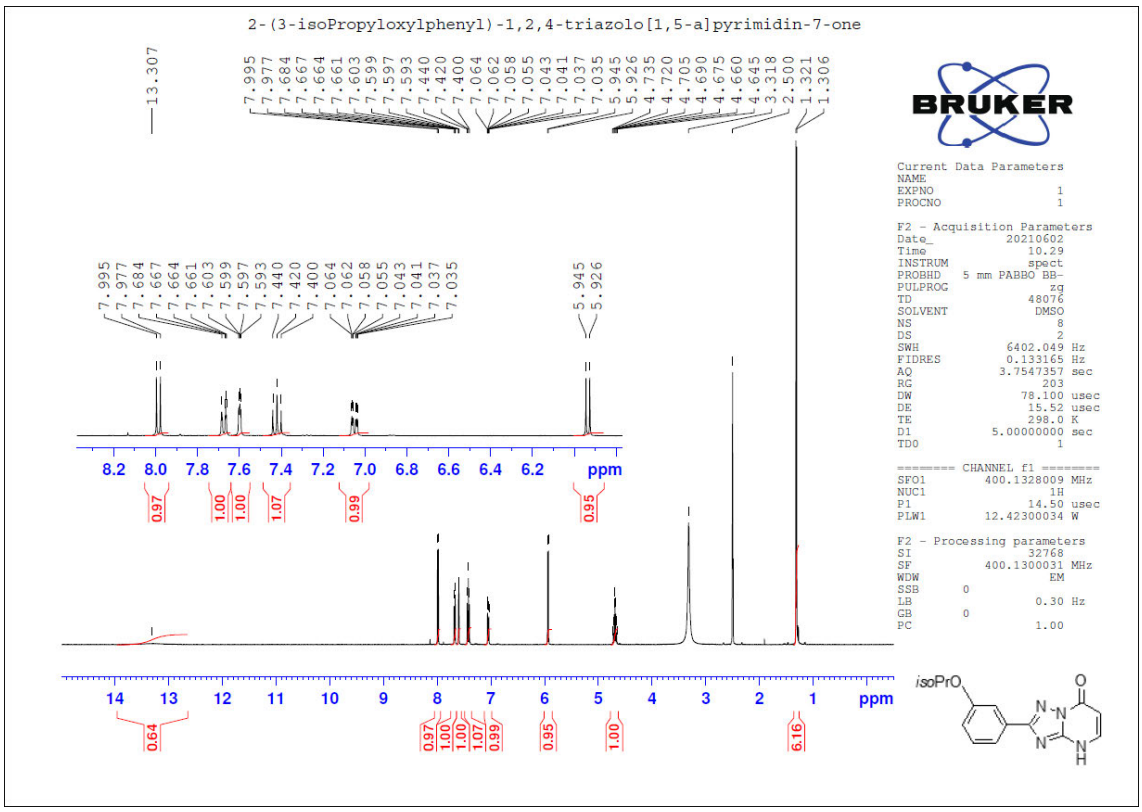

2-(4-Methoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
160.99
160.72
156.22
151.38
139.94
128.27
122.44
114.33
99.43
55.30
40.15
39.94
39.73
39.52
39.31
39.10
38.89
MeO
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210808
Time 1.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128146 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
160.99
160.72
162 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm

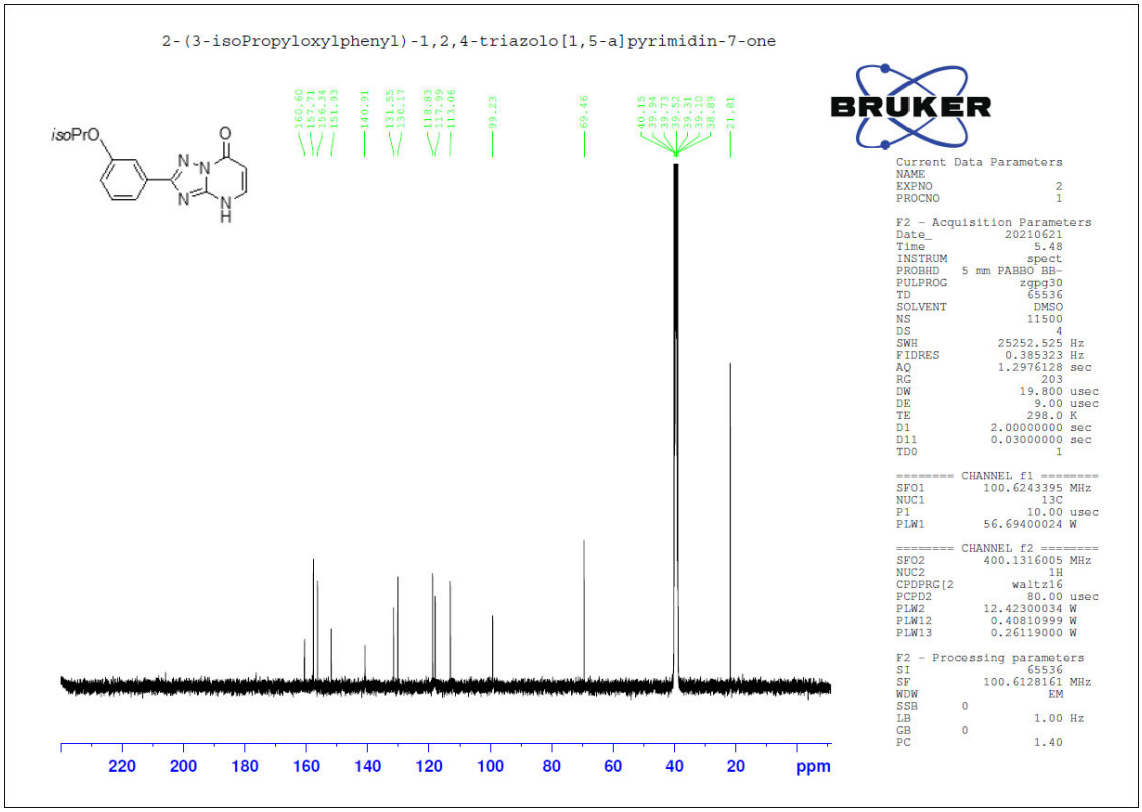

2-(3-Ethoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.327
8.010
7.992
7.705
7.702
7.699
7.686
7.683
7.680
7.612
7.609
7.606
7.603
7.455
7.434
7.415
7.084
7.082
7.077
7.075
7.063
7.061
7.057
7.055
5.974
5.956
4.143
4.126
4.108
4.091
3.327
2.505
2.500
2.495
1.386
1.368
1.351
EtO
O
N
N
N
H
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.96
1.00
1.02
0.98
1.04
1.00
0.96
2.05
3.05
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210715
Time 10.11
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

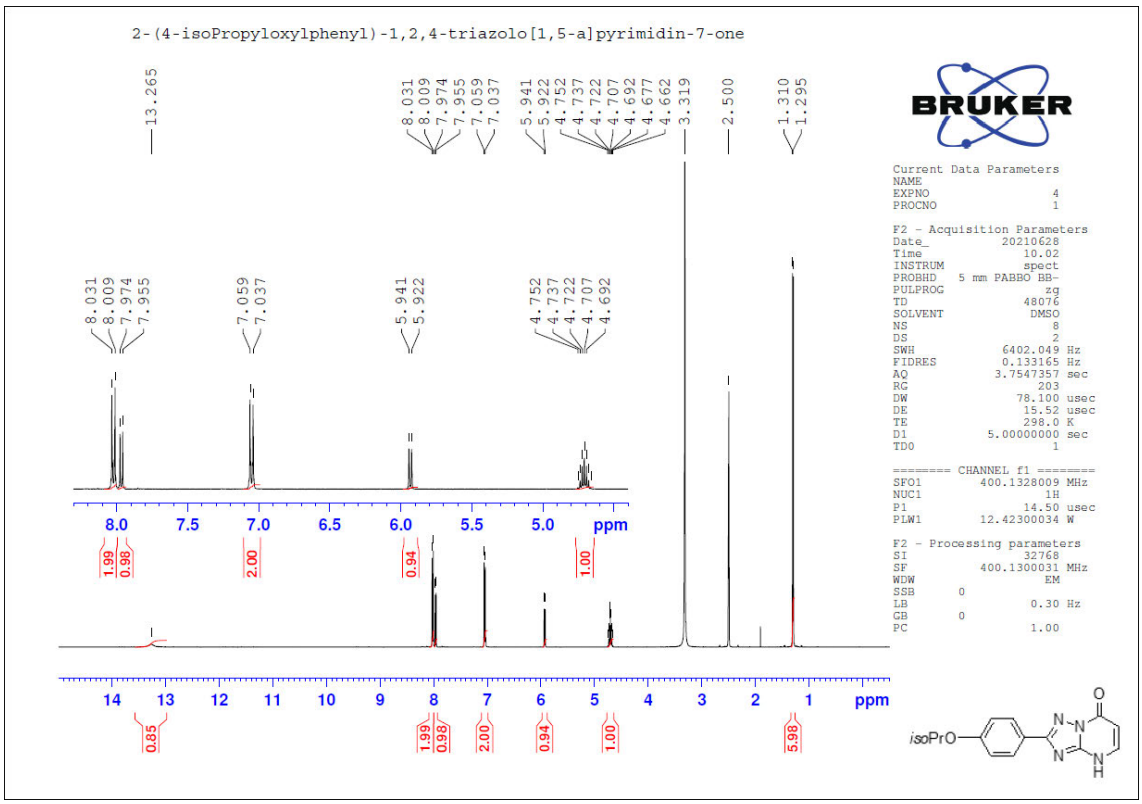

2-(3-Ethoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
160.66
158.77
156.21
151.41
140.14
131.35
130.12
118.93
116.92
111.81
99.45
63.17
40.15
39.94
39.73
39.52
39.31
39.10
38.89
14.65
EtO
O
N
N
N
H
220
200
180
160
140
120
100
80
60
40
20
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210719
Time 8.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128160 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

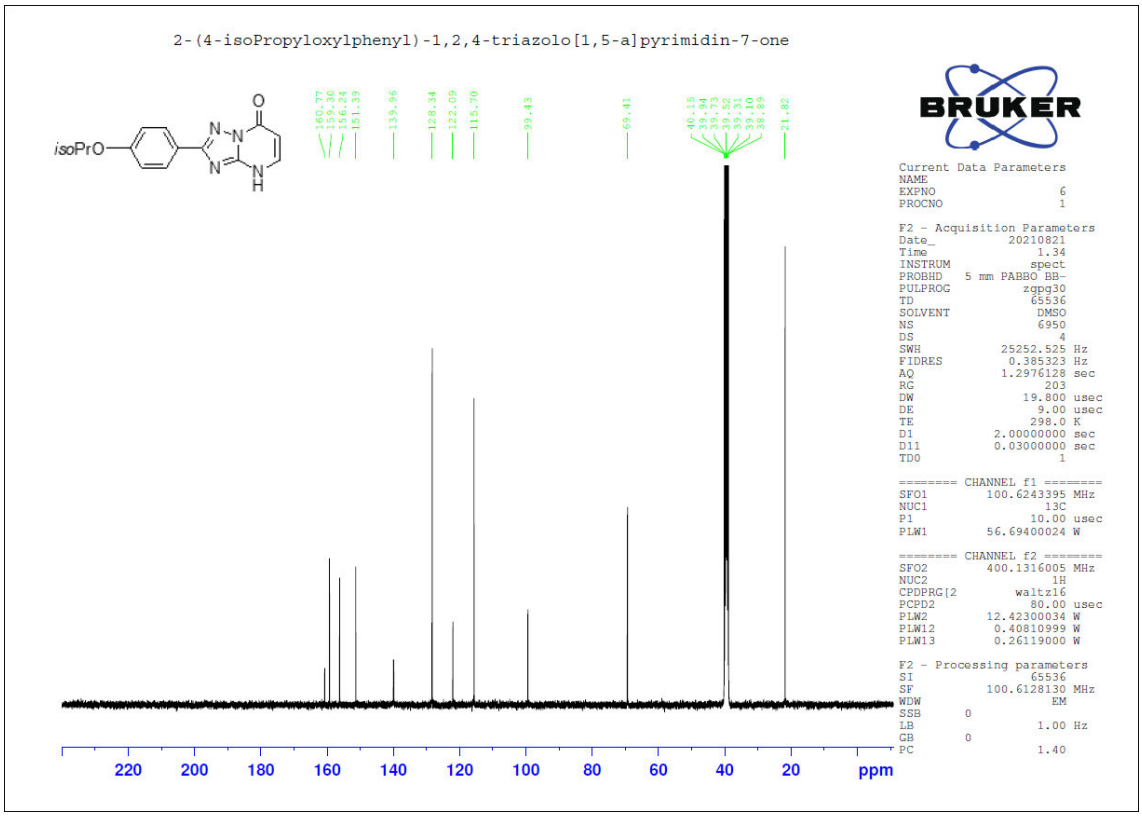

2-(4-Ethoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210728
Time 9.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
EtO

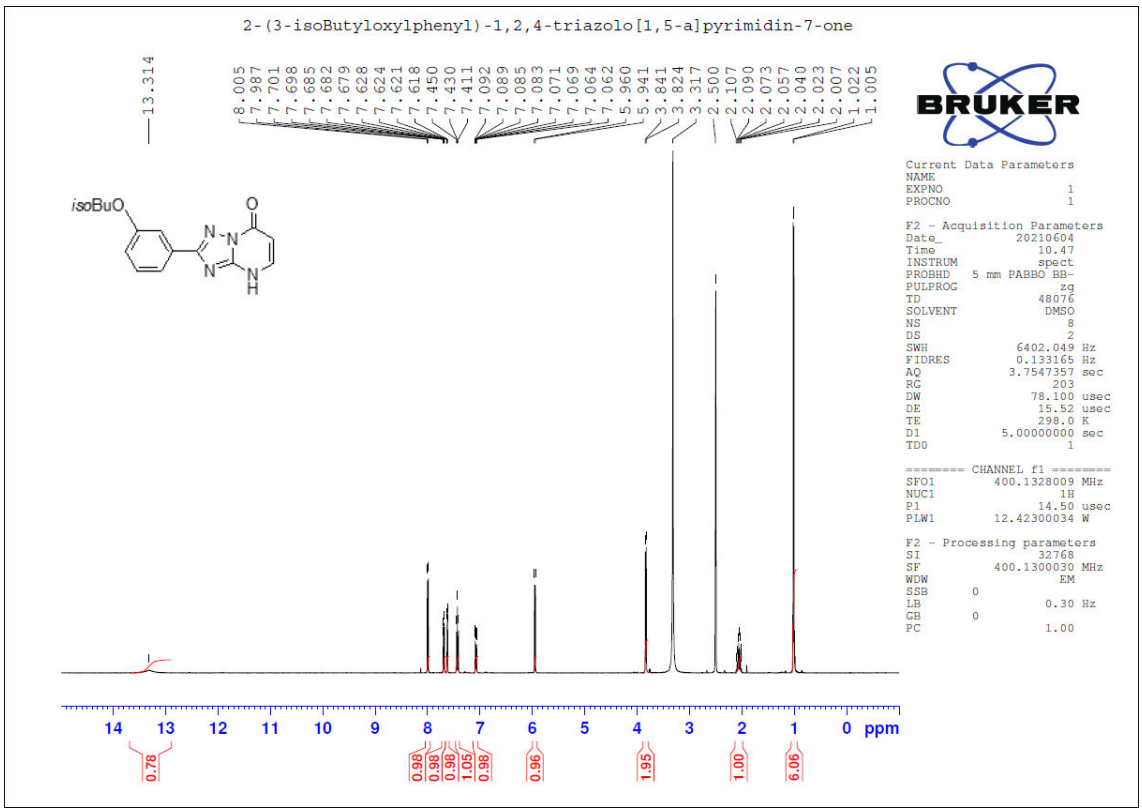

2-(4-Ethoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
EtO
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210921
Time 6.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 7680
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128134 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

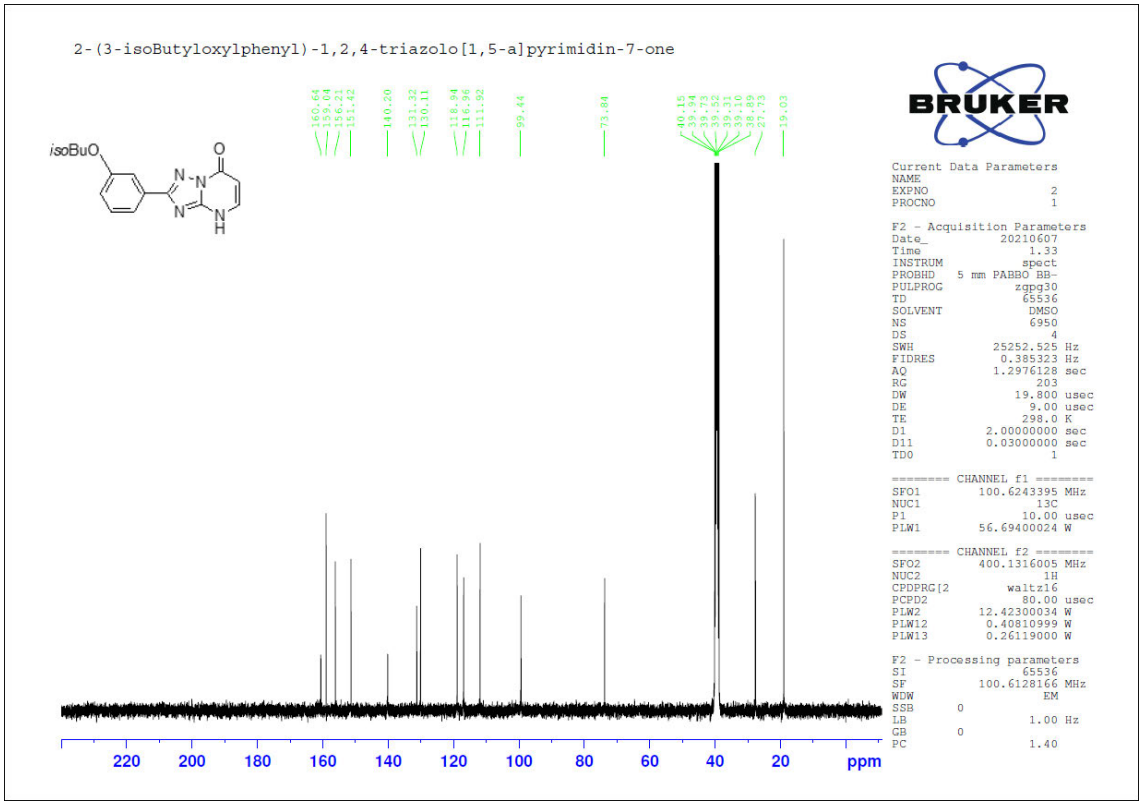

2-(3-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210602
Time 10.29
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
isoPrO

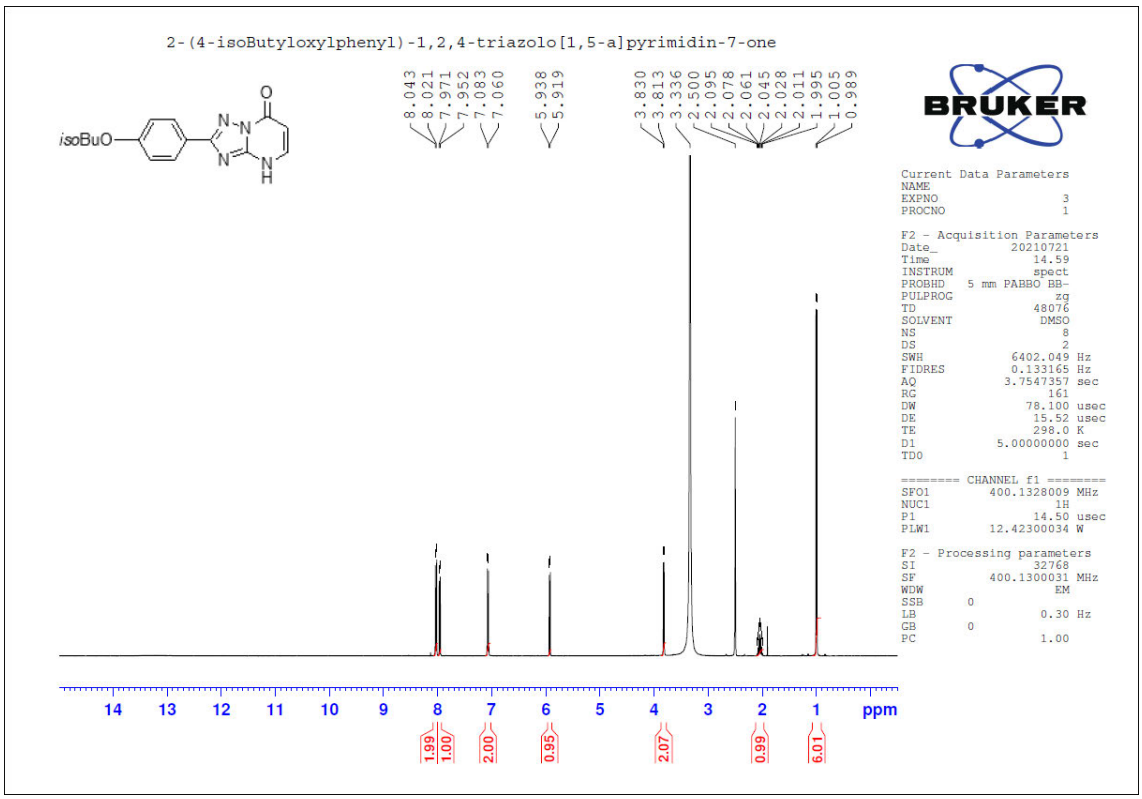

2-(3-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
isoPrO
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210621
Time 5.48
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 11500
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128161 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

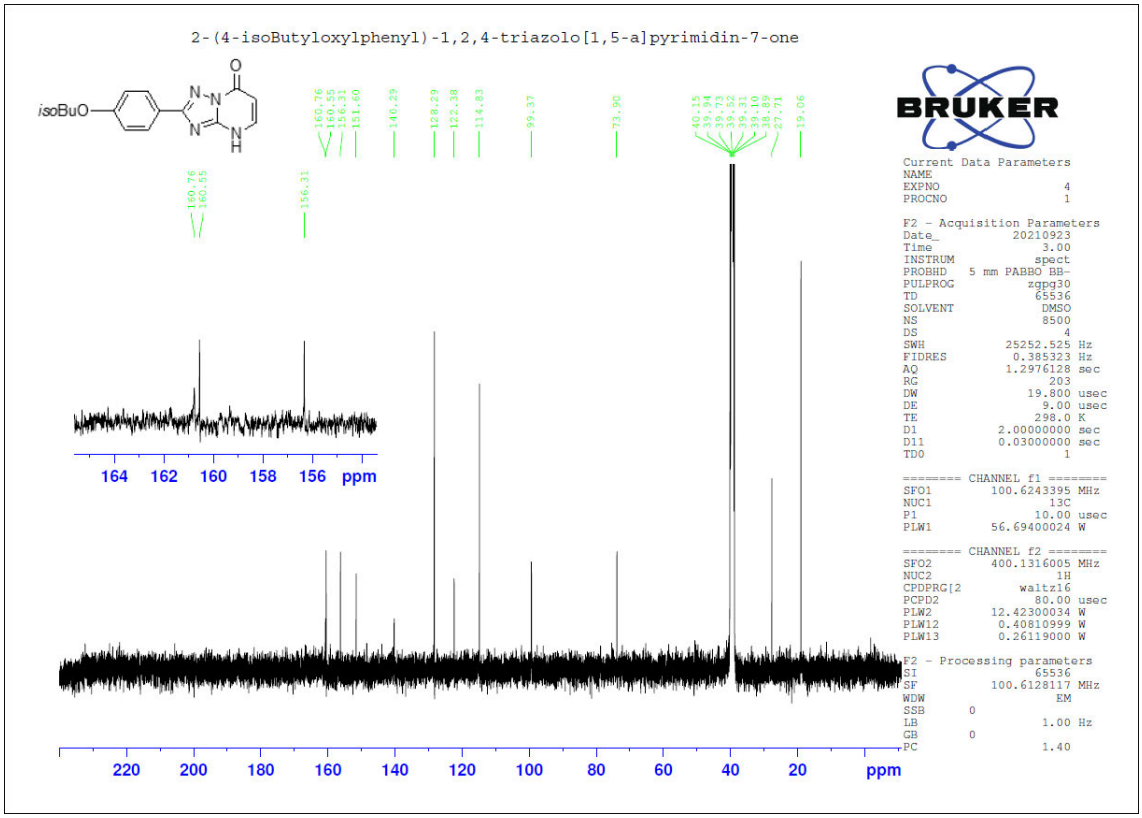

2-(4-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.265
8.031
8.009
7.974
7.955
7.059
7.037
5.941
5.922
4.752
4.737
4.722
4.707
4.692
4.677
4.662
3.319
2.500
1.310
1.295
BRUKER
Current Data Parameters
NAME
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210628
Time 10.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
isoPrO

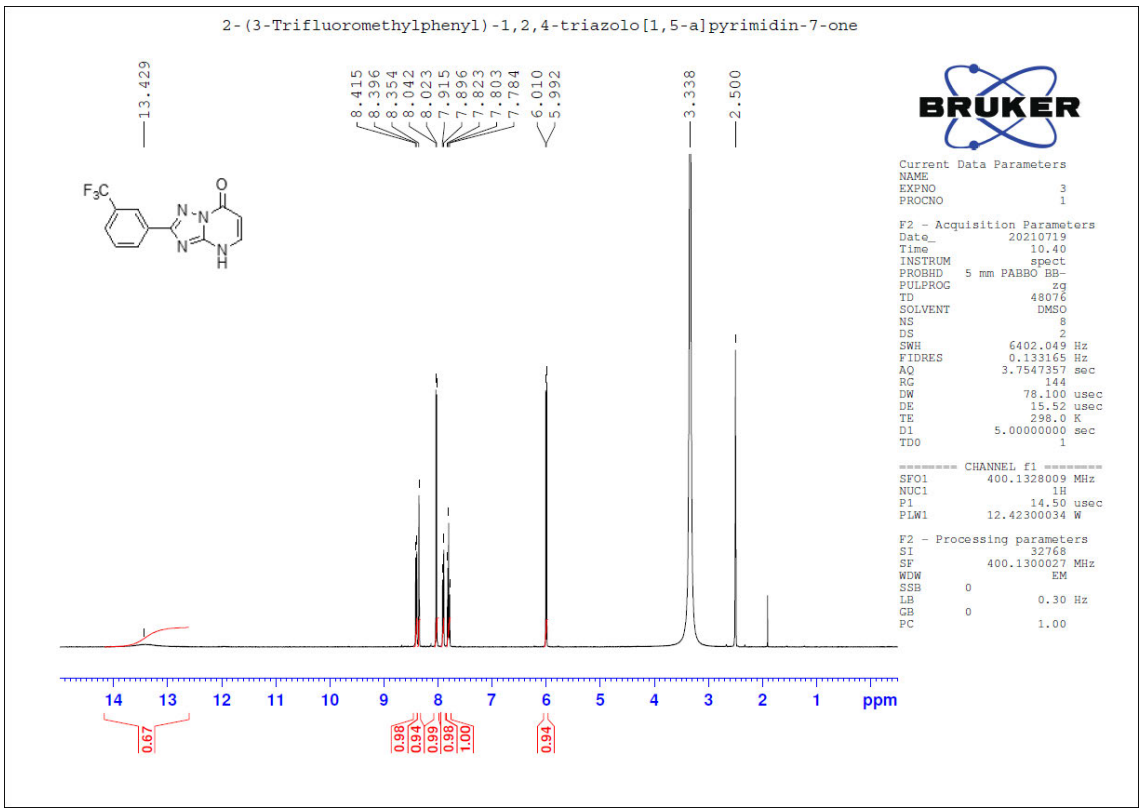

2-(4-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
isoPrO
160.77
159.30
156.24
151.39
139.96
128.34
122.09
115.70
93.43
69.41
40.15
39.94
39.73
39.52
39.31
39.10
38.89
21.82
BRUKER
Current Data Parameters
NAME
EXPNO 6
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210821
Time 1.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128130 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm

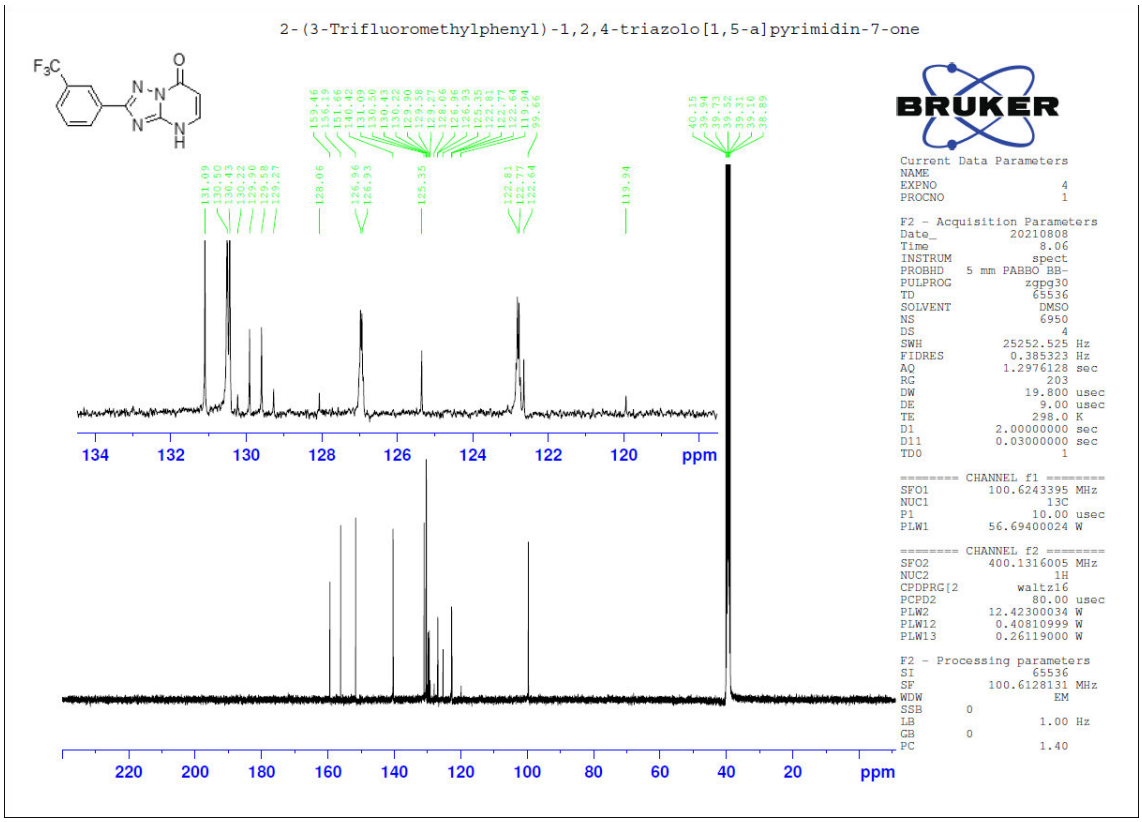

2-(3-isoButyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.314
8.005
7.987
7.701
7.698
7.685
7.682
7.679
7.628
7.624
7.621
7.618
7.450
7.430
7.411
7.092
7.089
7.085
7.083
7.071
7.069
7.064
7.062
5.960
5.941
3.841
3.824
3.317
2.500
2.107
2.090
2.073
2.057
2.040
2.023
2.007
1.022
1.005
isoBuO
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210604
Time 10.47
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.78
0.98
0.98
0.98
1.05
0.98
0.96
1.95
1.00
6.06

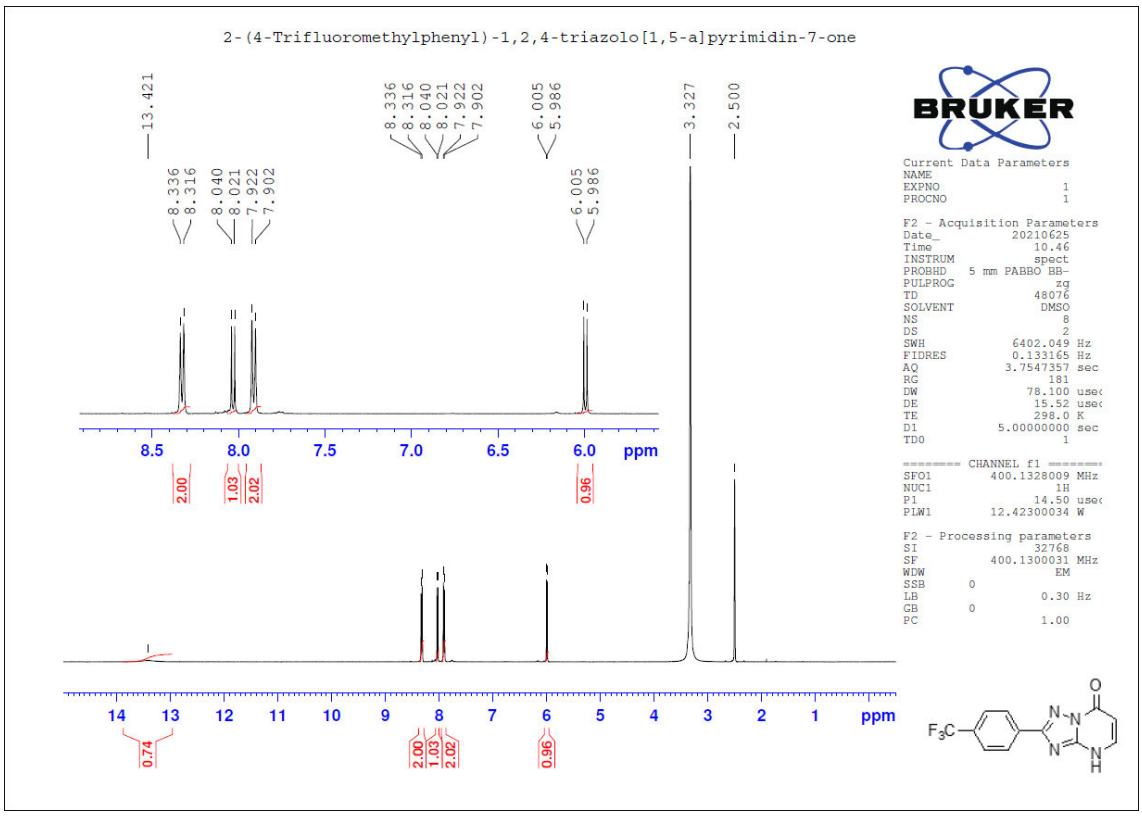

2-(3-isoButyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
160.64
159.04
156.21
151.42
140.20
131.32
130.11
118.94
116.96
111.92
99.44
73.84
40.15
39.94
39.73
39.52
39.31
39.10
38.89
27.73
19.03
isoBuO
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210607
Time 1.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128166 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

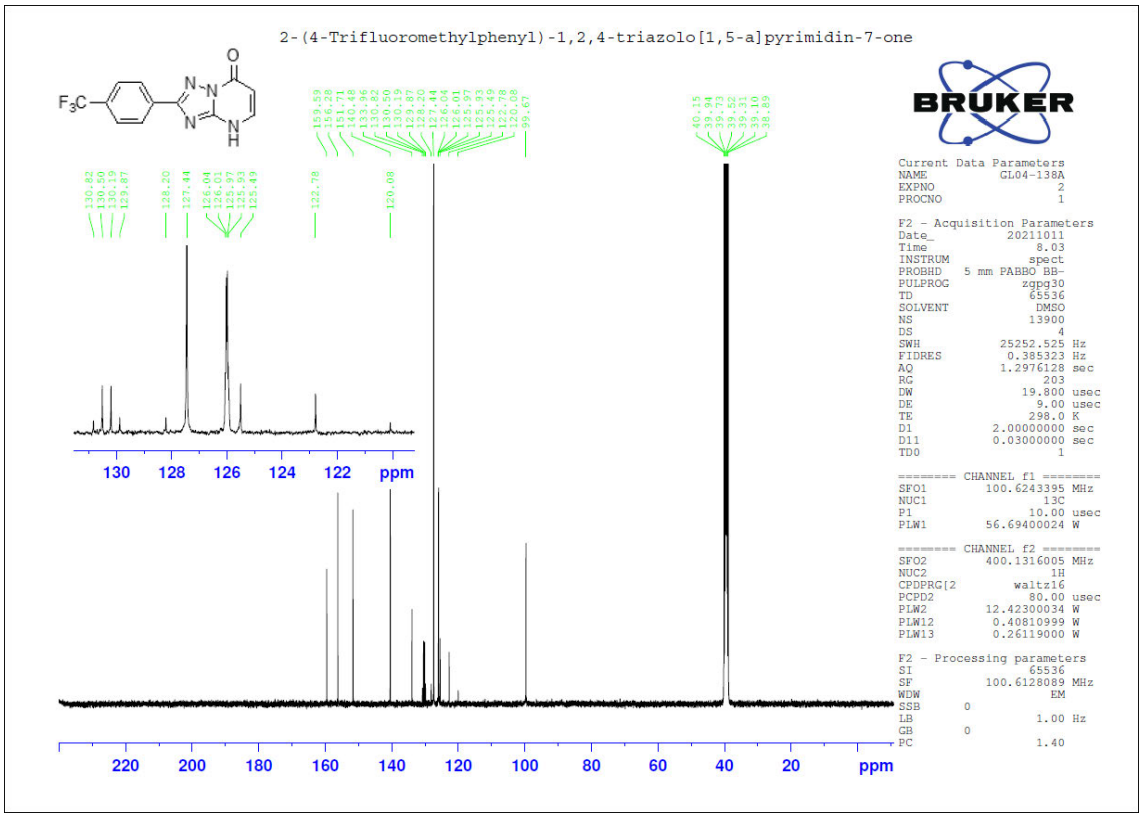

2-(4-isoButyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
isoBuO
8.043
8.021
7.971
7.952
7.083
7.060
5.938
5.919
3.830
3.813
3.336
2.500
2.095
2.078
2.061
2.045
2.028
2.011
1.995
1.005
0.989
BRUKER
Current Data Parameters
NAME
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210721
Time 14.59
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.99
1.00
2.00
0.95
2.07
0.99
6.01

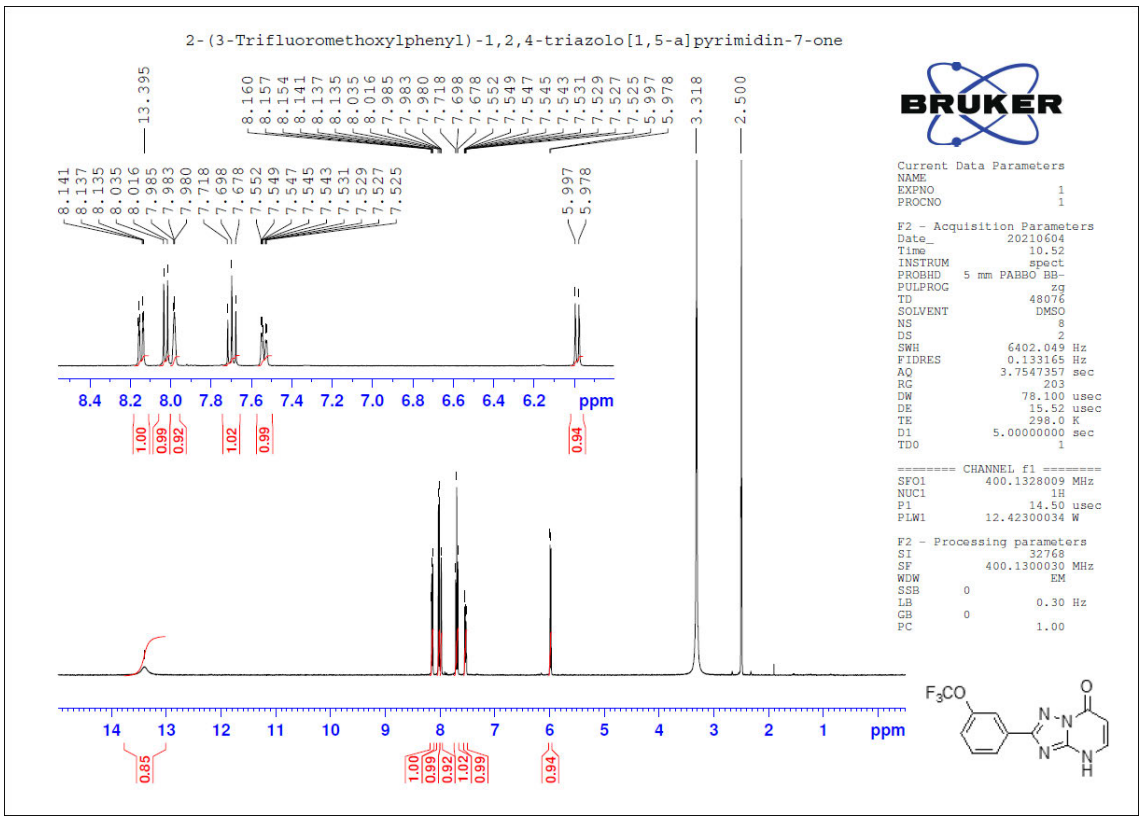

2-(4-isoButyloxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
isoBuO
160.76
160.55
156.31
151.60
140.29
128.29
122.38
114.83
89.37
73.90
40.15
39.94
39.73
39.52
39.31
39.10
38.89
27.71
19.06
160.76
160.55
156.31
164 162 160 158 156 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210923
Time 3.00
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 8500
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128117 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

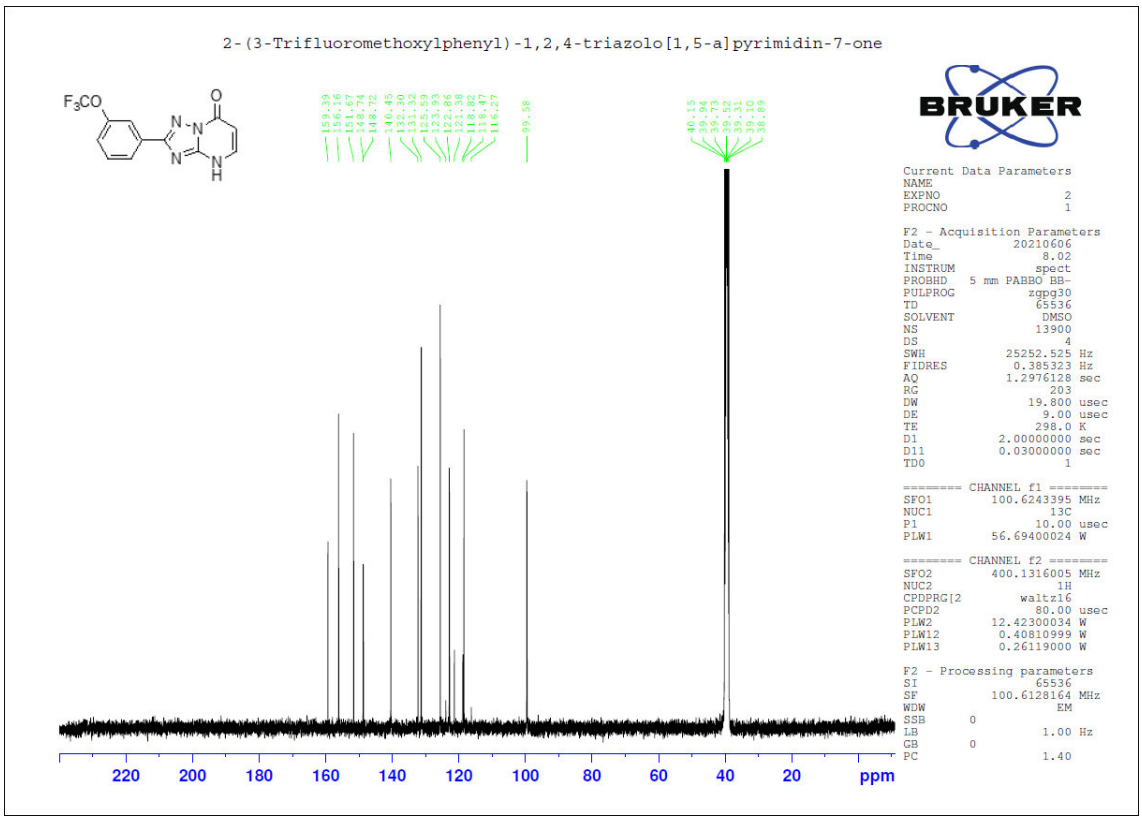

2-(3-Trifluoromethylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.429
8.415
8.396
8.354
8.042
8.023
7.915
7.896
7.823
7.803
7.784
6.010
5.992
3.338
2.500
BRUKER
Current Data Parameters
NAME
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210719
Time 10.40
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 144
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300027 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.67
0.98
0.94
0.99
0.98
1.00
0.94

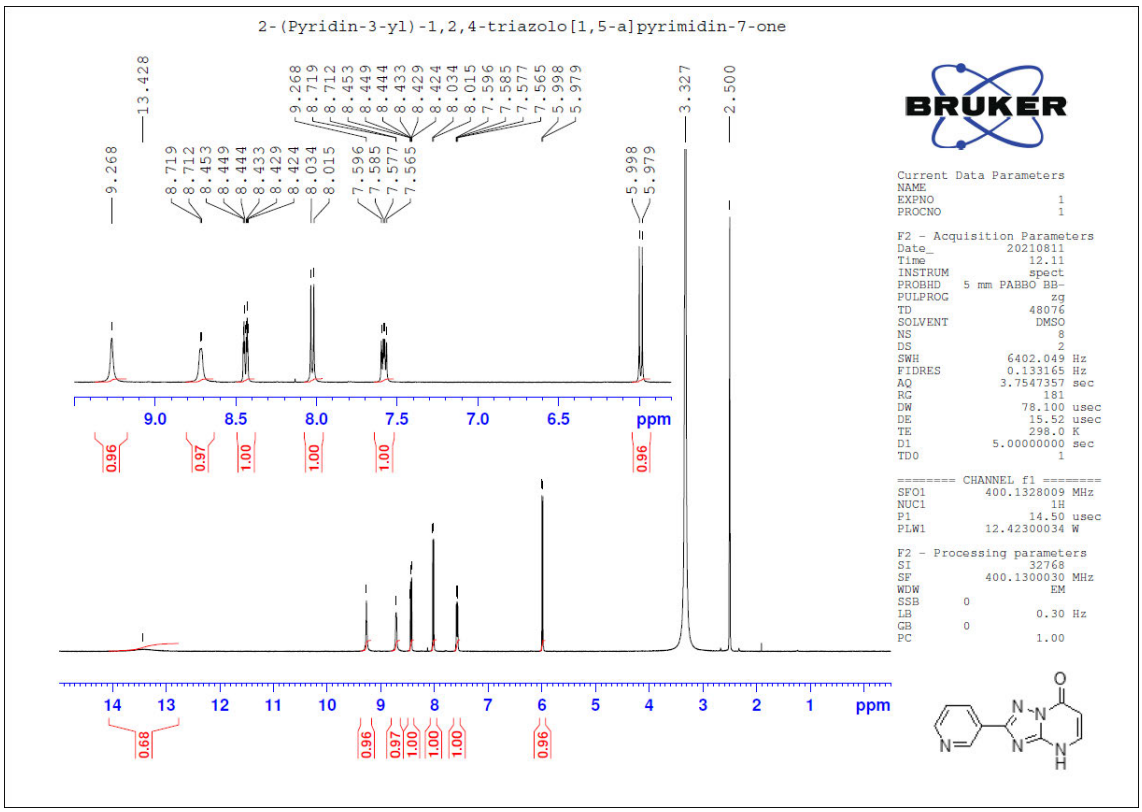

2-(3-Trifluoromethylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
153.46
156.19
151.66
140.42
131.09
130.50
130.43
130.22
129.90
129.58
129.27
128.06
126.96
126.93
125.35
122.81
122.77
122.64
119.94
99.66
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210808
Time 8.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
CHANNEL f2
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128131 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
134 132 130 128 126 124 122 120 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm

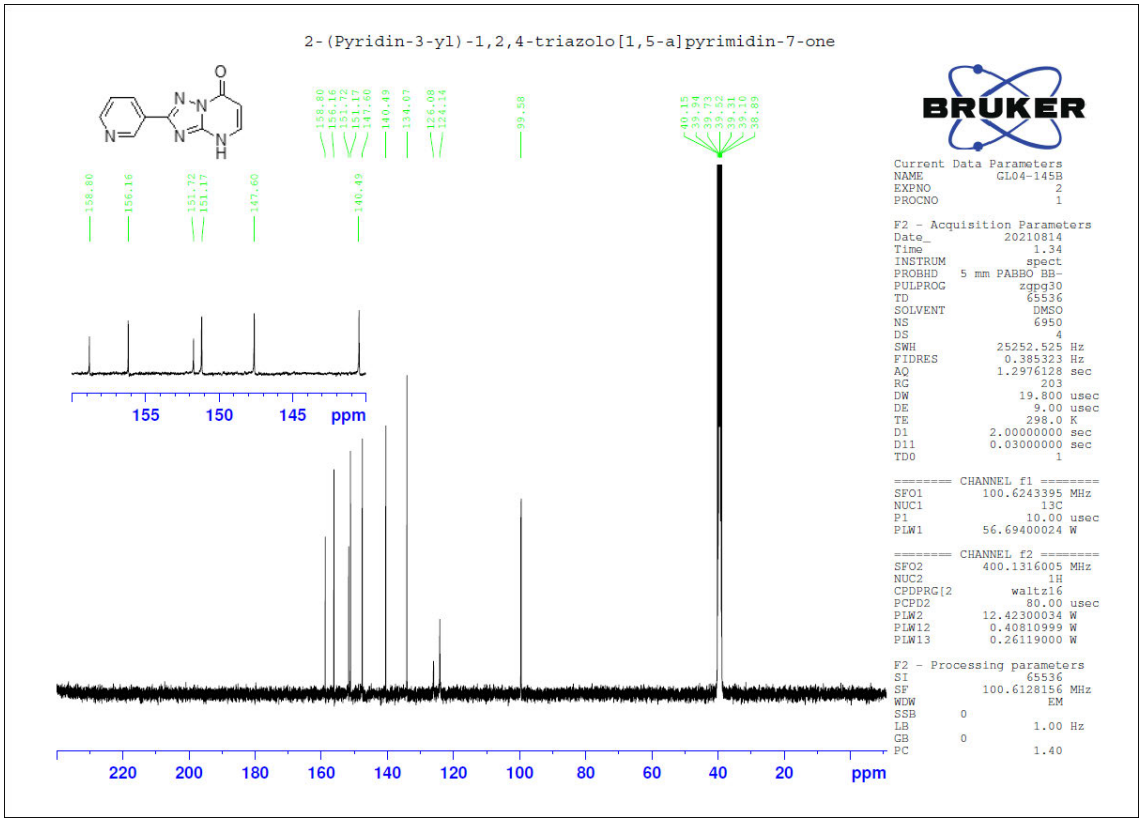

2-(4-Trifluoromethylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.421
8.336
8.316
8.040
8.021
7.922
7.902
6.005
5.986
3.327
2.500
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210625
Time 10.46
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

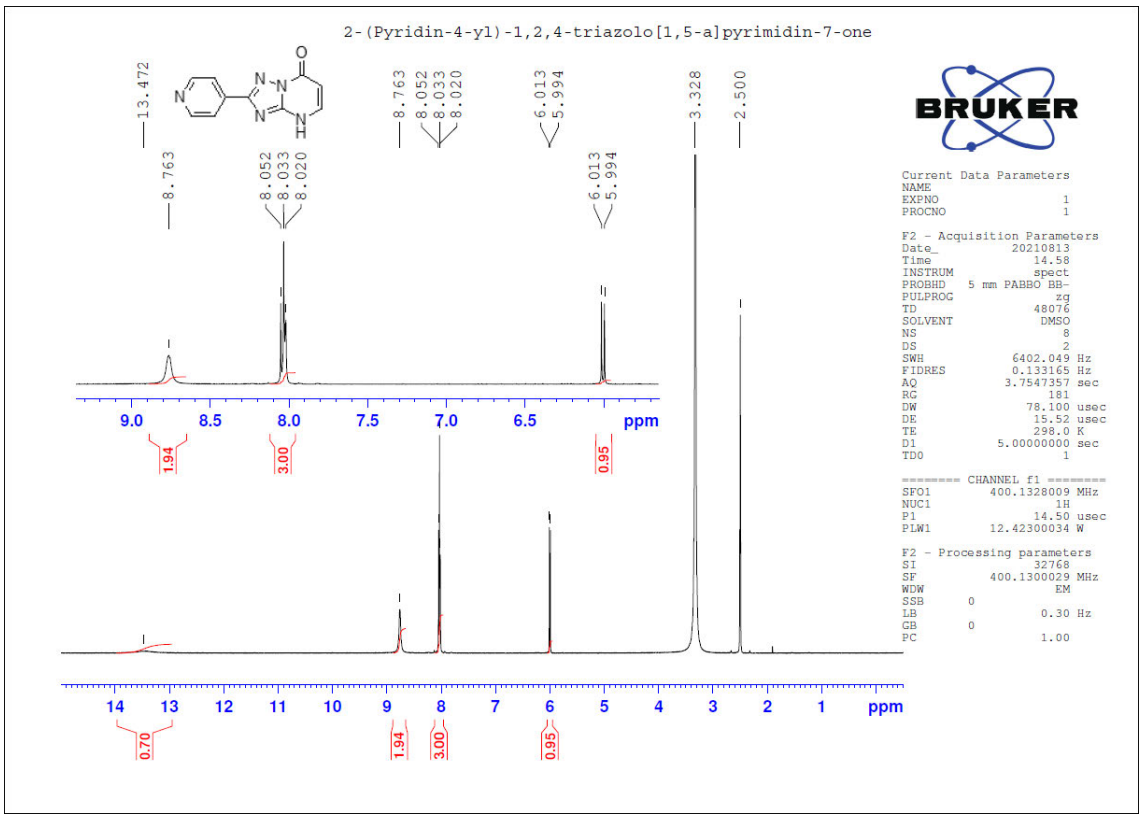

2-(4-Trifluoromethylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME GL04-138A
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20211011
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
CHANNEL f2
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128089 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm

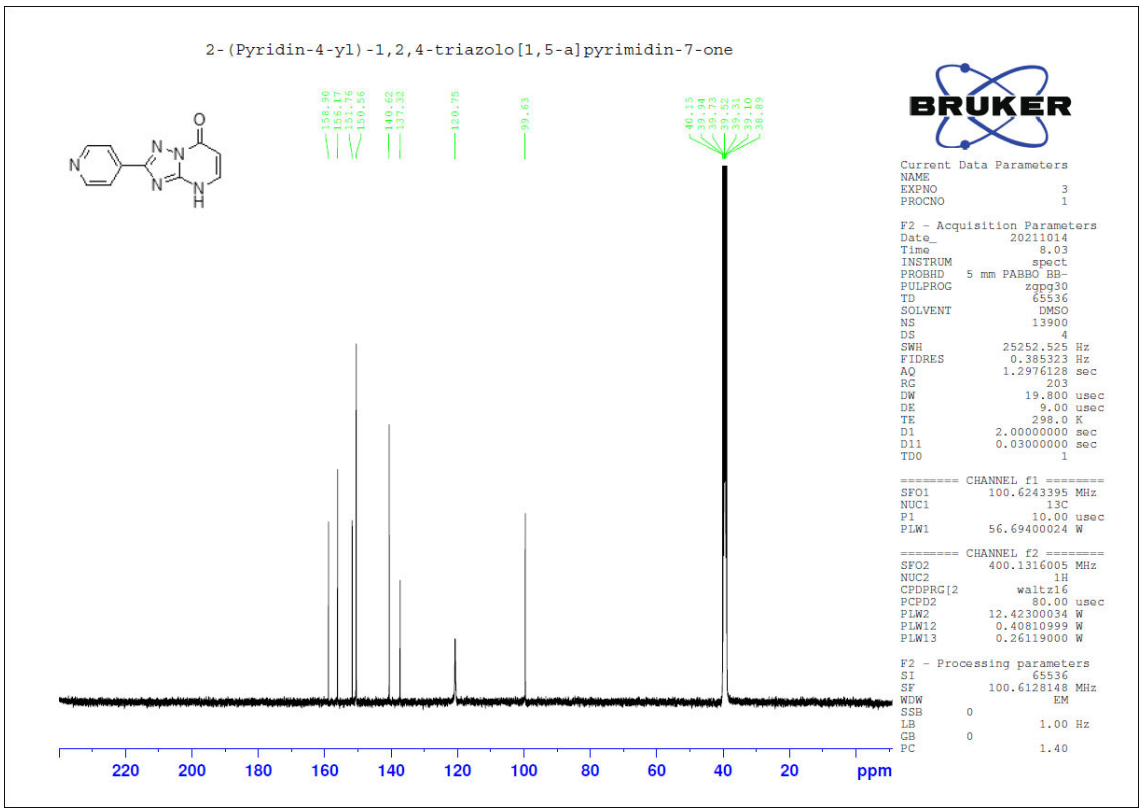

2-(3-Trifluoromethoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210604
Time 10.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

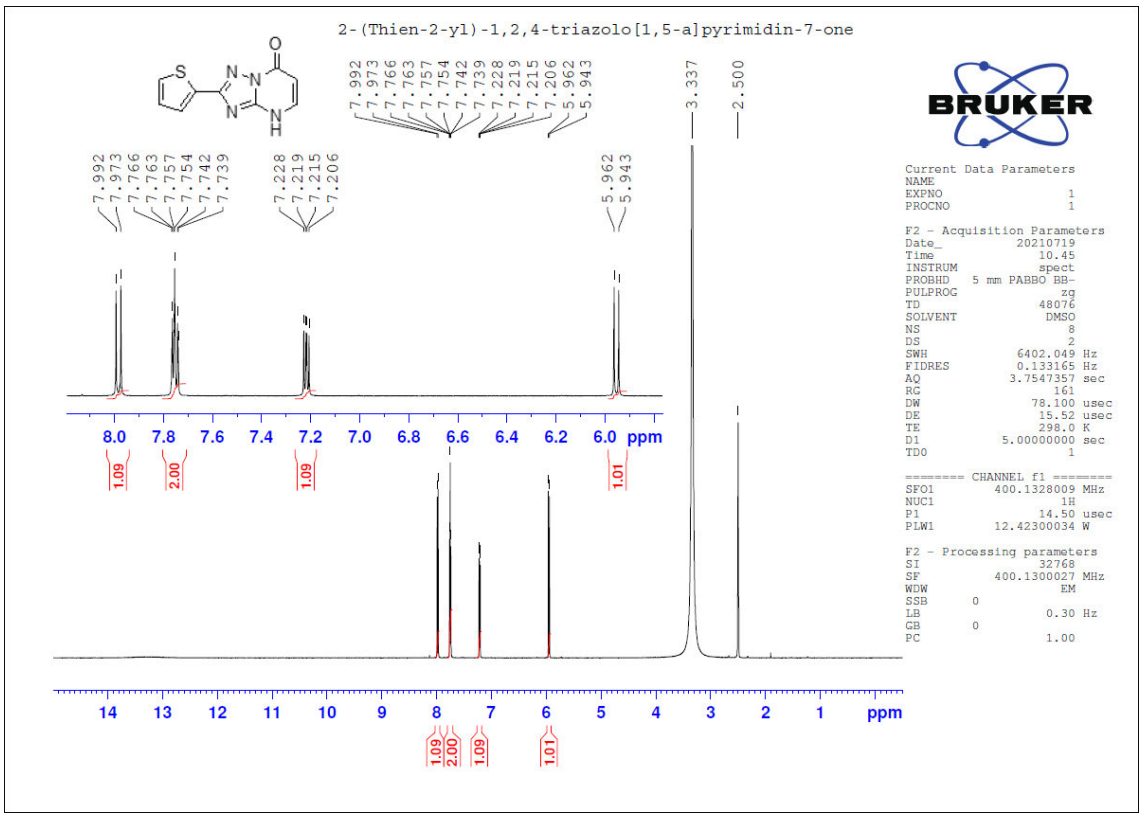

2-(3-Trifluoromethoxylphenyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210606
Time 8.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128164 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm

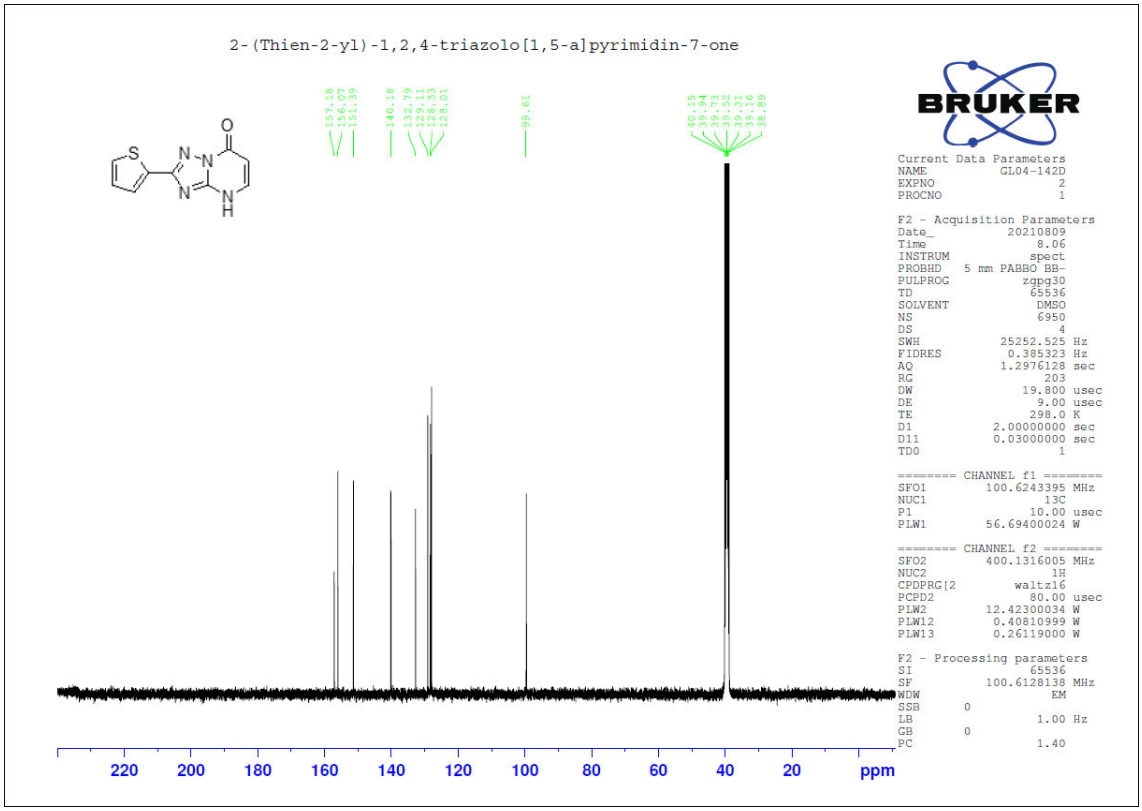

2-(Pyridin-3-yl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210811
Time 12.11
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(Pyridin-3-yl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
BRUKER
Current Data Parameters
NAME GL04-145B
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210814
Time 1.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128156 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

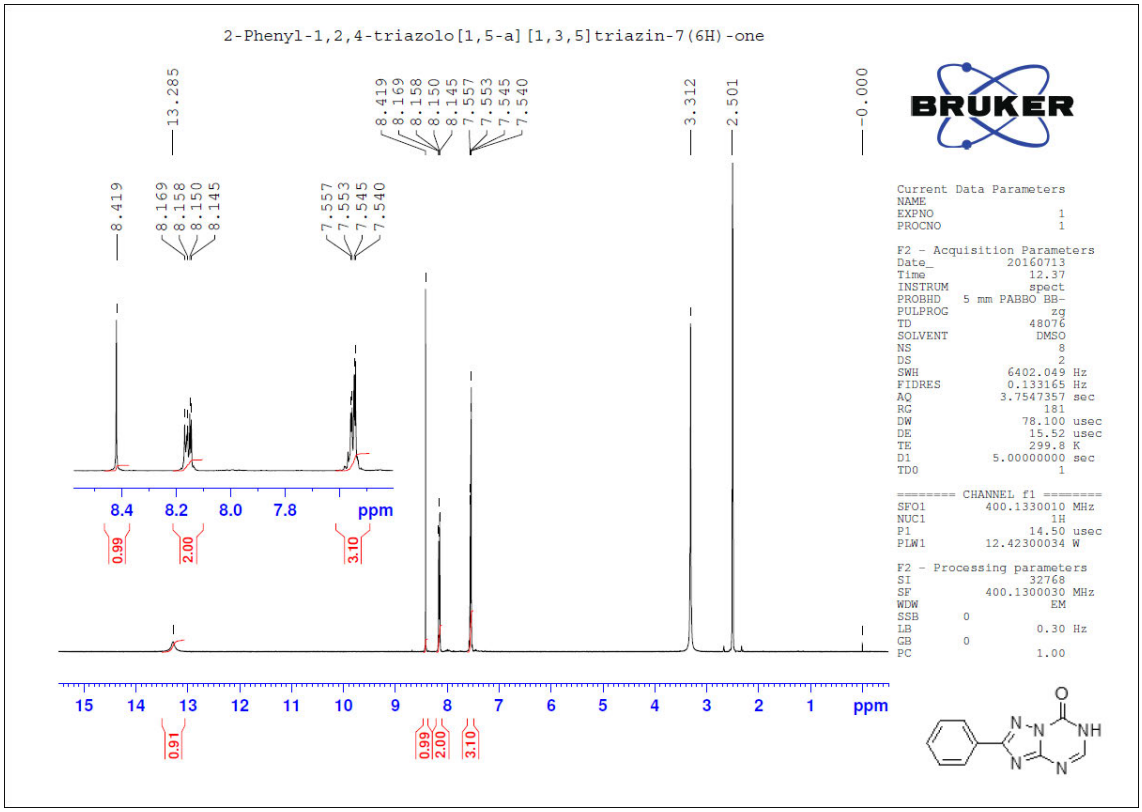

2-(Pyridin-4-yl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
13.472
8.763
8.052
8.033
8.020
6.013
5.994
3.328
2.500
ppm
1.94
3.00
0.95
0.70
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210813
Time 14.58
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300029 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

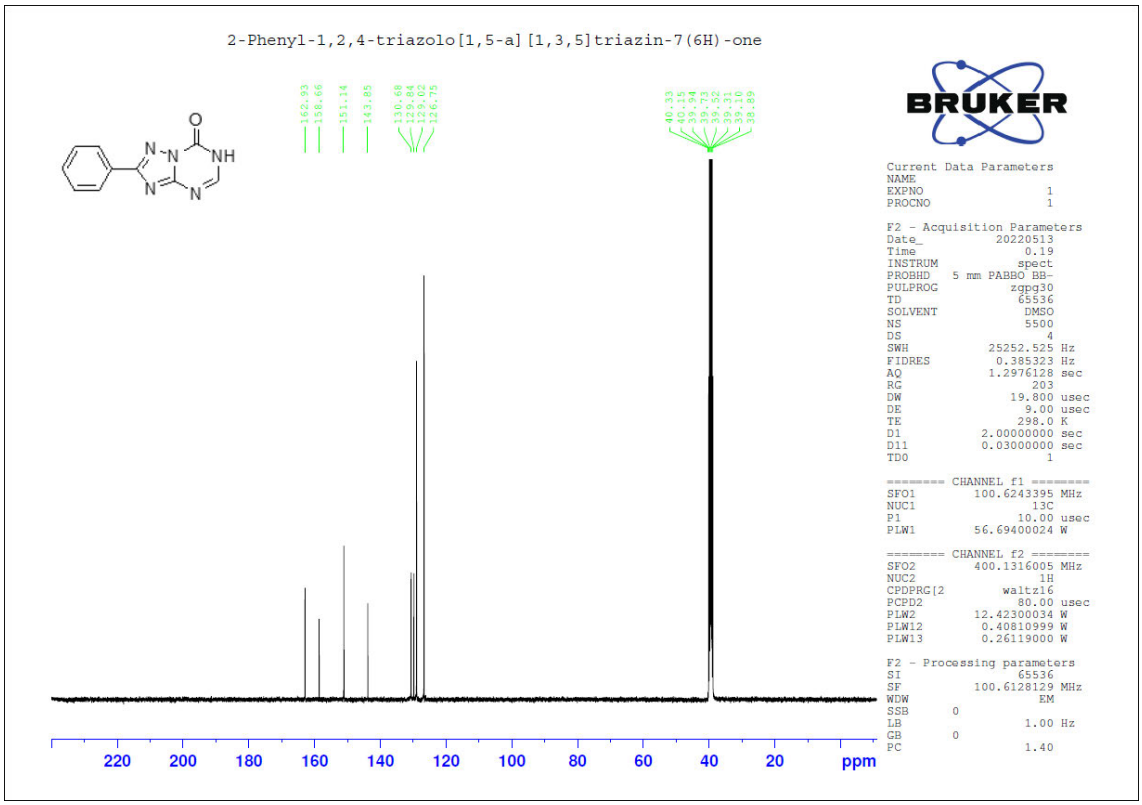

2-(Pyridin-4-yl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
158.90
156.17
151.76
150.56
140.62
137.82
120.75
99.63
40.15
39.94
39.73
39.52
39.31
39.10
38.89
ppm
Current Data Parameters
NAME
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20211014
Time 8.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13900
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128148 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

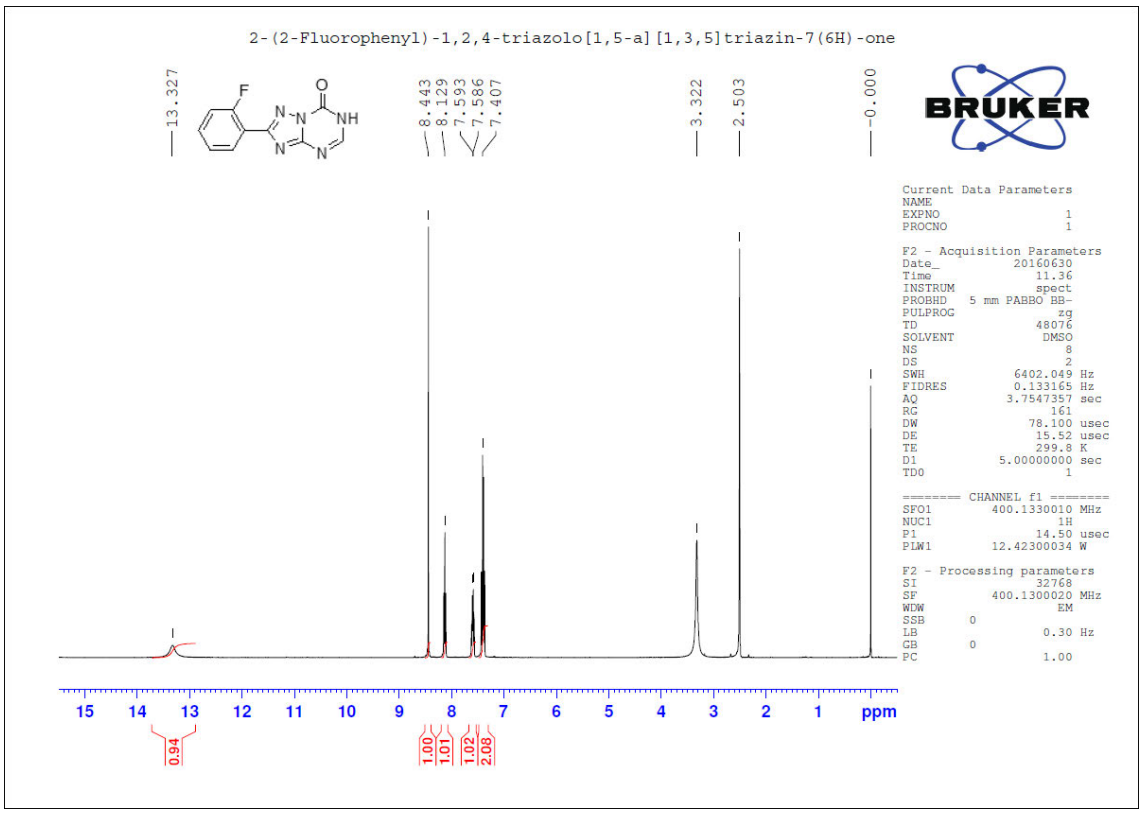

2-(Thien-2-yl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
7.992
7.973
7.766
7.763
7.757
7.754
7.742
7.739
7.228
7.219
7.215
7.206
5.962
5.943
3.337
2.500
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0 ppm
1.09
2.00
1.09
1.01
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210719
Time 10.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300027 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

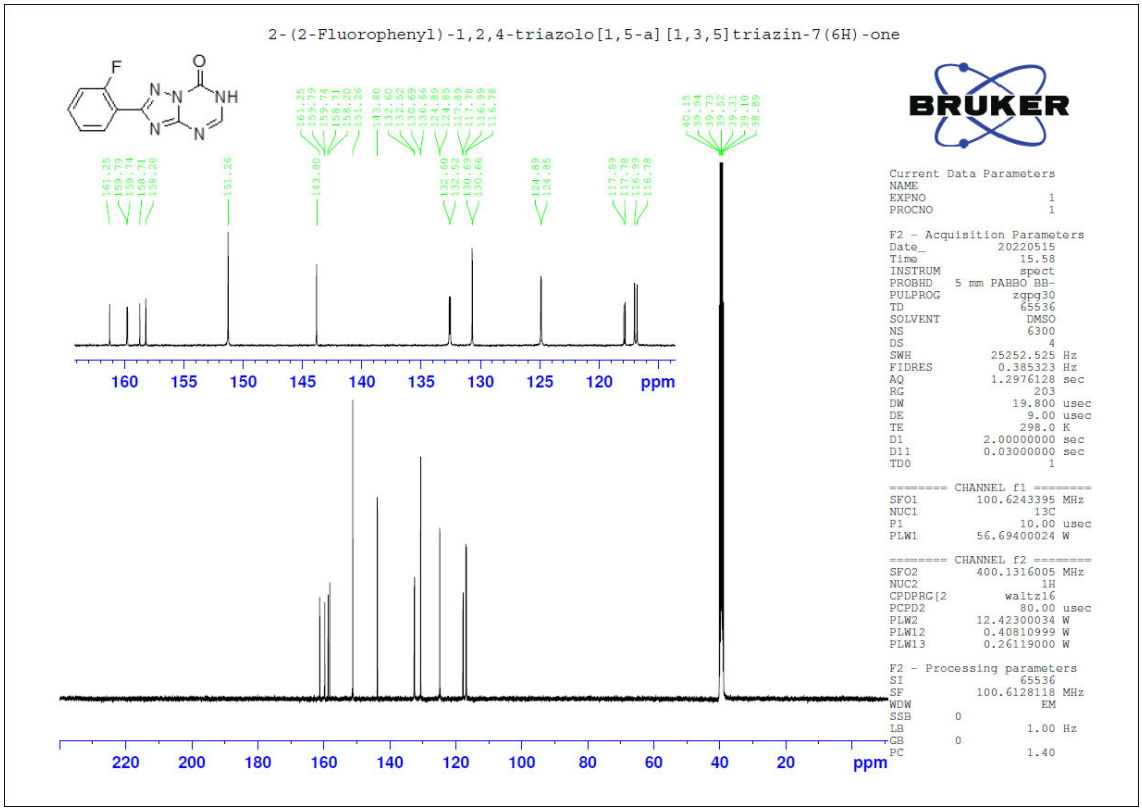

2-(Thien-2-yl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one
157.18
156.07
151.39
140.18
132.79
129.11
128.33
128.01
99.61
40.15
39.94
39.73
39.52
39.31
39.10
38.89
220
200
180
160
140
120
100
80
60
40
20
ppm
BRUKER
Current Data Parameters
NAME GL04-142D
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20210809
Time 8.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6950
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128138 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

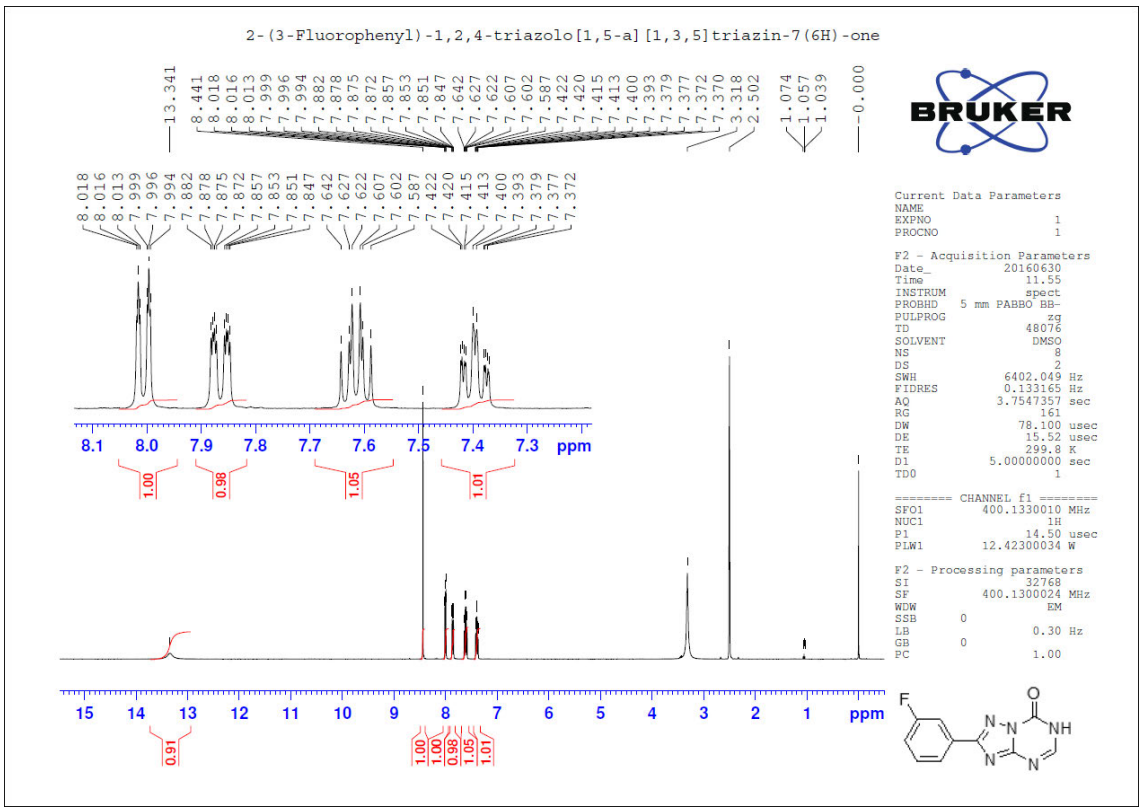
Appendix 5

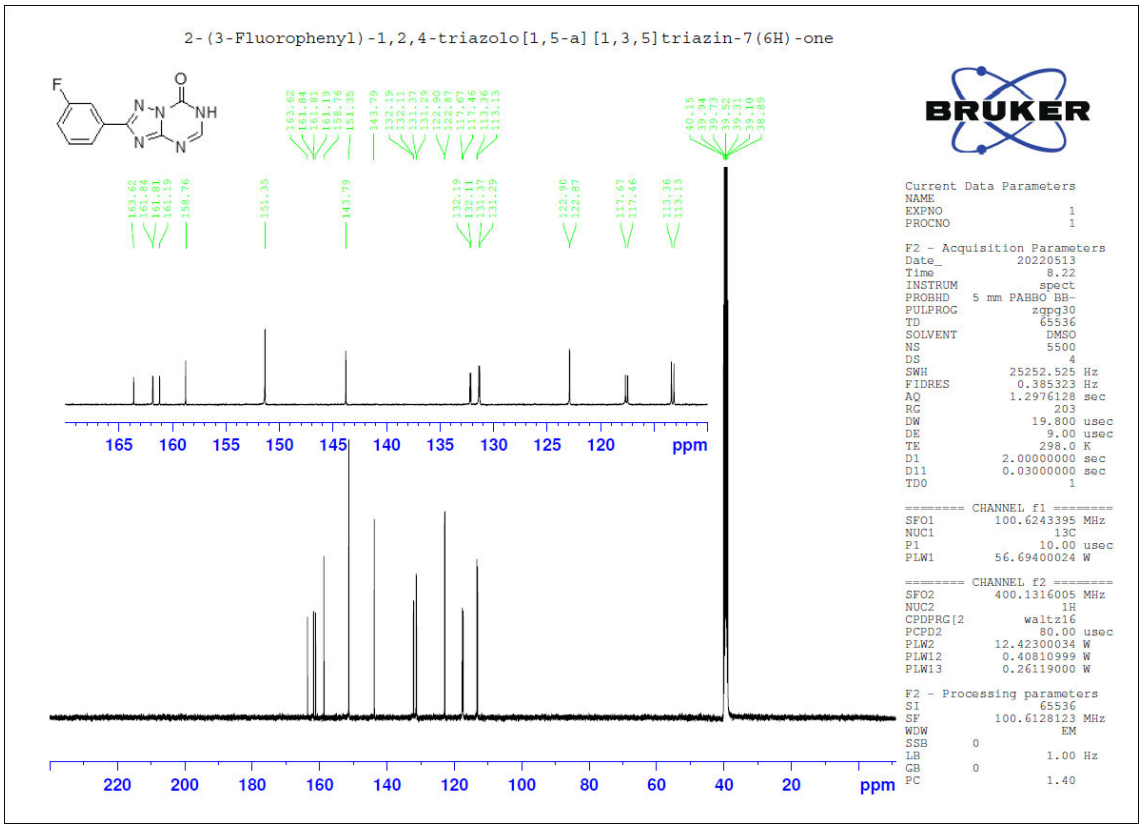

2-Phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.285
8.419
8.169
8.158
8.150
8.145
7.557
7.553
7.545
7.540
3.312
2.501
-0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160713
Time 12.37
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
0.99
2.00
3.10
0.91
NH

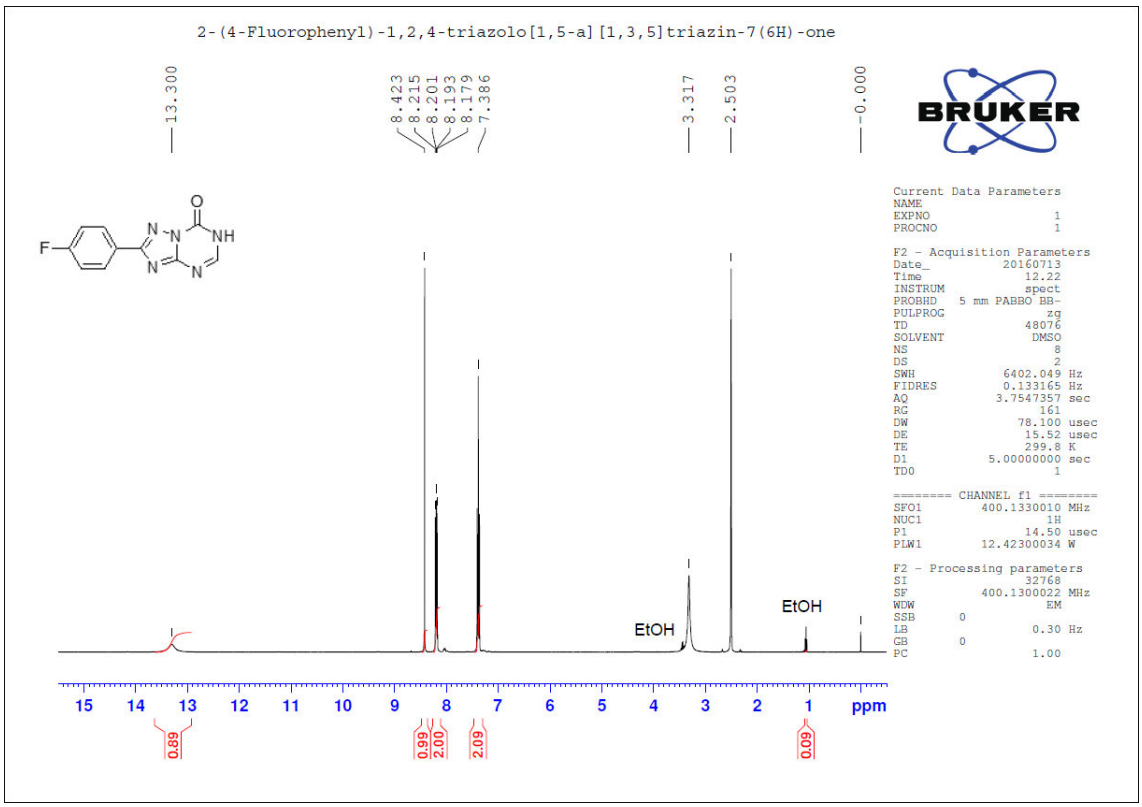

2-Phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
162.93
158.66
151.14
143.85
130.68
129.84
129.02
126.75
40.33
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220513
Time 0.19
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 5500
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128129 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm
NH

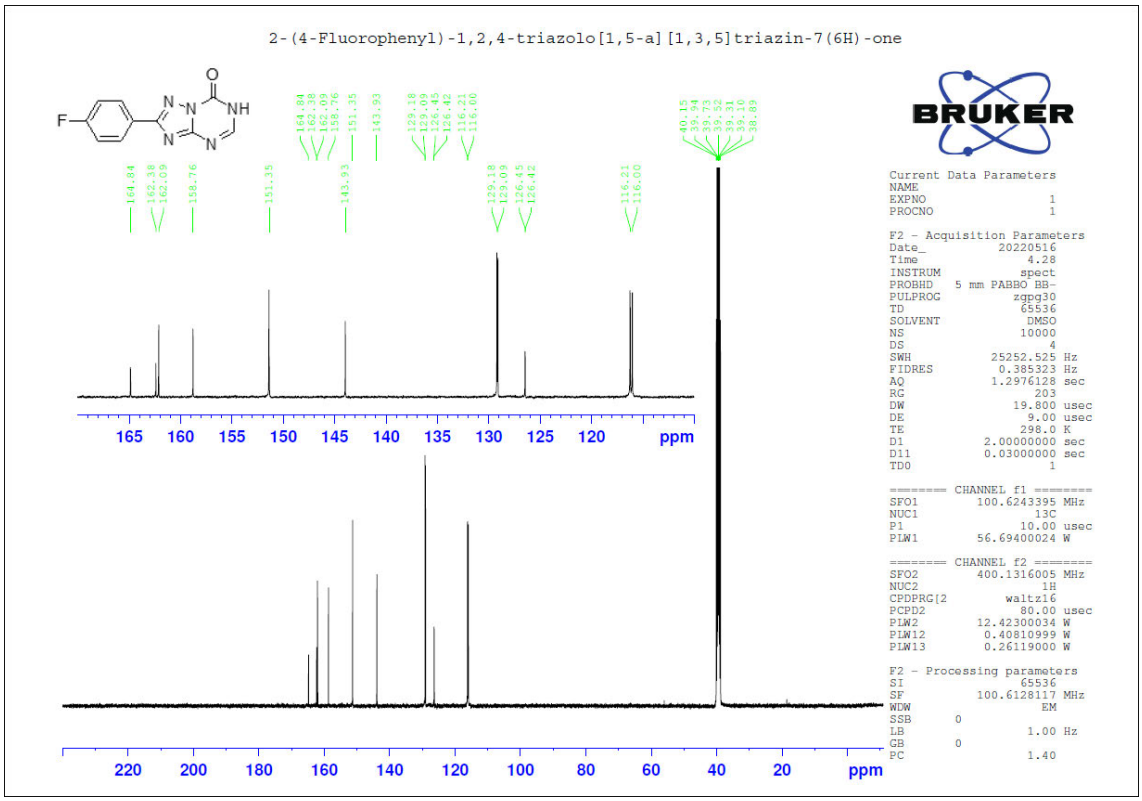

2-(2-Fluorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.327
8.443
8.129
7.593
7.586
7.407
3.322
2.503
0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160630
Time 11.36
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300020 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.94
1.00
1.01
1.02
2.08

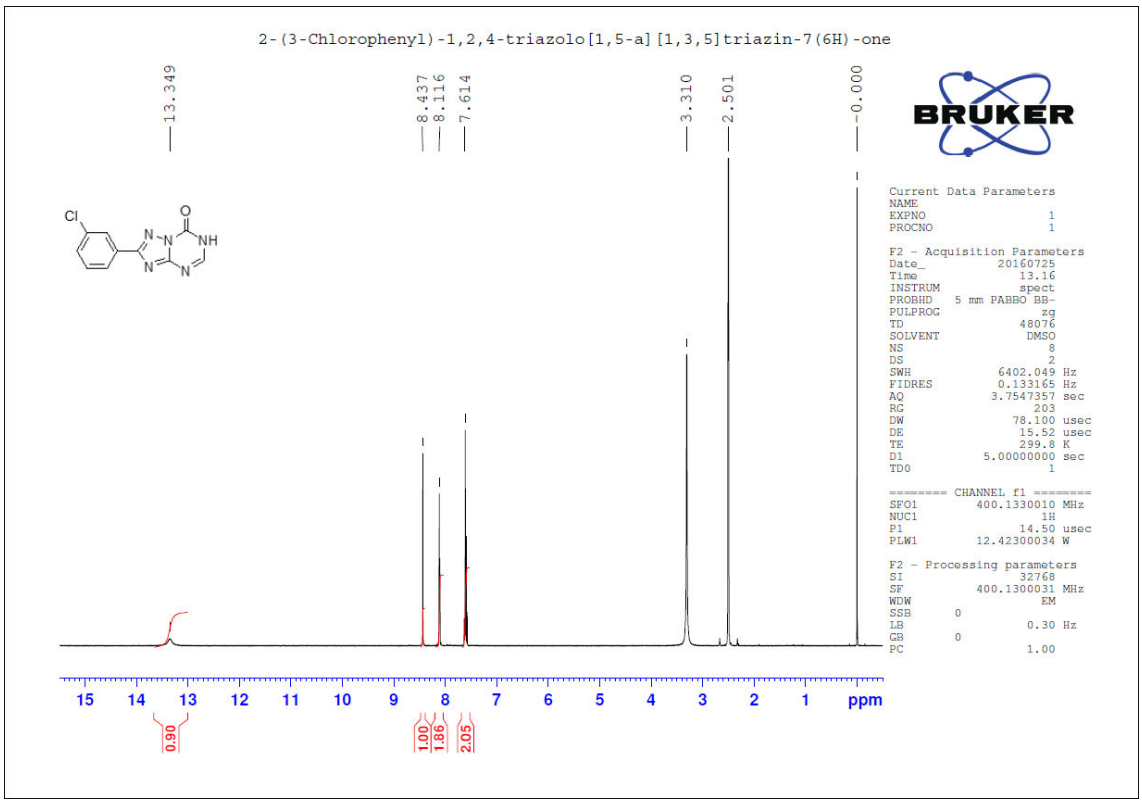

2-(2-Fluorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
161.25
159.79
159.74
158.71
158.20
151.26
143.80
132.60
132.52
130.69
130.66
124.89
124.85
117.89
117.78
116.99
116.78
40.15
39.94
39.73
39.52
39.31
39.10
38.89
160 155 150 145 140 135 130 125 120 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220515
Time 15.58
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6300
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128118 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

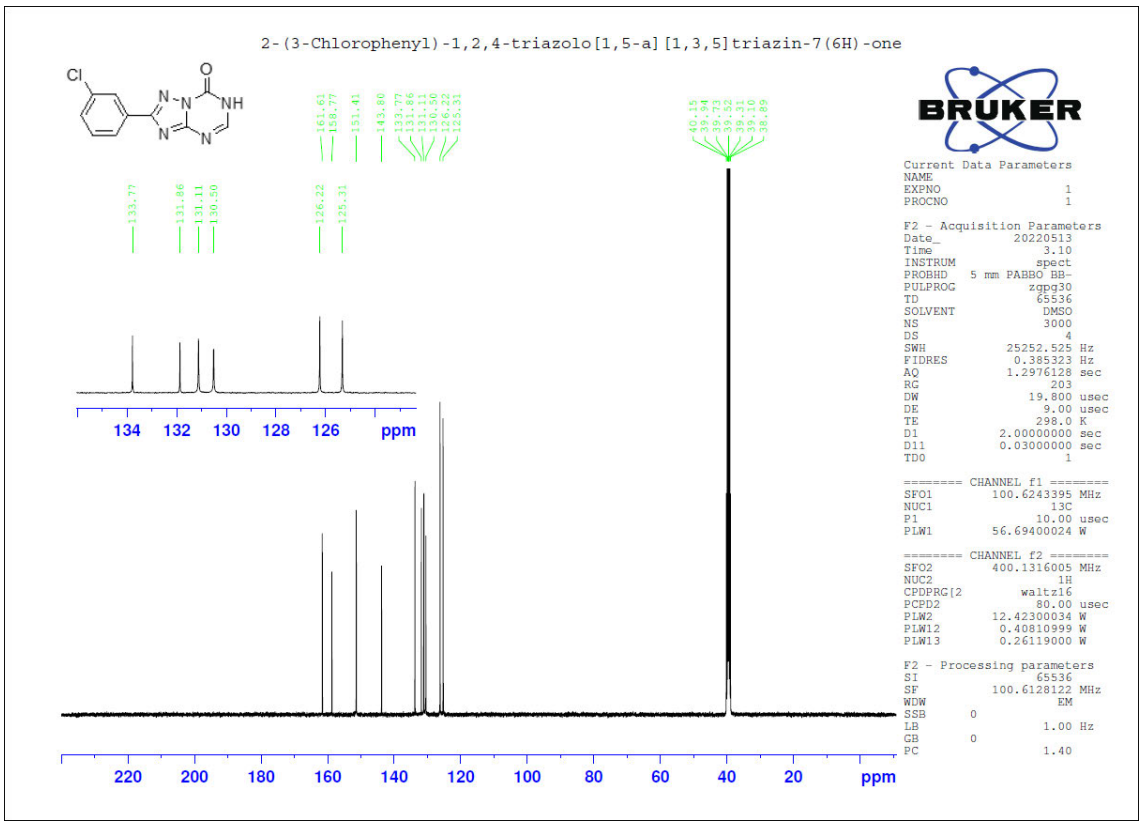

2-(3-Fluorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160630
Time 11.55
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300024 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

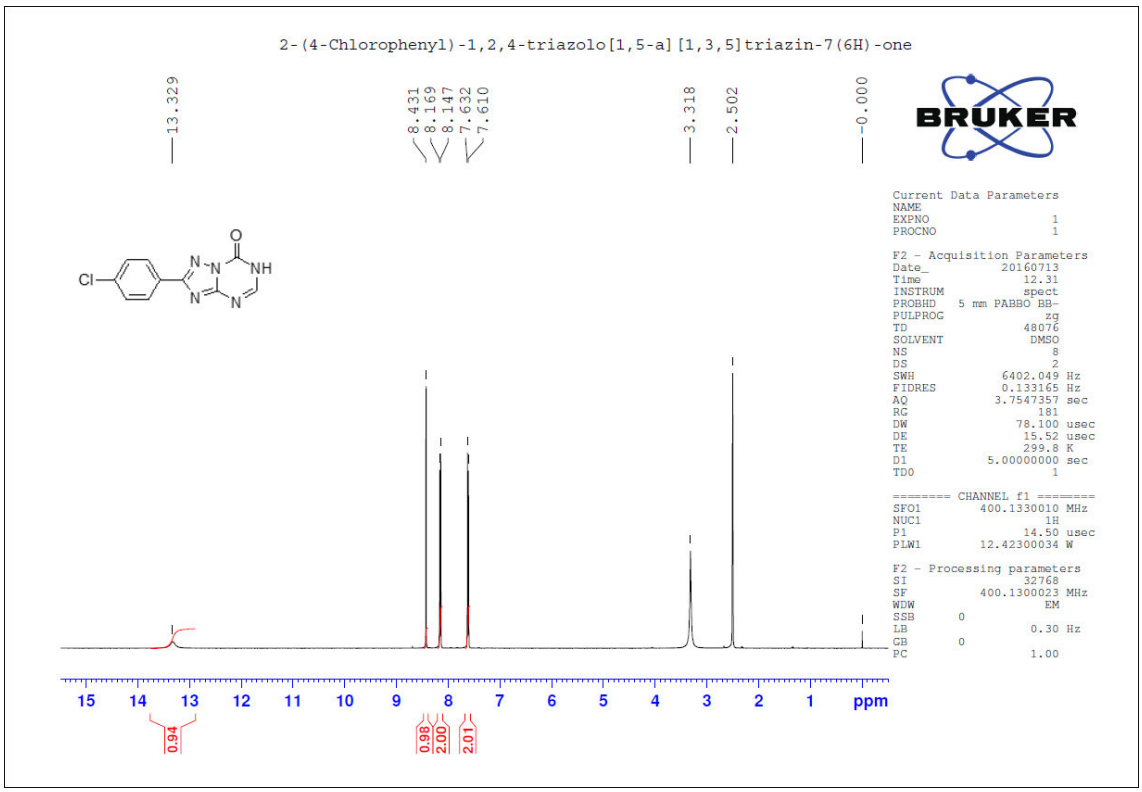

2-(3-Fluorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220513
Time 8.22
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 5500
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128123 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm

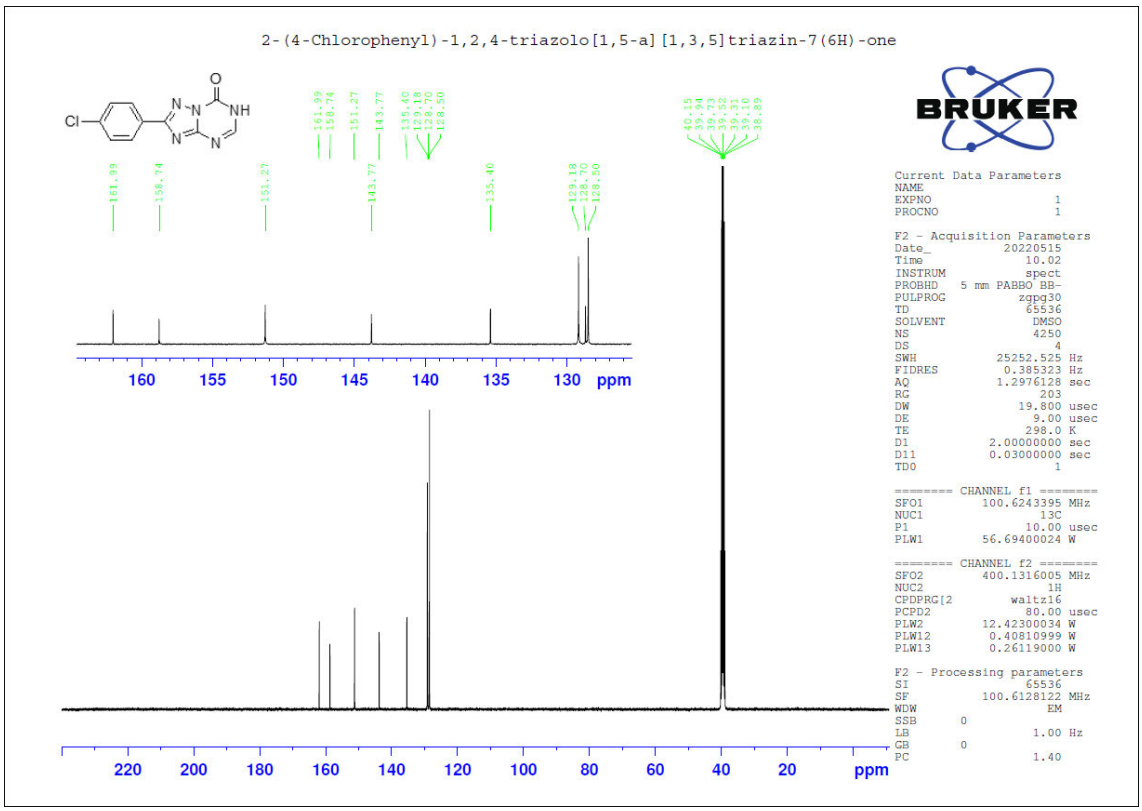

2-(4-Fluorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.300
8.423
8.215
8.201
8.193
8.179
7.386
3.317
2.503
0.000
EtOH
EtOH
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.89
0.99
2.00
2.09
0.09
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160713
Time 12.22
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300022 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

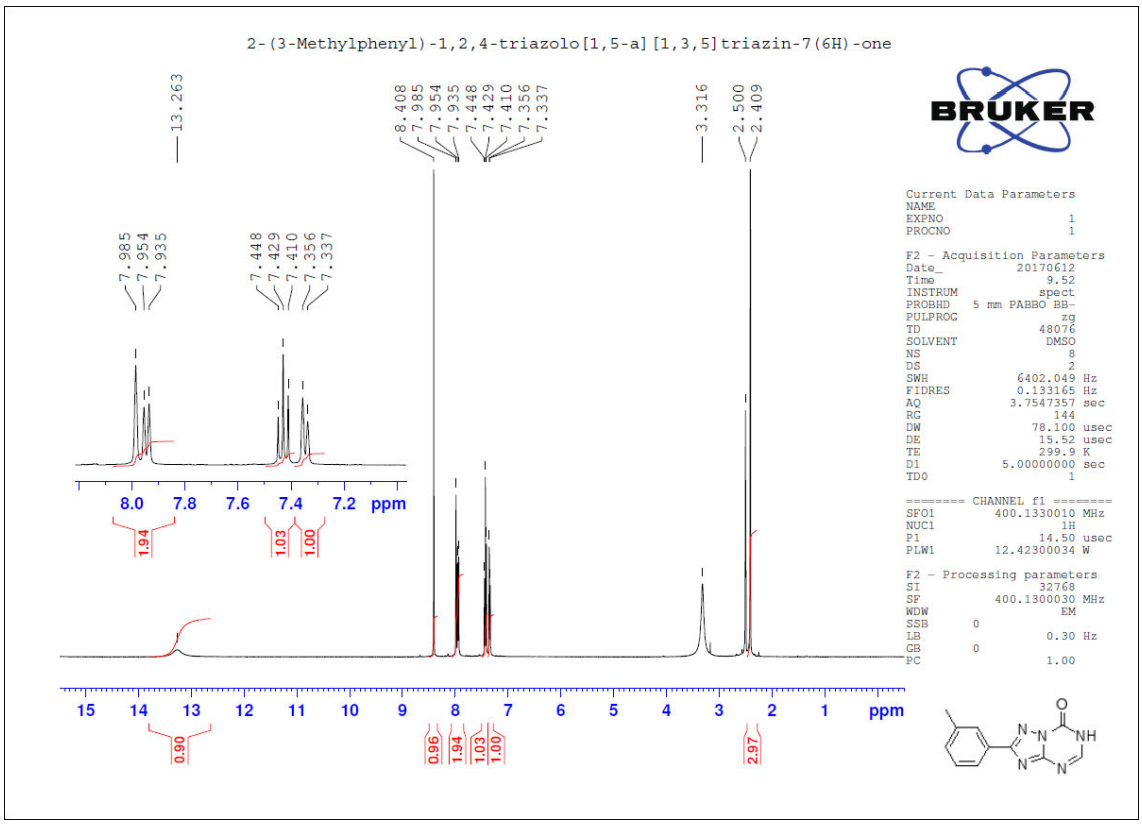

2-(4-Fluorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
164.84
162.38
162.09
158.76
151.35
143.93
129.18
129.09
126.45
126.42
116.21
116.00
40.15
39.94
39.73
39.52
39.31
39.10
38.89
165 160 155 150 145 140 135 130 125 120 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220516
Time 4.28
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 10000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128117 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

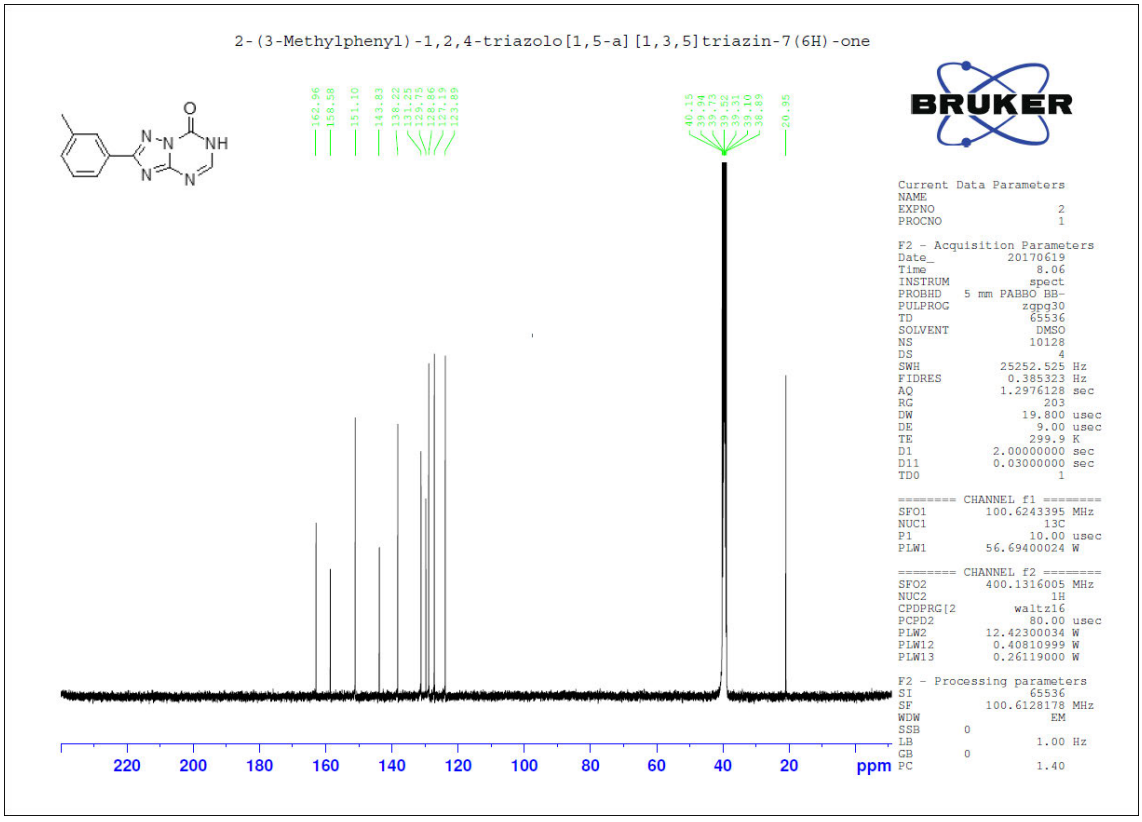

2-(3-Chlorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.349
8.437
8.116
7.614
3.310
2.501
-0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160725
Time 13.16
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.90
1.00
1.86
2.05

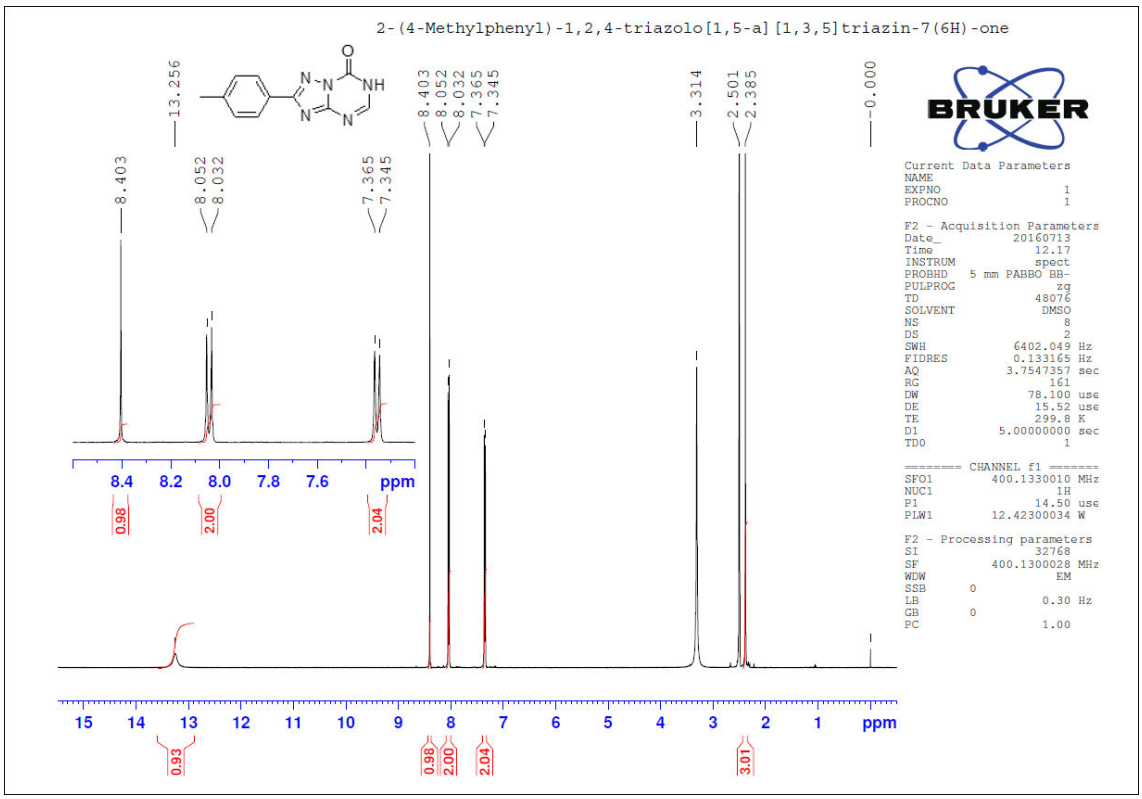

2-(3-Chlorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
161.61
158.77
151.41
143.80
133.77
131.86
131.11
130.50
126.22
125.31
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220513
Time 3.10
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 3000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128122 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
134 132 130 128 126 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm

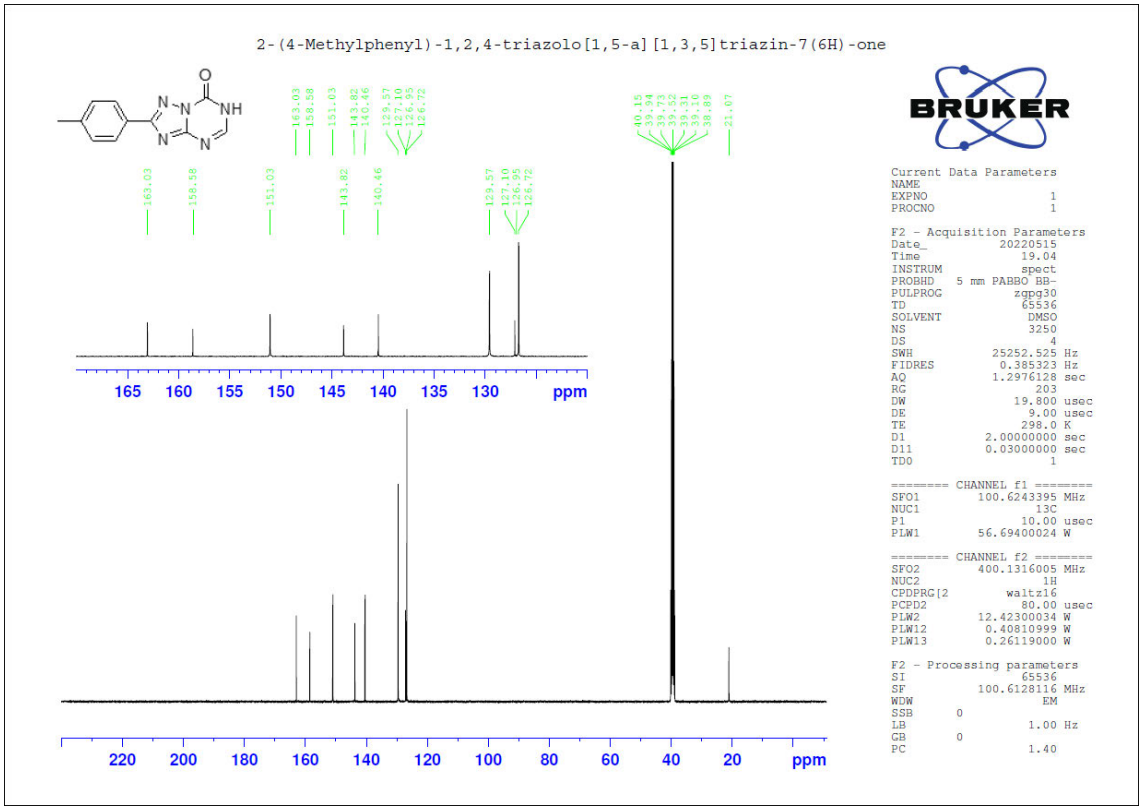

2-(4-Chlorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.329
8.431
8.169
8.147
7.632
7.610
3.318
2.502
0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160713
Time 12.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 181
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300023 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.94
0.98
2.00
2.01

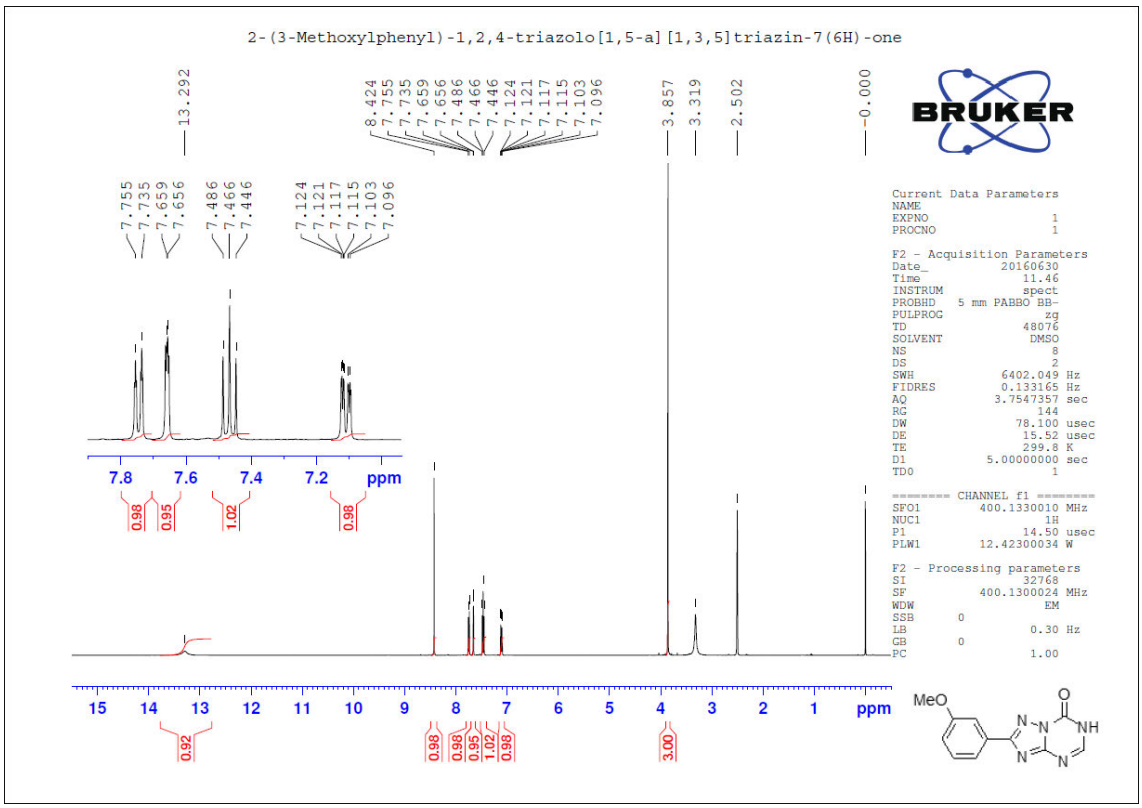

2-(4-Chlorophenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
161.99
158.74
151.27
143.77
135.40
129.18
128.70
128.50
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220515
Time 10.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 4250
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128122 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
160 155 150 145 140 135 130 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm

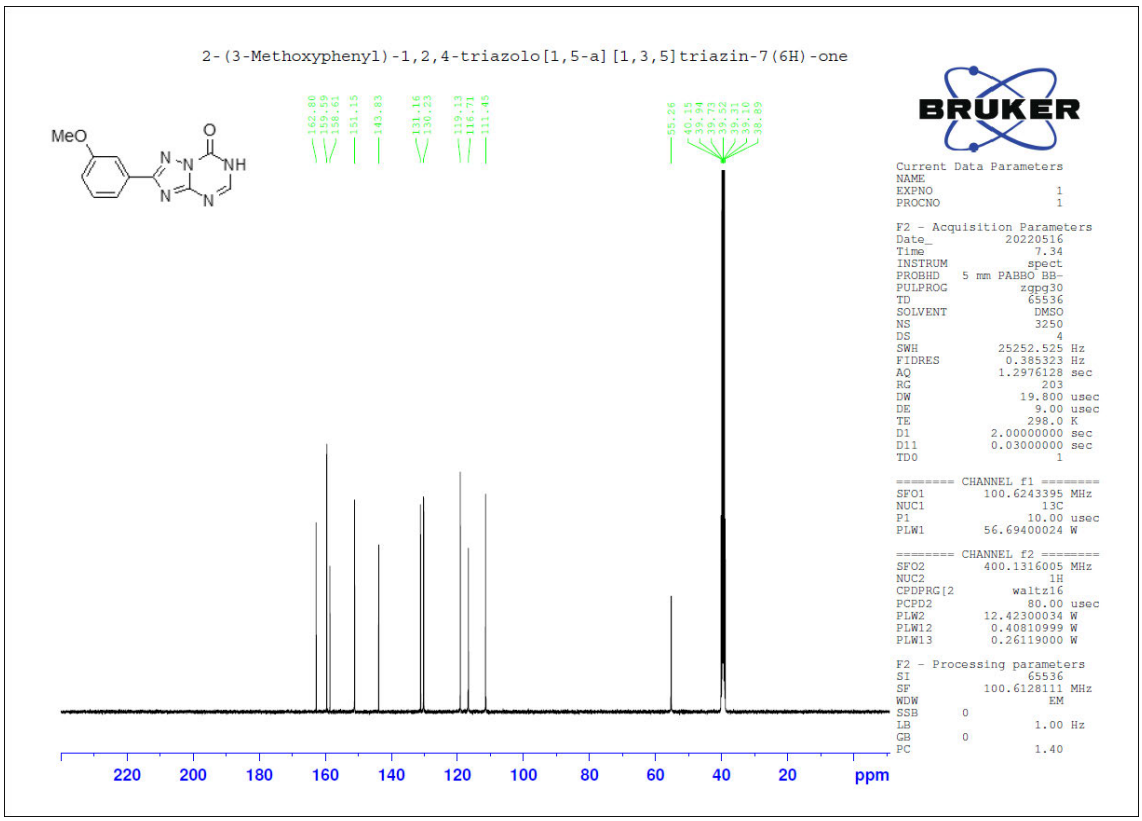

2-(3-Methylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
BRUKER
13.263
8.408
7.985
7.954
7.935
7.448
7.429
7.410
7.356
7.337
3.316
2.500
2.409
8.0
7.8
7.6
7.4
7.2
ppm
1.94
1.03
1.00
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.90
0.96
1.94
1.03
1.00
2.97
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170612
Time 9.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 144
DW 78.100 usec
DE 15.52 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
O
NH
N
N
N
N


2-(3-Methylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
BRUKER
O
NH
N
N
N
N
162.96
158.58
151.10
143.83
138.22
131.25
129.75
128.66
127.53
123.89
40.15
39.94
39.73
39.52
39.31
39.10
38.89
20.95
220
200
180
160
140
120
100
80
60
40
20
ppm
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170619
Time 8.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 10128
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128178 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

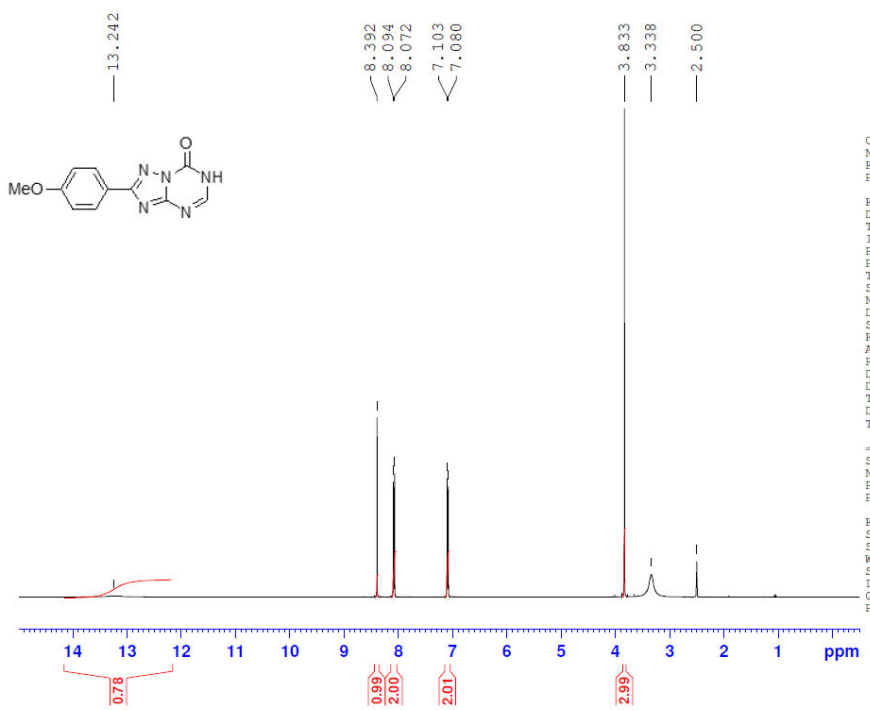

2-(4-Methylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.256
8.403
8.052
8.032
7.365
7.345
3.314
2.501
2.385
0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160713
Time 12.17
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 use
DE 15.52 use
TE 299.8 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 use
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300028 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
0.98
2.00
2.04
0.93
3.01


2-(4-Methylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
163.03
158.58
151.03
143.82
140.46
129.57
127.10
126.95
126.72
40.15
39.94
39.73
39.52
39.31
39.10
38.89
21.07
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220515
Time 19.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 3250
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
CHANNEL f2
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128116 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm


2-(3-Methoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.292
8.424
7.755
7.735
7.659
7.656
7.486
7.466
7.446
7.124
7.121
7.117
7.115
7.103
7.096
3.857
3.319
2.502
-0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160630
Time 11.46
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 144
DW 78.100 usec
DE 15.52 usec
TE 299.8 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300024 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
MeO
NH
ppm


2-(3-Methoxypheny1)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
162.80
159.59
158.61
151.15
143.83
131.16
130.23
119.13
116.71
111.45
55.26
40.15
39.94
39.73
39.52
39.31
39.10
38.89
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220516
Time 7.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 3250
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128111 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
MeO
NH
ppm

2-(4-Methoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one



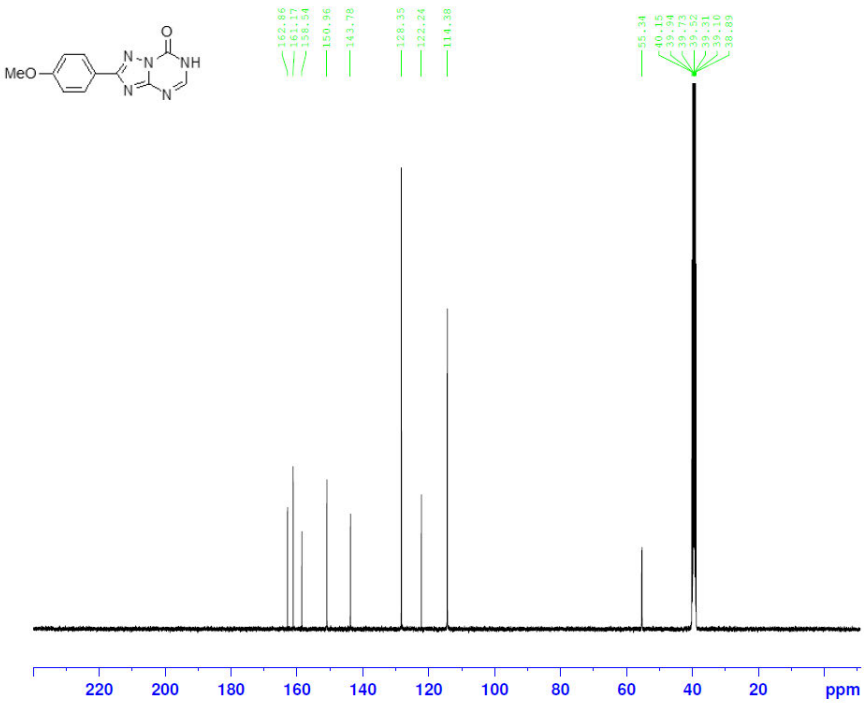

```
Current Data Parameters
NAME
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20220518
Time              19.06
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG              zg
TD                48076
SOLVENT            DMSO
NS                    8
DS                    2
SWH            6402.049 Hz
FIDRES         0.133165 Hz
AQ             3.7547357 sec
RG                  128
DW               78.100 usec
DE               15.52 usec
TE                298.0 K
D1           5.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1328009 MHz
NUC1                 1H
P1                14.50 usec
PLW1         12.42300034 W

F2 - Processing parameters
SI                32768
SF           400.1300033 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2-(4-Methoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one



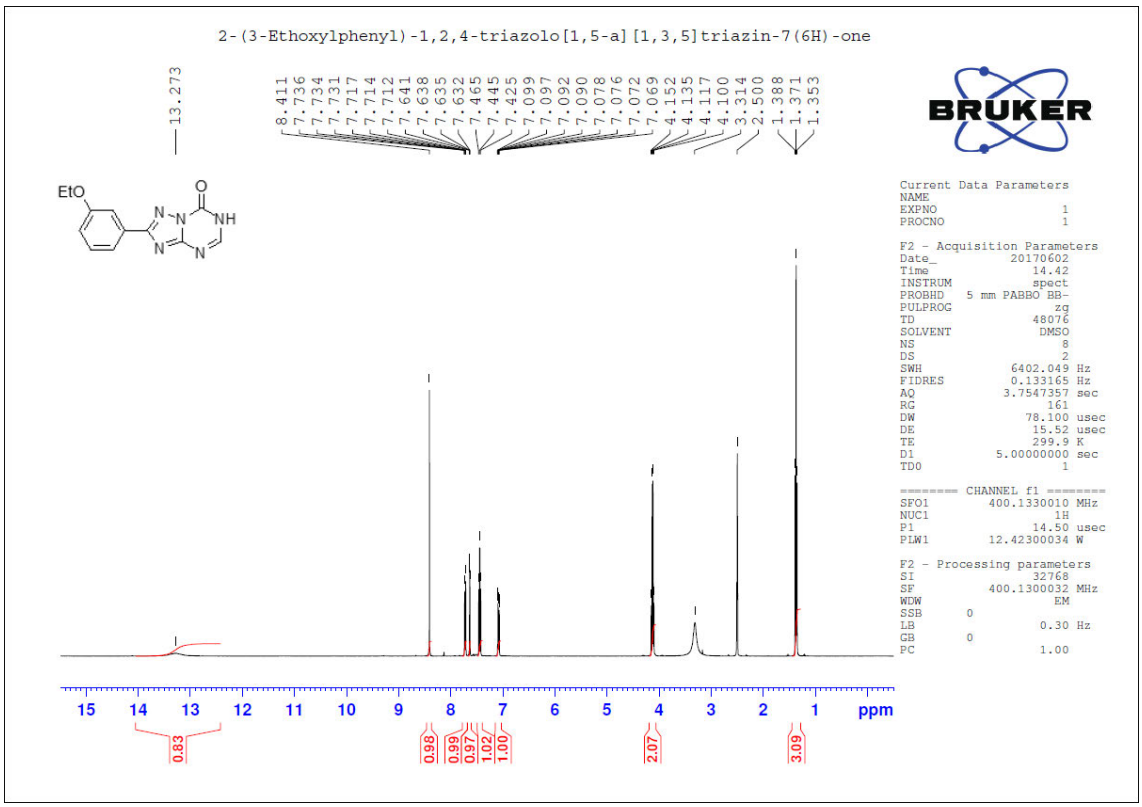

```
Current Data Parameters
NAME
EXPNO                  2
PROCNO                 1

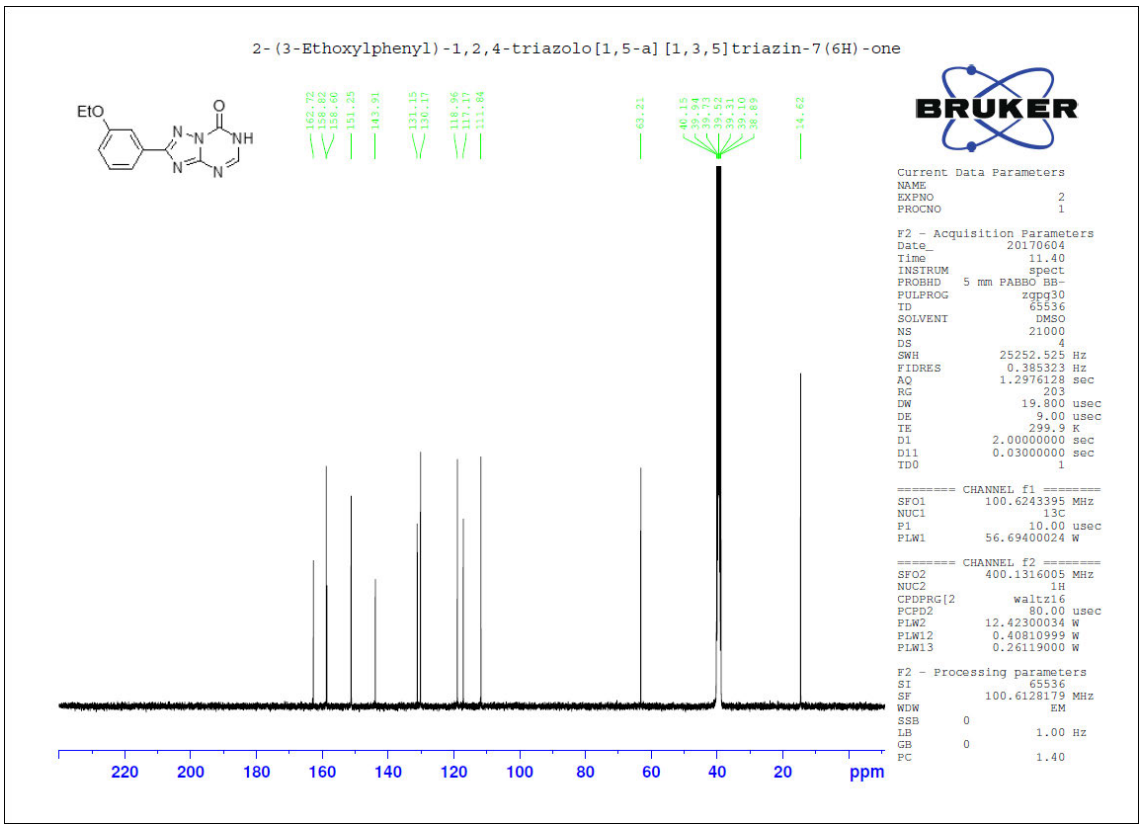
F2 - Acquisition Parameters
Date_           20220519
Time              1.30
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                65536
SOLVENT            DMSO
NS                 6800
DS                    4
SWH           25252.525 Hz
FIDRES         0.385323 Hz
AQ             1.2976128 sec
RG                  203
DW               19.800 usec
DE                9.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

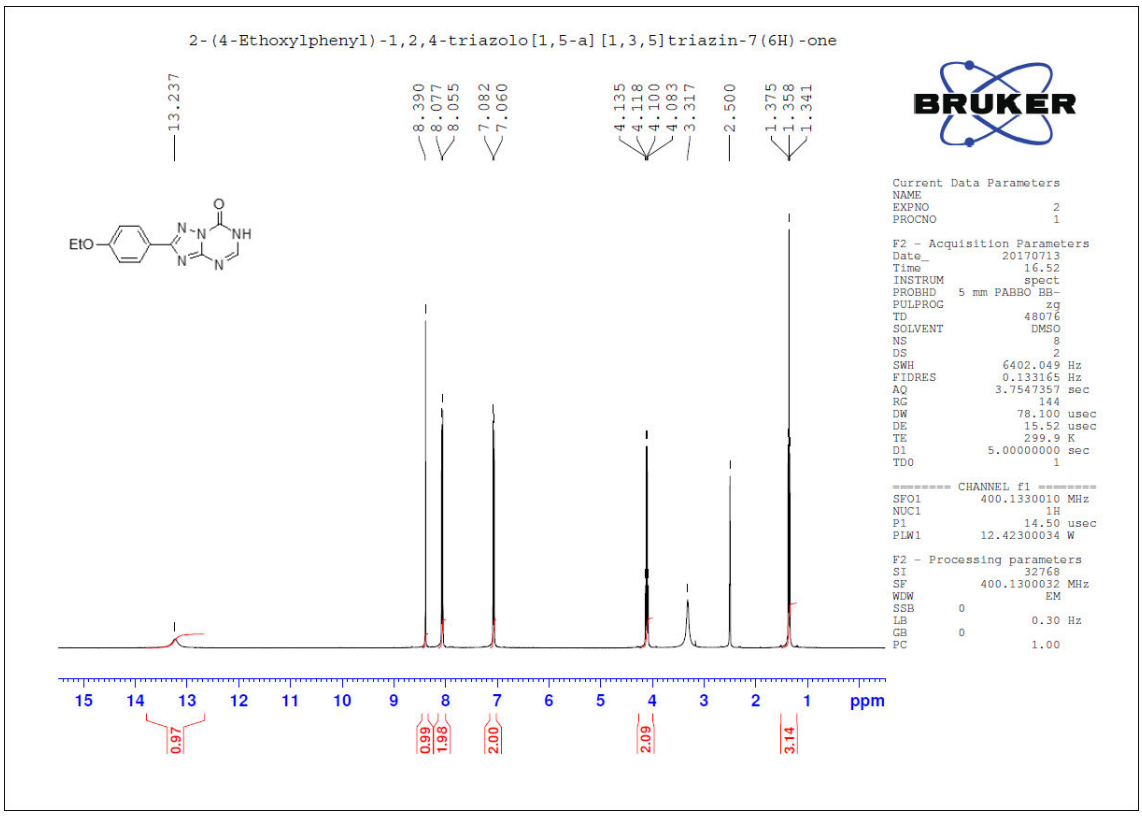
======== CHANNEL f1 ========
SFO1         100.6243395 MHz
NUC1                 13C
P1                10.00 usec
PLW1         56.69400024 W

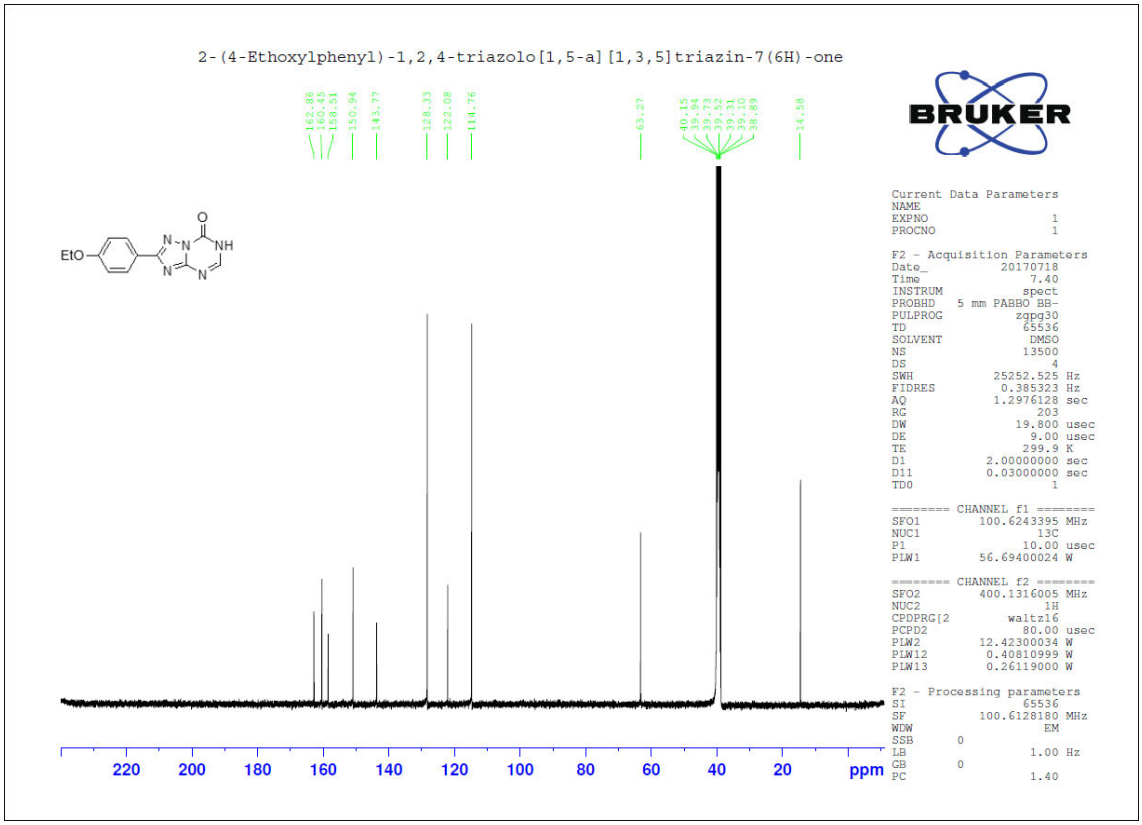
======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         12.42300034 W
PLW12         0.40810999 W
PLW13         0.26119000 W

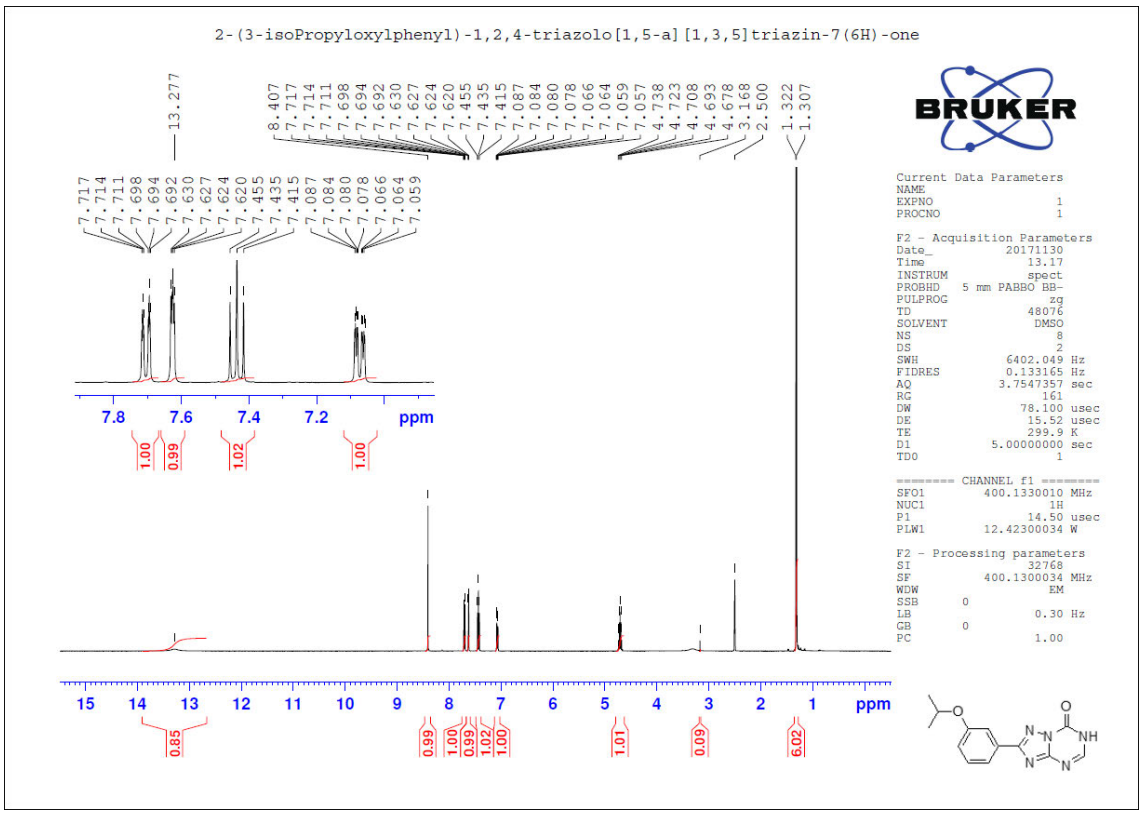
F2 - Processing parameters
SI                65536
SF           100.6128157 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

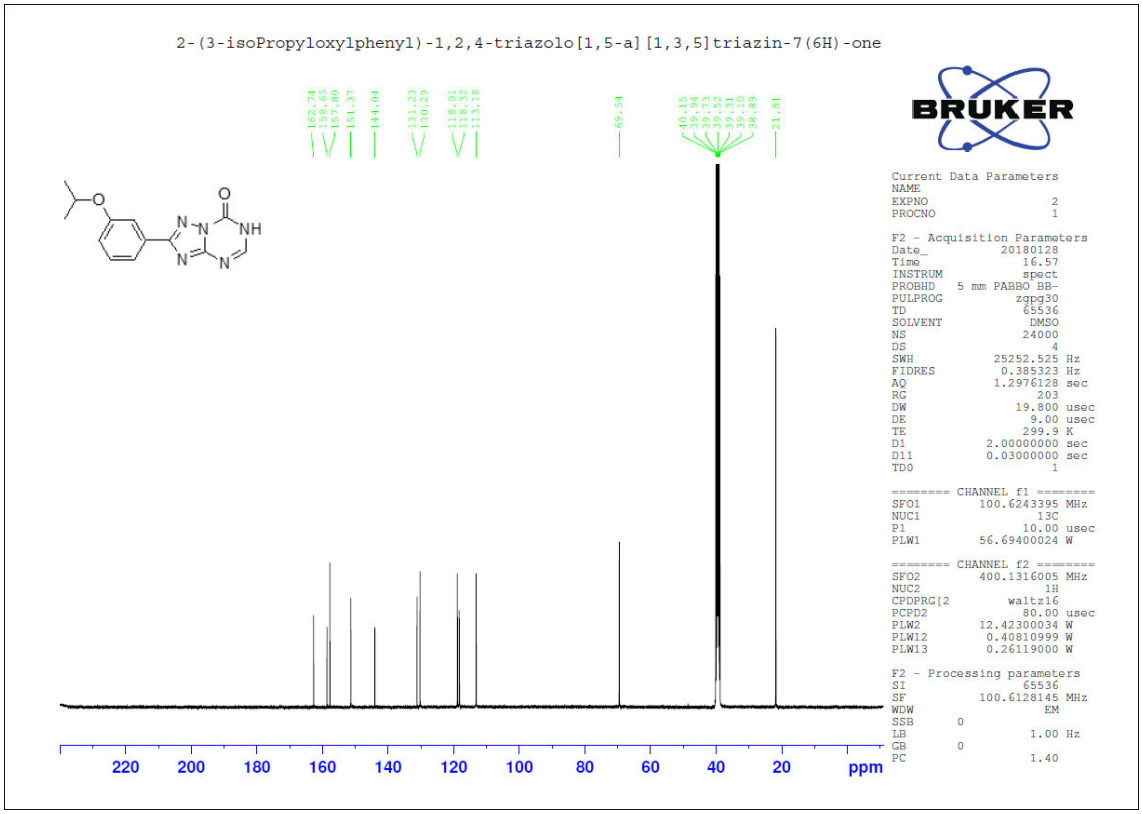

2-(3-Ethoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.273
8.411
7.736
7.734
7.731
7.717
7.714
7.712
7.641
7.638
7.635
7.632
7.465
7.445
7.425
7.099
7.097
7.092
7.090
7.078
7.076
7.072
7.069
4.152
4.135
4.117
4.100
3.314
2.500
1.388
1.371
1.353
EtO
O
NH
N
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170602
Time 14.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.83
0.98
0.99
0.97
1.02
1.00
2.07
3.09

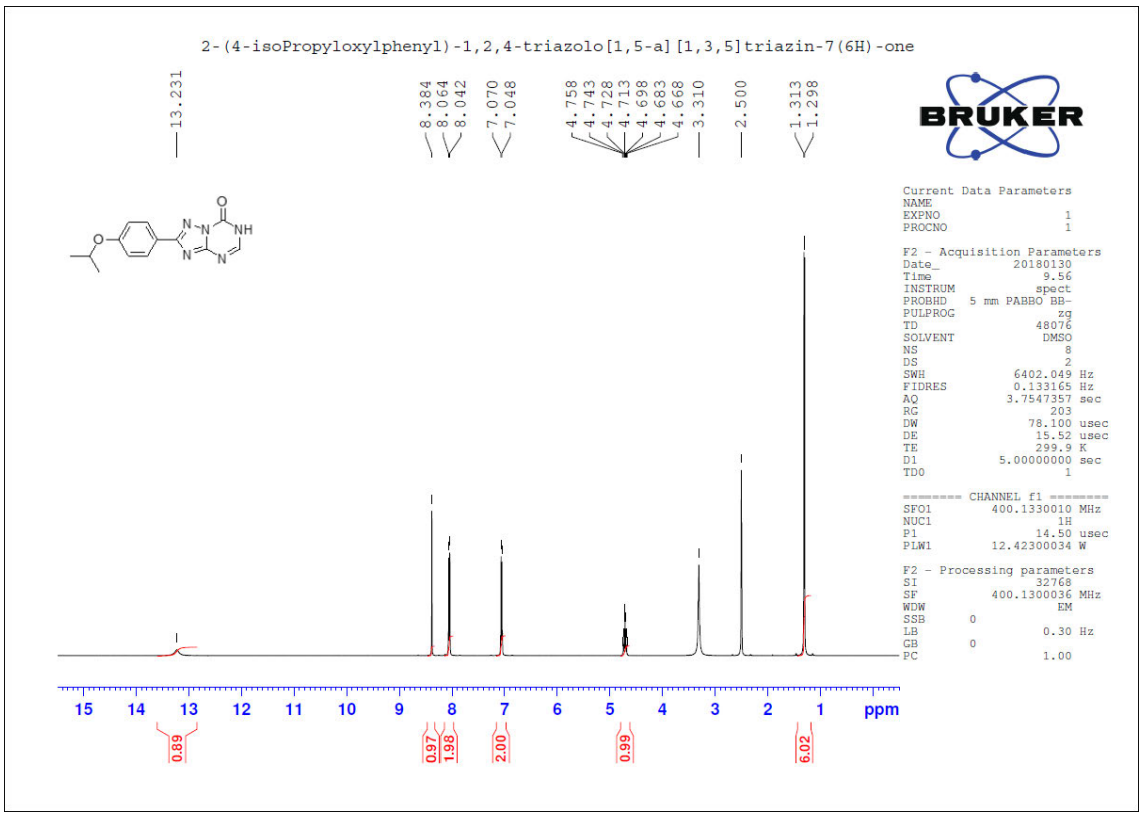

2-(3-Ethoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
EtO
O
NH
N
162.72
158.82
158.60
151.25
143.91
131.15
130.17
118.96
117.17
111.84
63.21
40.15
39.94
39.73
39.52
39.31
39.10
38.89
14.62
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170604
Time 11.40
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 21000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128179 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm

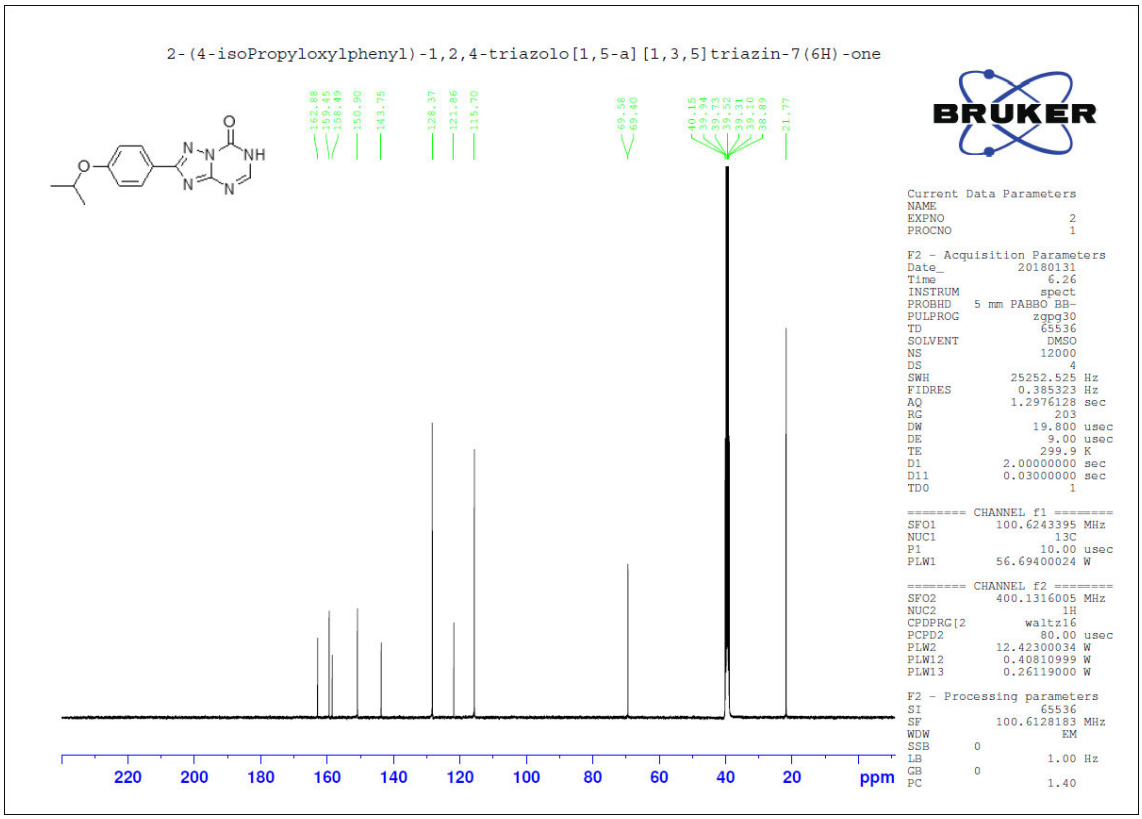

2-(4-Ethoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.237
8.390
8.077
8.055
7.082
7.060
4.135
4.118
4.100
4.083
3.317
2.500
1.375
1.358
1.341
EtO
NH
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.97
0.99
1.98
2.00
2.09
3.14
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170713
Time 16.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 144
DW 78.100 usec
DE 15.52 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

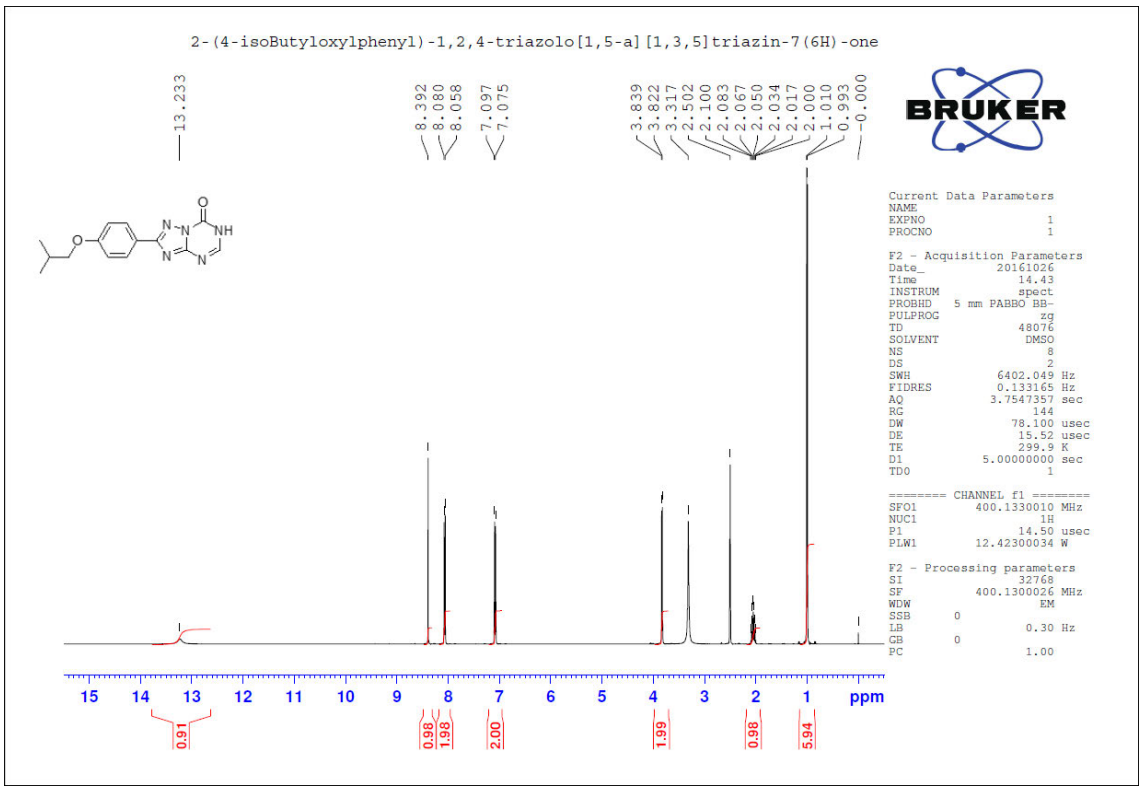

2-(4-Ethoxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
162.86
160.45
158.51
150.94
143.77
128.33
122.08
114.76
63.27
40.15
39.94
39.73
39.52
39.31
39.10
38.89
14.58
EtO
NH
220 200 180 160 140 120 100 80 60 40 20 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170718
Time 7.40
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 13500
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128180 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

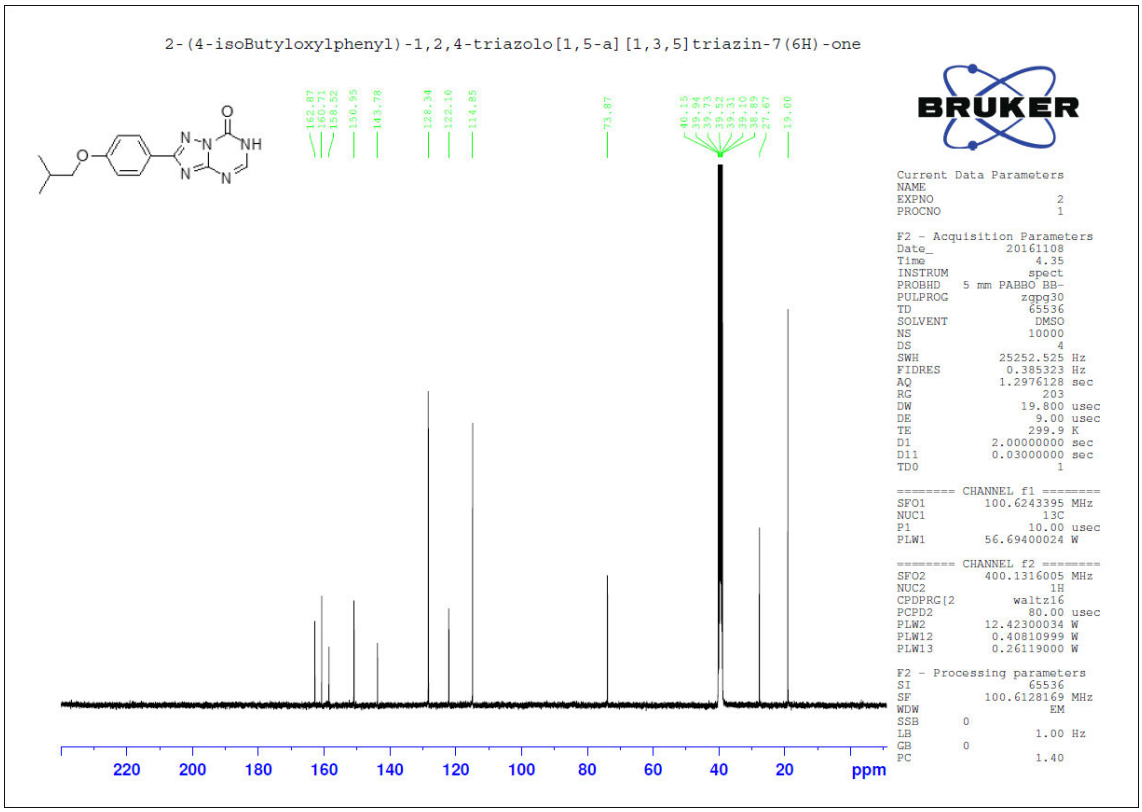

2-(3-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171130
Time 13.17
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300034 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

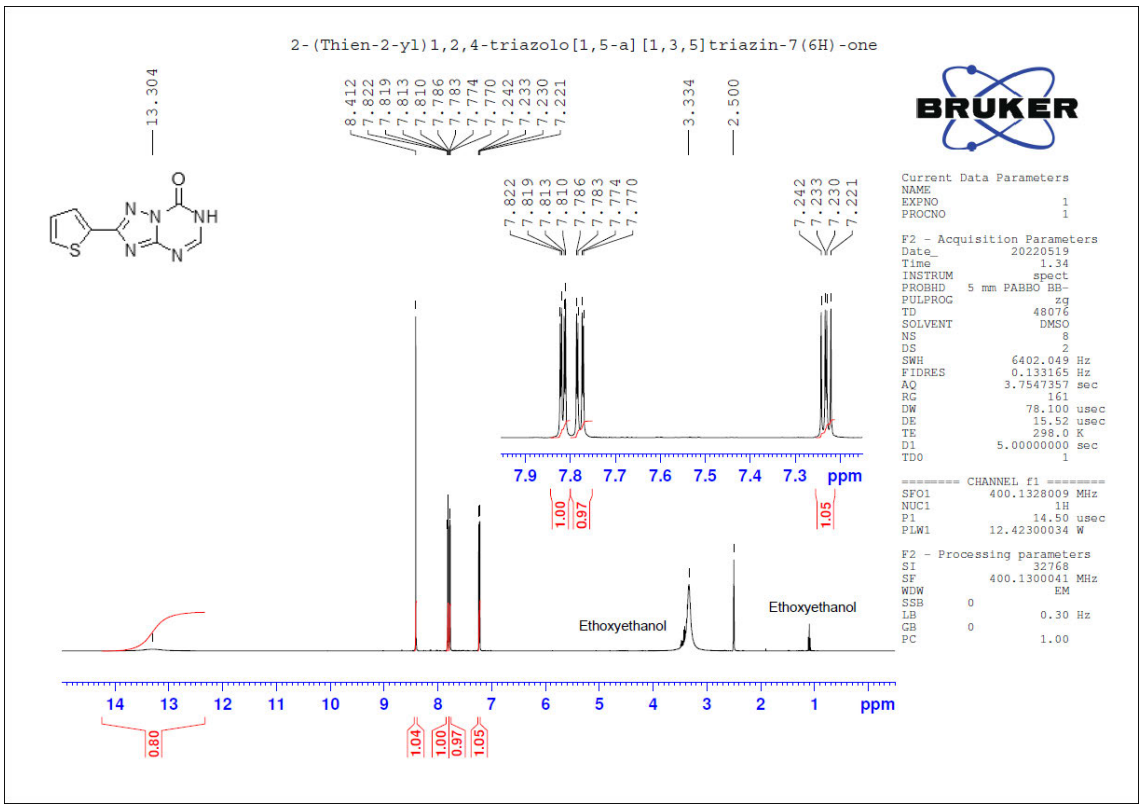

2-(3-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180128
Time 16.57
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 24000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128145 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm


2-(4-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.231
8.384
8.064
8.042
7.070
7.048
4.758
4.743
4.728
4.713
4.698
4.683
4.668
3.310
2.500
1.313
1.298
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180130
Time 9.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 203
DW 78.100 usec
DE 15.52 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300036 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.89 0.97 1.98 2.00 0.99 6.02


2-(4-isoPropyloxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
162.88
159.45
158.49
150.90
143.75
128.37
121.86
115.70
69.58
69.40
40.15
39.94
39.73
39.52
39.31
39.10
38.89
21.77
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180131
Time 6.26
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 12000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128183 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm


2-(4-isoButyloxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.233
8.392
8.080
8.058
7.097
7.075
3.839
3.822
3.317
2.502
2.100
2.083
2.067
2.050
2.034
2.017
2.000
1.010
0.993
-0.000
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161026
Time 14.43
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 144
DW 78.100 usec
DE 15.52 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300026 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.91
0.98
1.98
2.00
1.99
0.98
5.94


2-(4-isoButyloxylphenyl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
162.87
160.71
158.52
150.95
143.78
128.34
122.10
114.85
73.87
40.15
39.94
39.73
39.52
39.31
39.10
38.89
27.67
19.00
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161108
Time 4.35
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 10000
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128169 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm


2-(Thien-2-yl)1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.304
8.412
7.822
7.819
7.813
7.810
7.786
7.783
7.774
7.770
7.242
7.233
7.230
7.221
3.334
2.500
Ethoxyethanol
Ethoxyethanol
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220519
Time 1.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 6402.049 Hz
FIDRES 0.133165 Hz
AQ 3.7547357 sec
RG 161
DW 78.100 usec
DE 15.52 usec
TE 298.0 K
D1 5.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.1328009 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
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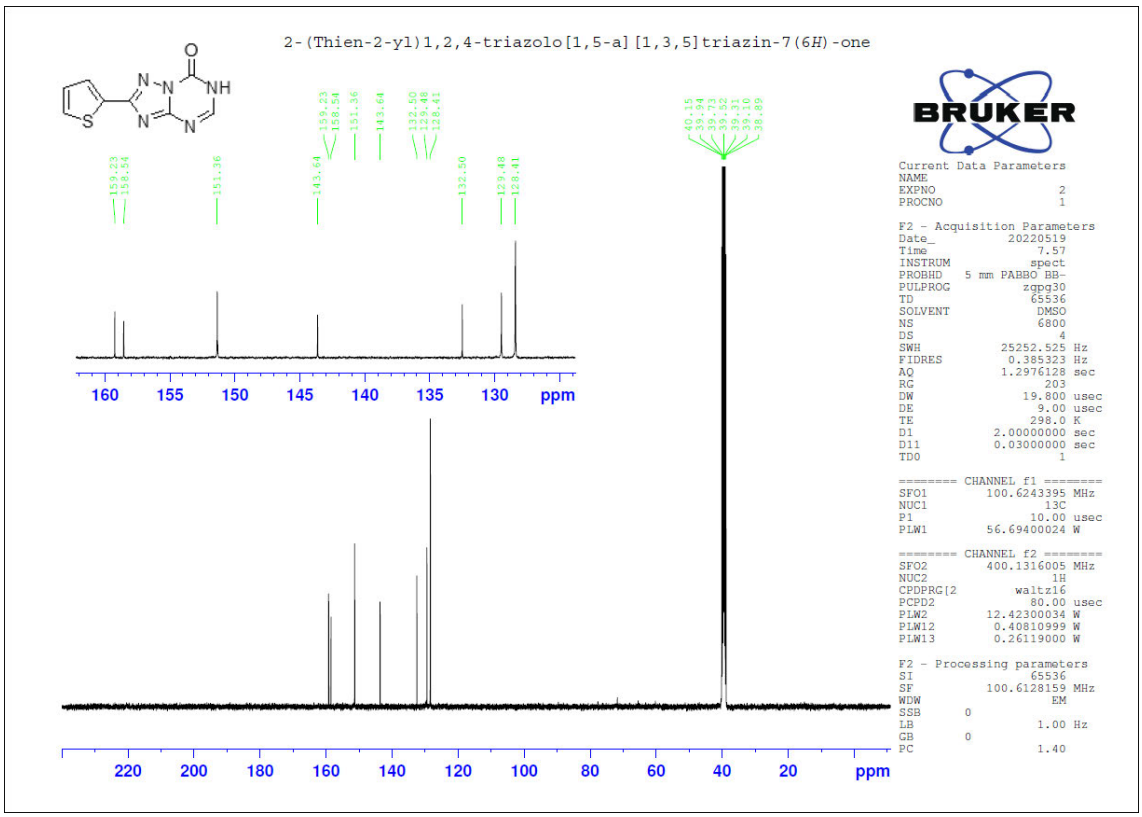
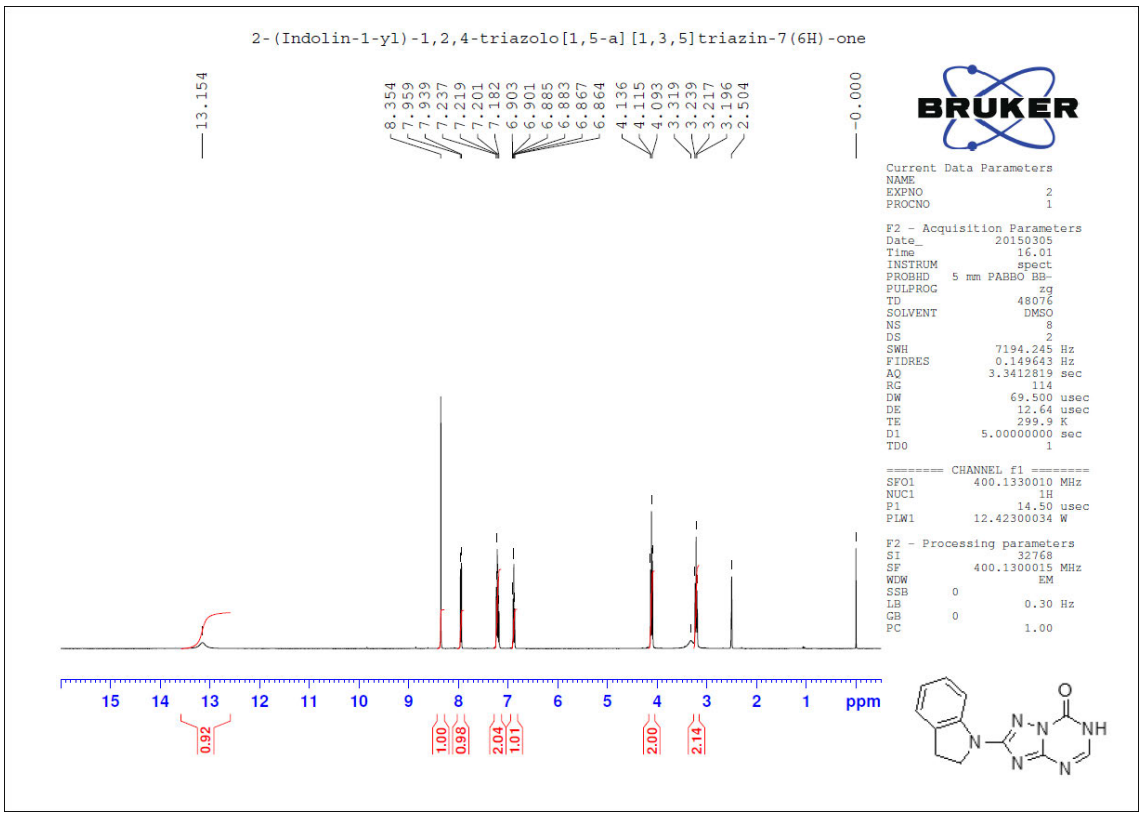
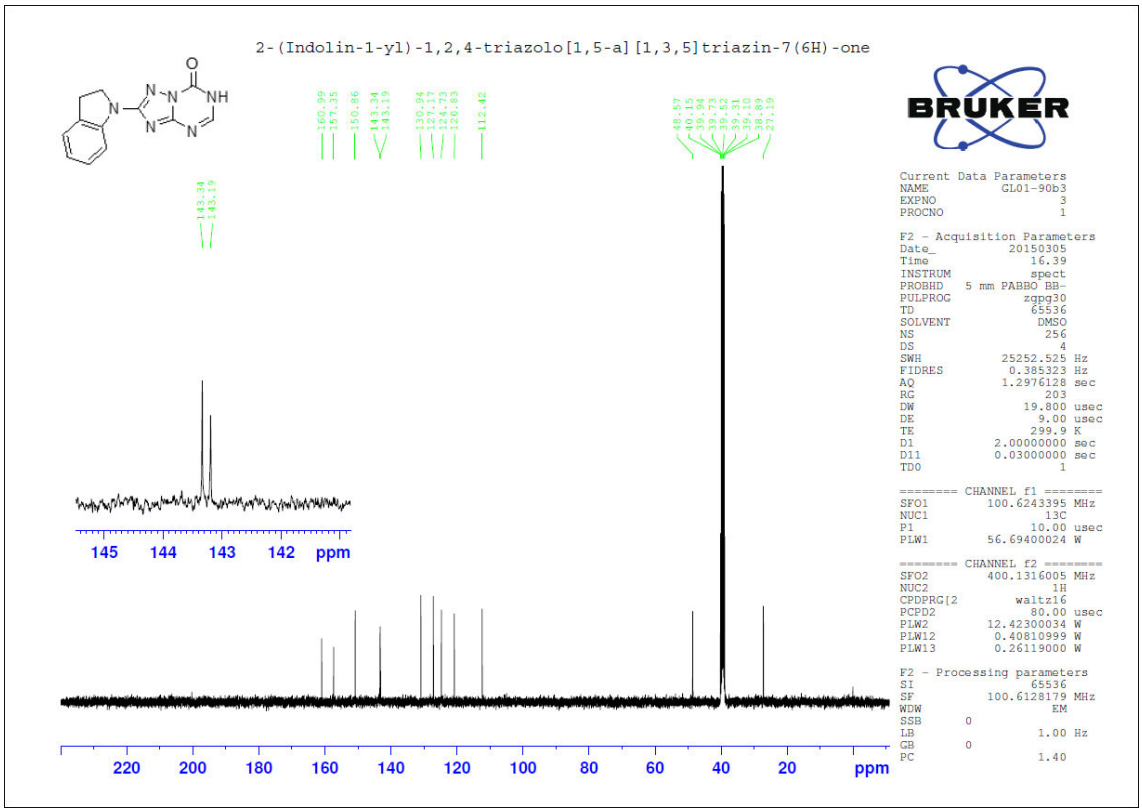
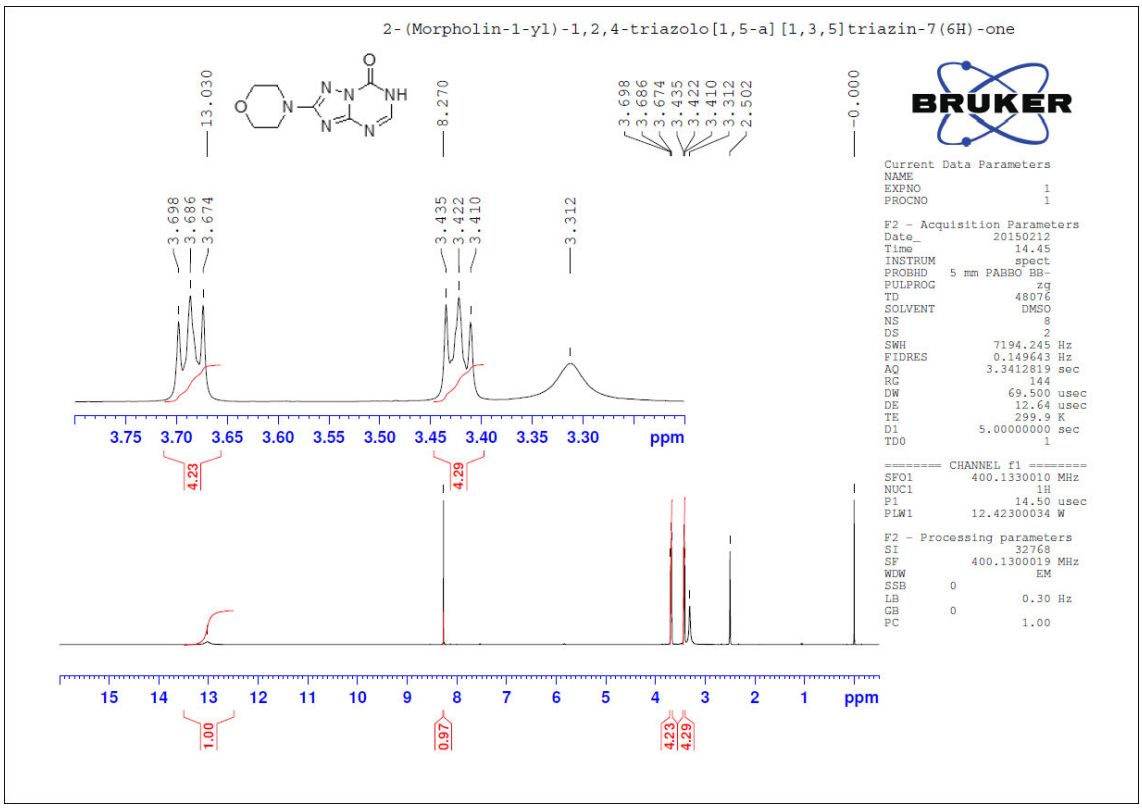
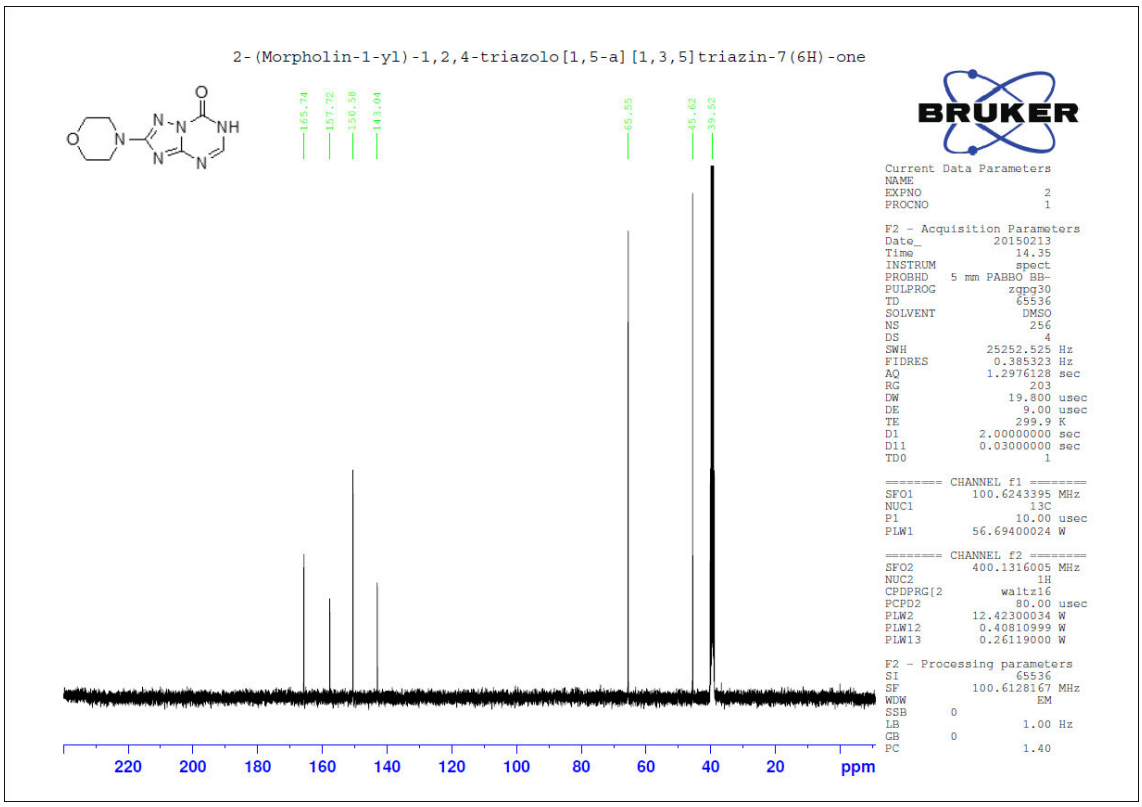
SF 400.1300041 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(Thien-2-yl)1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
159.23
158.54
151.36
143.64
132.50
129.48
128.41
40.15
39.94
39.73
39.52
39.31
39.10
38.89
ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220519
Time 7.57
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6800
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
CHANNEL f2
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128159 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


2-(Indolin-1-yl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.154
8.354
7.959
7.939
7.237
7.219
7.201
7.182
6.903
6.901
6.885
6.883
6.867
6.864
4.136
4.115
4.093
3.319
3.239
3.217
3.196
2.504
0.000
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.92
1.00
0.98
2.04
1.01
2.00
2.14
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150305
Time 16.01
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 7194.245 Hz
FIDRES 0.149643 Hz
AQ 3.3412819 sec
RG 114
DW 69.500 usec
DE 12.64 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300015 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(Indolin-1-yl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
160.99
157.35
150.86
143.34
143.19
130.94
127.17
124.23
120.83
112.42
48.57
40.15
39.94
39.73
39.52
39.31
39.10
38.89
27.19
145 144 143 142 ppm
220 200 180 160 140 120 100 80 60 40 20 ppm
BRUKER
Current Data Parameters
NAME GL01-90b3
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150305
Time 16.39
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 256
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128179 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


2-(Morpholin-1-yl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
13.030
8.270
3.698
3.686
3.674
3.435
3.422
3.410
3.312
2.502
-0.000
3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 ppm
4.23
4.29
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00
0.97
4.23
4.29
BRUKER
Current Data Parameters
NAME
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150212
Time 14.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg
TD 48076
SOLVENT DMSO
NS 8
DS 2
SWH 7194.245 Hz
FIDRES 0.149643 Hz
AQ 3.3412819 sec
RG 144
DW 69.500 usec
DE 12.64 usec
TE 299.9 K
D1 5.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1330010 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.42300034 W
F2 - Processing parameters
SI 32768
SF 400.1300019 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


2-(Morpholin-1-yl)-1,2,4-triazolo[1,5-a][1,3,5]triazin-7(6H)-one
165.74
157.72
150.38
143.04
65.55
45.62
39.52
220 200 180 160 140 120 100 80 60 40 20 ppm
BRUKER
Current Data Parameters
NAME
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150213
Time 14.35
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 256
DS 4
SWH 25252.525 Hz
FIDRES 0.385323 Hz
AQ 1.2976128 sec
RG 203
DW 19.800 usec
DE 9.00 usec
TE 299.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6243395 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.69400024 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 12.42300034 W
PLW12 0.40810999 W
PLW13 0.26119000 W
F2 - Processing parameters
SI 65536
SF 100.6128167 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

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