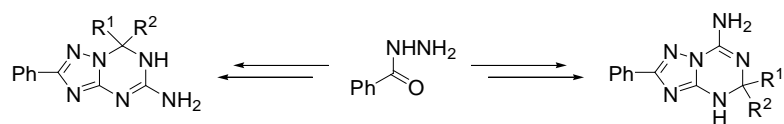


**NOTICE:** This is the author's version of a work that was accepted for publication in Tetrahedron. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Tetrahedron, 63, 52, 2007. DOI: 10.1016/j.tet.2007.10.046

## Graphical Abstract

### Practical Synthesis of Regioisomeric 5(7)-Amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines

Anton V. Dolzhenko,\* Anna V. Dolzhenko and Wai-Keung Chui



**Practical Synthesis of Regioisomeric  
5(7)-Amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines<sup>¶</sup>**

Anton V. Dolzhenko,<sup>a,\*</sup> Anna V. Dolzhenko<sup>b</sup> and Wai-Keung Chui<sup>a</sup>

<sup>a</sup>*Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4,  
Singapore 117543, Singapore*

<sup>b</sup>*Perm State Pharmaceutical Academy, 48 Lenin Street, Perm 614990, Russian Federation*

<sup>¶</sup> Part 8 in the series “Fused heterocyclic systems with *s*-triazine ring”, for part 7 see Ref. 1.

\* Corresponding author. Tel.: +65-6516-2657; fax: +65-6779-1554; e-mail: [phada@nus.edu.sg](mailto:phada@nus.edu.sg)

**Abstract** - A convenient and efficient synthesis of new 5-azapurine derivatives was developed. The regioisomeric 5-amino-6,7-dihydro- and 7-amino-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines were prepared in 3-4 steps from benzhydrazide *via* complementary and regiospecific routes as a part of our lead finding program. The molecular structures and tautomeric preferences of the compounds obtained were investigated using NMR spectral data and X-ray crystallography.

*Keywords:* Hydrazides, 1,2,4-Triazoles, Fused 1,3,5-triazines, Triazolotriazines, 5-Azapurines, Tautomerism

## 1. Introduction

Dihydrofolate reductase (DHFR) plays an essential role in cellular biochemistry and has been a well-recognized drug target for half a century. Antifolate drugs have been developed as anticancer, antibacterial, antifungal and antiparasitic agents.<sup>2</sup> 4,6-Diamino-1,2-dihydro-1,3,5-triazines (e.g. antimalarial drug cycloguanil and WR 99210) are known to be potent inhibitors of DHFR.<sup>2a,b</sup> Recently, dihydro-1,3,5-triazino[1,2-*a*]benzimidazoles (**1**) (Fig. 1) have also been found to possess antifolate activity.<sup>3</sup> Reports on other examples of fused dihydro-1,3,5-triazine with DHFR inhibitory activity are limited.

As a continuation to our investigation on fused 1,3,5-triazines as potential inhibitors of DHFR,<sup>3a,4</sup> we became interested in the aza-analogues of the purine system that carries a bridge nitrogen, particularly the 1,2,4-triazolo[1,5-*a*][1,3,5]triazines (5-azapurines).<sup>1</sup> The 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives have been shown to possess a wide range of biological activities.<sup>5</sup> However, antifolate activity of the compounds with this heterocyclic scaffold has not been investigated.

There are some common structural features which are unique to the triazine antifolates: 1) one of the carbon atoms of triazine ring should be in sp<sup>3</sup> hybridization (*gem*-dimethyl substitution is usually preferred); 2) the other two carbon atoms ought to be connected with nitrogen atoms of amino group or fused ring; 3) lipophilic aromatic moiety is required at the distal part of molecule. In this report we describe new practical synthesis of derivatives of 5-azapurine system, *viz.* hitherto unknown regioisomeric 5-amino-6,7-dihydro- and 7-amino-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2** and **3**). These compounds contain in the structure pharmacophoric fragments (Fig. 1) of the triazine DHFR inhibitors and potentially may interact with this enzyme.

We designed a 3-4 step synthesis of dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines **2** and **3** from benzhydrazide (**4**) with the subsequent formation of the 1,2,4-triazole and the 1,3,5-triazine rings using simple procedures, inexpensive and readily available reagents that make this synthetic approach very practical. The final step that leads to the annulation of the 1,3,5-triazine nucleus, can be successfully achieved *via* cyclization of the suitably substituted 1,2,4-triazoles, *i.e.* 5-guanidino-3-phenyl-1,2,4-triazole (**5**) and 5-amino-1-guanyl-3-phenyl-1,2,4-triazole (**6**) with aldehydes or ketones **7** to generate the libraries for biological screening (Scheme 1).

## 2. Results and discussion

The reactions of benzhydrazide (**4**) with cyanoguanidine in the presence of hydrochloric acid gave *N*-benzamidobiguanide (**8**) (Scheme 2). The treatment of *N*-benzamidobiguanide (**8**) with aqueous alkali resulted in 1,2,4-triazole ring closure and provided 5-guanidino-1,2,4-triazole **5**.

The heating of 5-guanidino-1,2,4-triazole **5** with carbonyl compounds **7** in ethanol in the presence of piperidine gave 5-amino-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines **2** (Scheme 3, Table 1). This heterocyclization was found to proceed smoothly with a variety of aldehydes and ketones. The reaction was regioselective and afforded only the products of the ring closure to nitrogen atom N-1 of the triazole **5**; the products of the ring closure to nitrogen atom N-4 (compounds **10**) as well as possible intermediates **9** were not isolated. The signal of sp<sup>3</sup>-hybridized carbon atom in <sup>13</sup>C NMR spectra at 62-80 ppm indicated that the triazine ring closure had occurred. The triazolotriazine structures **2** and **10** were differentiated using 2D NOESY experiments (no cross-peaks were found between R<sup>1</sup> or R<sup>2</sup> and phenyl group) and X-ray crystallography of compound **2h** (Fig. 2).<sup>6</sup>

The synthesis of the regioisomeric 7-amino-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**3**) was also initiated from benzhydrazide (**4**). The reactions of **4** with *S*-methyl isothioureia gave *N'*-benzamidoguanidine (**11**) (Scheme 4). The two signals of NH<sub>2</sub> groups in <sup>1</sup>H NMR spectra of the compound **11** indicated that tautomeric form **11**, rather than form **11'**, was preferred in DMSO solution ( $\Delta G_{316}^{\ddagger} = 67.6$  kJ). *N*-Benzamidoguanidine (**11**) was found to be stable: it could be recrystallized from water or aqueous ethanol and no changes were observed after drying at 140 °C under vacuum for 24 h. In general, *N*-benzamidoguanidine (**11**) could be cyclised to aminotriazoles with elimination of water molecule. The reported methods<sup>7</sup> required heating of **11** to above its mp (220 °C) or refluxing with sodium ethoxide. We found that the ring closure could be achieved easier and with higher yield. When *N*-benzamidoguanidine (**11**) was heated in water, the triazole **12** was obtained in almost quantitative yield (Scheme 4). The reaction was found to be clean and afforded **12** with excellent purity. <sup>1</sup>H NMR spectroscopy studies of the 1,2,4-triazoles **12** in DMSO solution concluded that 5-amino-3-phenyl-1,2,4-triazole (**12**) was predominant in the equilibrium, while 3-amino-5-phenyl-1,2,4-triazole (**12'**) was found to exist in minor proportion ( $K_T = 8.2$ ,  $\Delta G_{298} = -5.2$  kJ mol<sup>-1</sup>), whereas the 4*H*-form **12''** was not detected.

Benzotriazole has been shown to be a very effective auxiliary<sup>8</sup> and we attempted to apply 1-guanylbenzotriazole hydrochloride<sup>9</sup> as a guanylation agent<sup>10</sup> in the reaction with **12**. It was found that the guanylbenzotriazole selectively attacked endocyclic nitrogen N-1 of the triazole **12** affording **6** as hydrochloride which was converted to the base using sodium carbonate. The target 7-amino-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**3**) were prepared in good yields *via* (5+1) heterocyclization of **6** using aldehydes or ketones **7** as one-carbon inserting reagents (Scheme 5, Table 2).

Both 5-amino-6,7-dihydro- and 7-amino-5,6-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2** and **3**) might be involved in the annular tautomerism (Scheme 6). However, the indicated forms were found to

be predominant. The coupling of proton at  $sp^3$ -hybridized carbon and NH proton in  $^1H$  NMR spectra was observed for several compounds ( $J=0-1.5$  Hz). Despite  $J$  value was small and not always detectable, 2D NOESY experiments clearly indicated that NH proton located in the vicinity of  $sp^3$ -hybridized carbon. The same tautomeric preferences were found in solid state as confirmed by X-ray crystallography of **2h** and **3h** (Fig. 2 and 3).<sup>1,6</sup> Interestingly, two almost identical individual molecules with the intermediate between a twist boat and a half-boat conformation were identified for compound **3h** in the crystal (Fig. 3).

### 3. Conclusion

In summary, the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines with potential antifolate activity was successfully developed and their structures were investigated. The preparation method is practical: it required simple and easily available starting materials and provided derivatives of the interesting 5-azapurine system in 3-4 steps starting from benzhydrazide.

## 4. Experimental section

### 4.1. General

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. Analytical TLC were carried out on aluminum plates coated with Silica gel 60 F<sub>254</sub> (Merck) with detection by UV light. Mass spectra were obtained on a Finnigan MAT LCQ LC-MS mass spectrometer using atmospheric pressure chemical ionization (APCI) mode. IR spectra were recorded in KBr pellets using a Shimadzu IRPrestige-21 FTIR spectrophotometer.  $^1H$  and  $^{13}C$  NMR spectra were recorded on a Bruker DPX-300 spectrometer using DMSO-*d*<sub>6</sub> as a solvent and TMS as an internal reference. The energy of activation ( $\Delta G^\ddagger$ ) for the equilibrium between **11** and **11'** was estimated at the temperature of coalescence using dynamic  $^1H$  NMR experiments (0.1 M solution in DMSO-*d*<sub>6</sub>). The tautomeric preferences for **12** were investigated using  $^1H$  NMR spectral data (0.1 M solution in DMSO-*d*<sub>6</sub> at 27 °C).

### 4.2. *N*-Benzamidobiguanide hydrochloride (**8**).<sup>11</sup>

To the solution of benzhydrazide (**1**, 6.80 g, 50 mmol) in EtOH (35 ml), conc. HCl (5 ml, 50 mmol) and cyanoguanidine (4.62 g, 55 mmol) were added. The reaction mixture was heated under reflux with stirring for 4 h. After cooling on ice, the product was filtered, washed with cold EtOH and dried to give white powder. White crystalline powder; yield 80%, mp 172-173 °C (EtOH) [lit.<sup>11</sup> mp 169-170 °C].  $^1H$

NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  7.10 (br s, 4H, NH-C(=NH)NH<sub>2</sub>), 7.51 (t, 2H,  $^3J=7.3$  Hz, H-3 and H-5), 7.60 (t, 1H,  $^3J=7.2$  Hz, H-4), 7.67 (br s, 2H, NH<sub>2</sub>), 7.97 (d, 2H,  $^3J=7.2$  Hz, H-2 and H-6), 9.37 (br s, 1H, NH), 10.74 (br s, 1H, CONH).

#### 4.3. *N*-(3-Phenyl-1*H*-1,2,4-triazol-5-yl)guanidine (9).<sup>12</sup>

*N*-Benzamidobiguanide hydrochloride (**8**, 5.13 g, 20 mmol) was heated at 80 °C in 10% aqueous sodium hydroxide solution (10 ml) for 6 h. After cooling, the product was filtered, washed with cold water and dried. White crystalline powder; yield 68%, mp 242-243 °C [lit.<sup>12</sup> mp 244-246 °C]. TLC (silica gel, EtOH):  $R_f$  0.16. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  6.58 (br s, 4H, NH-C(=NH)NH<sub>2</sub>), 7.33 (t, 1H,  $^3J=7.1$  Hz, H-4'), 7.41 (t, 2H,  $^3J=7.1$  Hz, H-3' and H-5'), 7.96 (d, 2H,  $^3J=7.1$  Hz, H-2' and H-6'), 12.40 (br s, 1H, NH). <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  125.2 (2C), 128.0, 128.3 (2C), 139.2, 157.0, 157.7, 160.5.

#### 4.4. *N*-Benzamidoguanidine (11).<sup>7</sup>

The mixture of benzhydrazide (**1**, 13.6 g, 100 mmol) and *S*-methylisothiuronium sulfate (13.9 g, 50 mmol) in 1% aqueous sodium hydroxide solution (400 ml) was stirred at rt for 72 h and then heated to 50 °C for another 3 h. After cooling, the precipitated product was filtered, washed with ice-cold water and dried. White powder; yield 86%, mp 176 °C [lit.<sup>7</sup> mp 184 °C]. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  6.97 (br s, 2H, NH<sub>2</sub>), 7.16 (br s, 2H, NH<sub>2</sub>), 7.23-7.35 (m, 3H,  $W_{1/2}=9$  Hz, H-3, H-4 and H-5), 7.89-8.01 (m, 2H,  $W_{1/2}=12$  Hz, H-2 and H-6), 11.01 (br s, 1H, NH). <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  126.5 (2C), 127.3 (2C), 128.0, 138.5, 152.9, 160.5.

#### 4.5. 3(5)-Phenyl-1,2,4-triazol-5(3)-amine (12).<sup>13</sup>

*N*-Benzamidoguanidine (**11**, 8.90 g, 50 mmol) was heated under reflux in 80 ml of water for 4 h. After cooling, the precipitated amino-1,2,4-triazole **12** was filtered, washed with ice-cold water and dried. Colorless crystals; yield 97%, mp 186-187 °C [lit.<sup>13</sup> mp 186-187 °C]. TLC (silica gel, EtOH):  $R_f$  0.59. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  5.29\* and 6.05 (two br s, 2H, NH<sub>2</sub>), 7.32 (t, 1H,  $^3J=7.2$  Hz, H-4'), 7.39 (t, 2H,  $^3J=7.2$  Hz, H-3' and H-5'), 7.89 (d, 2H,  $^3J=6.8$  Hz, H-2' and H-6'), 12.04 and 13.20\* (two br s, 1H, NH). <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  125.2 (2C), 128.0, 128.2 (2C), 132.3, 157.2, 158.3.

\* - signals of the minor tautomeric form

#### 4.6. 1-Guanylbenzotriazole hydrochloride.<sup>14</sup>



To the stirred mixture of benzotriazole (8.9 g, 75 mmol) and cyanamide (6.3 g, 150 mmol) at 100 °C, conc. HCl (8.5 ml, 85 mmol) was added. When vigorous reaction was completed, the mixture was heated for 15 min at 100 °C. Then, 1 M HCl (12 ml) was added. After cooling, the precipitated 1-guanylbenzotriazole hydrochloride was filtered, washed with ice-cold 1 M HCl and dried. White powder; yield 68%, mp 194-196 °C [lit.<sup>14</sup> mp 195-197 °C]. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 7.67 (t, 1H, <sup>3</sup>*J*=7.7 Hz, H-3), 7.87 (t, 1H, <sup>3</sup>*J*=7.7 Hz, H-5), 8.08 (d, 1H, <sup>3</sup>*J*=8.3 Hz, H-4), 8.32 (d, 1H, <sup>3</sup>*J*=8.3 Hz, H-7), 10.23 (s, 4H, C(=NH)NH<sub>2</sub>·HCl). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 112.8, 120.2, 126.5, 130.4, 130.5, 145.6, 152.1.

#### 4.7. 1-Guanyl-3-phenyl-1,2,4-triazol-5-amine hydrochloride (6·HCl).<sup>9b</sup>

To the solution of 3(5)-phenyl-1,2,4-triazol-5(3)-amine (**12**, 6.80 g, 40 mmol) in EtOH (35 ml), 1-guanylbenzotriazole hydrochloride (7.88 g, 40 mmol) was added. The reaction mixture was heated under reflux with stirring for 30 min. After cooling, the product was filtered, washed with cold EtOH and dried. White crystalline powder; yield 62%, mp 212 °C [lit.<sup>9b</sup> mp 210 °C]. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 7.46-7.56 (m, 3H, H-3', H-4' and H-5'), 7.71 (s, 2H, NH<sub>2</sub>), 8.00 (dd, 2H, <sup>3</sup>*J*=6.4, <sup>4</sup>*J*=3.0 Hz, H-2' and H-6'), 9.47 (s, 4H, C(=NH)NH<sub>2</sub>·HCl). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 126.4 (2C), 128.7 (2C), 129.4, 130.3, 151.9, 157.0, 160.8.

#### 4.8. 1-Guanyl-3-phenyl-1,2,4-triazol-5-amine (6).<sup>9b</sup>

The sodium carbonate solution (10%, 30 ml) was added to the solution of 1-guanyl-3-phenyl-1,2,4-triazol-5-amine hydrochloride (6·HCl, 5.95 g, 25 mmol) in water (15 ml). After cooling, the product was filtered, washed with cold water and dried. White powder; yield 92%, mp 168 °C [lit.<sup>9b</sup> mp 165 °C]. TLC (silica gel, EtOH): *R*<sub>f</sub> 0.59. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 6.36 (s, 1H, NH), 6.54 (s, 2H, NH<sub>2</sub>), 7.37-7.53 (m, 3H, H-3', H-4' and H-5'), 7.88 (s, 2H, NH<sub>2</sub>), 7.98 (dd, 2H, <sup>3</sup>*J*=7.3, <sup>4</sup>*J*=1.7 Hz, H-2' and H-6'). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 125.9 (2C), 128.4 (2C), 129.2, 130.7, 152.7, 156.6, 156.8.

#### 4.9. General method for preparation of 7-(het)aryl-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines (2a-g).

The solution of *N*-(3-phenyl-1*H*-1,2,4-triazol-5-yl)guanidine (**5**, 0.50 g, 2.5 mmol), appropriate aldehyde (**7a-g**, 2.5 mmol) and piperidine (0.10 ml, 1.0 mmol) in EtOH (7-10 ml) was heated under reflux for 3-18 h. After cooling, the product was filtered, washed with cold EtOH, dried and recrystallized from EtOH, DMF or their mixture.

**4.9.1. 2,7-Diphenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2a).** White crystalline powder; yield 92%; mp 300-301 °C. TLC (silica gel, EtOH):  $R_f$  0.57. LC-MS (APCI)  $m/z$  291 (MH<sup>+</sup>). Anal. Calcd for C<sub>16</sub>H<sub>14</sub>N<sub>6</sub>: C 66.19; H 4.86; N 28.95. Found: C 66.02; H 4.95; N 28.73%. IR (KBr):  $\nu$  3461, 3311, 3237, 3135, 1653, 1646, 1597, 1548, 1522 (m), 1474, 1460, 1444, 1434, 1411, 1371, 764, 747, 713, 691 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  6.42 (s, 2H, NH<sub>2</sub>), 6.69 (s, 1H, H-7), 7.29-7.47 (m, 8H, H-3', H-4', H-5' and 5-Ph), 7.86 (dd, 2H, <sup>3</sup>*J*=7.9, <sup>4</sup>*J*=1.5 Hz, H-2' and H-6'), 7.93 (d, 1H, <sup>3</sup>*J*=1.5 Hz, NH). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  68.3, 125.3 (2C), 126.2 (2C), 128.3 (2C), 128.4, 128.7 (2C), 129.0, 131.7, 140.4, 155.5, 156.4, 159.2.

**4.9.2. 7-(4-Methoxyphenyl)-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2b).** White crystalline powder; yield 91%, mp 291 °C. TLC (silica gel, EtOH):  $R_f$  0.54. LC-MS (APCI)  $m/z$  321 (MH<sup>+</sup>). Anal. Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>O: C 63.74; H 5.03; N 4.99. Found: C 63.70; H 5.12; N 4.91%. IR (KBr):  $\nu$  3451, 3309, 3227, 3110, 1653, 1647, 1594, 1523 (m), 1477, 1435, 1409, 1369, 1254, 1178, 815, 746, 688 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  3.75 (s, 3H, OMe), 6.39 (s, 2H, NH<sub>2</sub>), 6.63 (d, 1H, <sup>3</sup>*J*=0.8 Hz, H-7), 6.97 (d, 2H, <sup>3</sup>*J*=8.7 Hz, H-3'' and H-5''), 7.28 (d, 2H, <sup>3</sup>*J*=8.7 Hz, H-2'' and H-6''), 7.35 (t, 2H, <sup>3</sup>*J*=7.5 Hz, H-3' and H-5'), 7.37 (t, 1H, <sup>3</sup>*J*=7.5 Hz, H-4'), 7.86 (d, 2H, <sup>3</sup>*J*=7.5 Hz, H-2' and H-6'), 7.87 (s, 1H, NH). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  55.1, 68.0, 114.0 (2C), 125.3 (2C), 127.6 (2C), 128.3 (2C), 128.4, 131.8, 132.5, 155.5, 156.3, 159.1, 159.7.

**4.9.3. 7-(4-Methylphenyl)-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2c).** White crystalline powder; yield 90%, mp 281 °C. TLC (silica gel, EtOH):  $R_f$  0.57. LC-MS (APCI)  $m/z$  305 (MH<sup>+</sup>). Anal. Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>: C 67.09; H 5.30; N 27.61. Found: C 66.92; H 5.34; N 27.55%. IR (KBr):  $\nu$  3462, 3311, 3231, 3111, 1652, 1648, 1599, 1522 (m), 1477, 1444, 1435, 1409, 1365, 805, 745, 689 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.29 (s, 3H, Me), 6.40 (s, 2H, NH<sub>2</sub>), 6.65 (d, 1H, <sup>3</sup>*J*=1.5 Hz, H-7), 7.20-7.25 (m, 4H, H-2'', H-3'', H-5'' and H-6''), 7.32-7.41 (m, 3H, H-3', H-4' and H-5'), 7.86 (dd, 2H, <sup>3</sup>*J*=8.1, <sup>4</sup>*J*=1.7 Hz, H-2' and H-6'), 7.89 (d, 1H, <sup>3</sup>*J*=1.5 Hz, NH). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  20.7, 68.2, 125.3 (2C), 126.2 (2C), 128.3 (2C), 128.4, 129.2 (2C), 131.8, 137.6, 138.5, 155.5, 156.3, 159.1.

**4.9.4. 7-(4-Chlorophenyl)-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2d).** White crystalline powder; yield 73%, mp 290 °C. TLC (silica gel, EtOH):  $R_f$  0.56. LC-MS (APCI)  $m/z$  325, 327 (MH<sup>+</sup>). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>ClN<sub>6</sub>: C 59.17; H 4.03; N 25.88. Found: C 59.02; H 4.19; N

25.73%. IR (KBr):  $\nu$  3446, 3309, 3237, 3111, 1653, 1647, 1595, 1525 (m), 1472, 1433, 1396, 1363, 745, 689  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  6.48 (s, 2H,  $\text{NH}_2$ ), 6.74 (s, 1H, H-7), 7.29-7.44 (m, 5H, H-2'', H-6'', H-3', H-4' and H-5'), 7.51 (d, 2H,  $^3J=8.3$  Hz, H-3'' and H-5''), 7.87 (d, 2H,  $^3J=7.5$  Hz, H-2' and H-6'), 7.97 (s, 1H, NH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  67.6, 125.4 (2C), 128.2 (2C), 128.3 (2C), 128.5, 128.8 (2C), 131.7, 133.6, 139.3, 155.4, 156.4, 159.4.

**4.9.5. 7-(4-Fluorophenyl)-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2e).**

White crystalline powder; yield 76%, mp 280-281 °C. TLC (silica gel, EtOH):  $R_f$  0.56. LC-MS (APCI)  $m/z$  309 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{16}\text{H}_{13}\text{FN}_6$ : C 62.33; H 4.25; N 27.26. Found: C 62.21; H 4.34; N 27.19%. IR (KBr):  $\nu$  3469, 3310, 3236, 3112, 1653, 1647, 1601, 1524 (m), 1476, 1436, 1409, 1363, 1239, 821, 746, 688  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  6.45 (s, 2H,  $\text{NH}_2$ ), 6.73 (s, 1H, H-7), 7.27 (dd, 2H,  $^3J_{\text{HF}}=8.9$ ,  $^3J=8.9$  Hz, H-3'' and H-5''), 7.32-7.47 (m, 5H, H-3', H-4', H-5', H-2'' and H-6''), 7.86 (d, 2H,  $^3J=7.5$  Hz, H-2' and H-6'), 7.94 (d, 1H,  $^3J=1.5$  Hz, NH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  67.6, 115.6 (d,  $^2J_{\text{CF}}=21.8$  Hz, 2C), 125.4 (2C), 128.3 (2C), 128.5, 127.5 (d,  $^3J_{\text{CF}}=8.8$  Hz, 2C), 131.7, 136.7 (d,  $^4J_{\text{CF}}=2.9$  Hz), 155.5, 156.4, 159.4, 162.3 (d,  $^1J_{\text{CF}}=245.2$  Hz).

**4.9.6. 7-(2-Furyl)-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2f).**

Beige crystalline powder; yield 72%, mp 272 °C. TLC (silica gel, EtOH):  $R_f$  0.56. LC-MS (APCI)  $m/z$  281 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{14}\text{H}_{12}\text{N}_6\text{O}$ : C 59.99; H 4.32; N 29.98. Found: C 59.78; H 4.43; N 29.89%. IR (KBr):  $\nu$  3472, 3448, 3313, 3232, 3105, 1655, 1651, 1603, 1523 (m), 1477, 1436, 1395, 1364, 830, 750, 693  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  6.45 (s, 2H,  $\text{NH}_2$ ), 6.47 (dd, 1H,  $^3J=3.1$ ,  $^3J=1.7$  Hz, H-4''), 6.54 (d, 1H,  $^3J=3.1$  Hz, H-3''), 6.81 (s, 1H, H-7), 7.30-7.45 (m, 3H, H-3', H-4' and H-5'), 7.68 (d, 1H,  $^3J=1.7$  Hz, H-5''), 7.88 (dd, 2H,  $^3J=7.5$ ,  $^4J=1.5$  Hz, H-2' and H-6'), 7.92 (s, 1H, NH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  62.2, 108.7, 110.5, 125.4 (2C), 128.3 (2C), 128.5, 131.7, 143.8, 151.5, 155.5, 156.3, 159.3.

**4.9.7. 2-Phenyl-7-(4-pyridyl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2g).**

Yellowish crystalline powder; yield 78%, mp 242-244 °C. TLC (silica gel, EtOH):  $R_f$  0.30. LC-MS (APCI)  $m/z$  292 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{13}\text{N}_7$ : C 61.84; H 4.50; N 33.66. Found: C 61.63; H 4.62; N 33.50%. IR (KBr):  $\nu$  3447, 3324, 3107, 3079, 1653, 1647, 1624, 1606, 1521 (m), 1437, 1420, 1362, 1295, 741, 685  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  6.56 (s, 2H,  $\text{NH}_2$ ), 6.77 (s, 1H, H-7), 7.23-7.48 (m, 5H, H-2'', H-6'', H-3', H-4' and H-5'), 7.89 (d, 2H,  $^3J=7.5$  Hz, H-2' and H-6'), 8.06 (s, 1H, NH),

8.64 (d, 2H,  $^3J=5.3$  Hz, H-3'' and H-5'').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  67.0, 120.9 (2C), 125.4 (2C), 128.3 (2C), 128.6, 131.6, 148.2, 150.2 (2C), 155.5, 156.4, 159.6.

**4.10. 7,7-Dimethyl-2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (2h).** The mixture of *N*-(3-phenyl-1*H*-1,2,4-triazol-5-yl)guanidine (**5**, 0.50 g, 2.5 mmol) and piperidine (0.10 ml, 1.0 mmol) in acetone (7 ml) was heated under reflux for 18 h. After cooling, the precipitated product was filtered, washed with acetone, dried and recrystallized from EtOH. White crystalline powder; yield 98%, mp 294 °C. TLC (silica gel, EtOH):  $R_f$  0.50. LC-MS (APCI)  $m/z$  243 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{N}_6$ : C 59.49; H 5.82; N 34.69. Found: C 59.45; H 5.86; N 34.64%. IR (KBr):  $\nu$  3473, 3308, 3230, 3055, 2977, 1654, 1651, 1616, 1559, 1528 (m), 1476, 1460, 1444, 1420, 1362, 1172, 748, 699, 691, 476  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  1.64 (s, 6H,  $\text{Me}_2$ ), 6.21 (s, 2H,  $\text{NH}_2$ ), 7.36-7.40 (m, 3H, H-3', H-4' and H-5'), 7.58 (s, 1H, NH), 7.92 (dd, 2H,  $^3J=8.3$ ,  $^4J=1.5$  Hz, H-2' and H-6').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  28.7 (2C), 70.1, 125.3 (2C), 128.3 (3C), 132.1, 155.5, 155.8, 158.7.

**4.11. General method for preparation of the spiro derivatives of 2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines (2i,j).** The solution of *N*-(3-phenyl-1*H*-1,2,4-triazol-5-yl)guanidine (**9**, 0.50 g, 2.5 mmol), appropriate cyclic ketone (**7i,j**, 5.0 mmol) and piperidine (0.10 ml, 1.0 mmol) in EtOH (8 ml) was heated under reflux for 16-24 h. After cooling, the product was filtered, washed with cold EtOH, dried and recrystallized from EtOH/DMF.

**4.11.1. 2'-Phenyl-6'*H*-spiro[cyclopentane-1,7'-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin]-5'-amine (2i).** White crystalline powder; yield 96%, mp 300 °C. TLC (silica gel, EtOH):  $R_f$  0.54. LC-MS (APCI)  $m/z$  269 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{14}\text{H}_{16}\text{N}_6$ : C 62.67; H 6.01; N 31.32. Found: C 62.46; H 6.17; N 31.19%. IR (KBr):  $\nu$  3471, 3280, 3204, 3123, 2962, 1653, 1635, 1630, 1598, 1523 (m), 1473, 1435, 1419, 1362, 753, 703  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  1.71-1.97 (m, 6H,  $(\text{CH}_2)_2$ , H-2*a* and H-5*a*), 2.21-2.37 (m, 2H, H-2*e* and H-5*e*), 6.20 (s, 2H,  $\text{NH}_2$ ), 7.34 (t, 1H,  $^3J=7.0$  Hz, H-4'), 7.46 (t, 2H,  $^3J=7.2$  Hz, H-3' and H-5'), 7.73 (s, 1H, NH), 7.92 (d, 2H,  $^3J=7.2$  Hz, H-2' and H-6').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  23.0 (2C), 39.6 (2C), 79.6, 125.3 (2C), 128.3 (3C), 132.0, 155.5, 156.2, 158.7.

**4.11.2. 2'-Phenyl-6'*H*-spiro[cyclohexane-1,7'-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin]-5'-amine (2j).** White crystalline powder; yield 72%, mp 283 °C. TLC (silica gel, EtOH):  $R_f$  0.58. LC-MS (APCI)  $m/z$  283 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{18}\text{N}_6$ : C 63.81; H 6.43; N 29.77. Found: C 63.72; H 6.65; N 29.58%. IR (KBr):  $\nu$  3463, 3363, 3309, 3134, 2933, 1653, 1647, 1636, 1587, 1558, 1534, 1521 (m), 1473, 1444,

1409, 1356, 753, 710, 693  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  1.22-1.71 (m, 8H,  $(\text{CH}_2)_3$ , H-2a and H-6a), 2.00-2.18 (m, 2H, H-2e and H-6e), 6.28 (s, 2H,  $\text{NH}_2$ ), 7.30-7.45 (m, 3H, H-3', H-4' and H-5'), 7.45 (s, 1H, NH), 7.92 (dd, 2H,  $^3J=8.0$ ,  $^4J=1.5$  Hz, H-2' and H-6').  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  20.8 (2C), 24.0, 36.4 (2C), 71.2, 125.3 (2C), 128.3 (3C), 132.1, 155.5, 155.8, 158.5.

#### 4.12. General method for preparation of 5-(het)aryl-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-amines (3a-g).

The solution of 1-guanyl-3-phenyl-1,2,4-triazol-5-amine (**6**, 0.50 g, 2.5 mmol), appropriate aldehyde (**7a-g**, 2.5 mmol) and piperidine (0.10 ml, 1.0 mmol) in EtOH (7-10 ml) was heated under reflux for 2-12 h. After cooling, the product was filtered, washed with cold EtOH, dried and recrystallized from EtOH, DMF or their mixture.

**4.12.1. 2,5-Diphenyl-4,5-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-amine (3a).** White crystalline powder; yield 78%, mp 204-205  $^\circ\text{C}$ . TLC (silica gel, EtOH):  $R_f$  0.62. LC-MS (APCI)  $m/z$  291 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{16}\text{H}_{14}\text{N}_6$ : C 66.19; H 4.86; N 28.95. Found: C 66.05; H 5.07; N 28.73%. IR (KBr):  $\nu$  3437, 3231, 3133, 1706, 1697, 1626, 1531, 1495, 1450, 1434, 1340, 1026, 730, 701, 685  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  5.91 (s, 1H, H-5), 6.60 (s, 2H,  $\text{NH}_2$ ), 7.25-7.53 (m, 8H, H-3', H-4', H-5' and 5-Ph), 8.00 (dd, 2H,  $^3J=7.3$ ,  $^4J=2.4$  Hz, H-2' and H-6'), 8.68 (s, 1H, NH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  68.2, 126.2 (2C), 126.3 (2C), 127.7, 128.1 (2C), 128.5 (2C), 129.6, 130.5, 142.9, 143.4, 156.1, 159.8.

**4.12.2. 5-(4-Methoxyphenyl)-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-amine (3b).** White crystalline powder; yield 87%, mp 212  $^\circ\text{C}$ . TLC (silica gel, EtOH):  $R_f$  0.55. LC-MS (APCI)  $m/z$  321 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_6\text{O}$ : C 63.74; H 5.03; N 4.99. Found: C 63.60; H 5.23; N 4.81%. IR (KBr):  $\nu$  3439, 3231, 3126, 1706, 1697, 1624, 1534, 1511, 1499, 1451, 1437, 1343, 1252, 1036, 737, 690  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  3.74 (s, 3H, OMe), 5.84 (s, 1H, H-5), 6.54 (s, 2H,  $\text{NH}_2$ ), 6.92 (d, 2H,  $^3J=8.7$  Hz, H-3'' and H-5''), 7.37 (d, 2H,  $^3J=8.7$  Hz, H-2'' and H-6''), 7.42-7.52 (m, 3H, H-3', H-4' and H-5'), 7.98 (dd, 2H,  $^3J=7.2$ ,  $^4J=2.3$  Hz, H-2' and H-6'), 8.58 (s, 1H, NH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  55.0, 67.8, 113.4 (2C), 126.2 (2C), 127.5 (2C), 128.5 (2C), 129.6, 130.5, 135.0, 143.4, 156.1, 158.7, 159.8.

**4.12.3. 5-(4-Methylphenyl)-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-amine (3c).** White crystalline powder; yield 72%, mp 213-214  $^\circ\text{C}$ . TLC (silica gel, EtOH):  $R_f$  0.58. LC-MS (APCI)

$m/z$  305 ( $MH^+$ ). Anal. Calcd for  $C_{17}H_{16}N_6$ : C 67.09; H 5.30; N 27.61. Found: C 66.86; H 5.52; N 27.53%. IR (KBr):  $\nu$  3440, 3224, 3125, 1702, 1696, 1624, 1532, 1496, 1452, 1437, 1343, 1034, 758, 735, 686  $cm^{-1}$ .  $^1H$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  2.29 (s, 3H, Me), 5.86 (s, 1H, H-5), 6.56 (s, 2H,  $NH_2$ ), 7.17 (d, 2H,  $^3J=7.9$  Hz, H-3'' and H-5''), 7.32 (d, 2H,  $^3J=7.9$  Hz, H-2'' and H-4''), 7.41-7.51 (m, 3H, H-3', H-4' and H-5'), 7.98 (dd, 2H,  $^3J=7.2$ ,  $^4J=2.2$  Hz, H-2' and H-6'), 8.61 (s, 1H, NH).  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  20.6, 68.0, 126.1 (2C), 126.2 (2C), 128.5 (2C), 128.6 (2C), 129.6, 130.5, 136.8, 140.0, 143.4, 156.1, 159.8.

#### 4.12.4. 5-(4-Chlorophenyl)-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (3d).

White crystalline powder; yield 70%, mp 220-221 °C. TLC (silica gel, EtOH):  $R_f$  0.60. LC-MS (APCI)  $m/z$  325, 327 ( $MH^+$ ). Anal. Calcd for  $C_{16}H_{13}ClN_6$ : C 59.17; H 4.03; N 25.88. Found: C 58.94; H 4.32; N 25.62%. IR (KBr):  $\nu$  3444, 3228, 3124, 1705, 1697, 1628, 1533, 1498, 1490, 1454, 1437, 1343, 1018, 773, 737, 689  $cm^{-1}$ .  $^1H$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  5.92 (s, 1H, H-5), 6.62 (s, 2H,  $NH_2$ ), 7.39-7.54 (m, 7H, H-3', H-4', H-5', H-2'', H-3'', H-5'' and H-6''), 7.99 (dd, 2H,  $^3J=7.2$ ,  $^4J=1.9$  Hz, H-2' and H-6'), 8.67 (s, 1H, NH).  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  67.6, 126.2 (2C), 128.1 (2C), 128.2 (2C), 128.5 (2C), 129.6, 130.4, 132.2, 141.9, 143.6, 156.0, 159.9.

#### 4.12.5. 5-(4-Fluorophenyl)-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (3e).

White crystalline powder; yield 78%, mp > 360 °C. TLC (silica gel, EtOH):  $R_f$  0.63. LC-MS (APCI)  $m/z$  309 ( $MH^+$ ). Anal. Calcd for  $C_{16}H_{13}FN_6$ : C 62.33; H 4.25; N 27.26. Found: C 62.02; H 4.47; N 27.11%. IR (KBr):  $\nu$  3441, 3225, 3134, 1706, 1697, 1627, 1601, 1532, 1507, 1496, 1449, 1436, 1340, 1227, 1034, 771, 736, 687  $cm^{-1}$ .  $^1H$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  5.93 (s, 1H, H-5), 6.63 (s, 2H,  $NH_2$ ), 7.20 (dd, 2H,  $^3J_{HF}=8.9$ ,  $^3J=8.9$  Hz, H-3'' and H-5'') 7.43-7.54 (m, 5H, H-3', H-4', H-5', H-2'' and H-6''), 8.00 (dd, 2H,  $^3J=7.3$ ,  $^4J=2.4$  Hz, H-2' and H-6'), 8.68 (s, 1H, NH).  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  67.6, 114.9 (d,  $^2J_{CF}=21.8$  Hz, 2C), 126.2 (2C), 128.4 (d,  $^3J_{CF}=8.2$  Hz, 2C), 128.5 (2C), 129.6, 130.5, 139.1 (d,  $^4J_{CF}=2.9$  Hz), 143.6, 156.1, 159.9, 161.6 (d,  $^1J_{CF}=243.4$  Hz).

#### 4.12.6. 5-Furyl-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (3f). Beige

crystalline powder; yield 70%, mp 210 °C. TLC (silica gel, EtOH):  $R_f$  0.62. LC-MS (APCI)  $m/z$  281 ( $MH^+$ ). Anal. Calcd for  $C_{14}H_{12}N_6O$ : C 59.99; H 4.32; N 29.98. Found: C 59.83; H 4.46; N 29.84%. IR (KBr):  $\nu$  3305, 3240, 3159, 1702, 1698, 1637, 1529, 1457, 1438, 1342, 1233, 1015, 755  $cm^{-1}$ .  $^1H$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  5.94 (d, 1H,  $^3J=1.1$  Hz, H-5), 6.29 (d, 1H,  $^3J=3.0$  Hz, H-5''), 6.40 (dd, 1H,  $^3J=3.0$ ,  $^3J=1.8$  Hz, H-4''), 6.63 (s, 2H,  $NH_2$ ), 7.41-7.52 (m, 3H, H-3', H-4' and H-5'), 7.59 (d, 1H,

$^3J=1.8$  Hz, H-3''), 7.99 (dd, 2H,  $^3J=7.3$ ,  $^4J=2.4$  Hz, H-2' and H-6'), 8.74 (d, 1H,  $^3J=1.1$  Hz, NH).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  62.3, 105.8, 110.1, 126.1 (2C), 128.5 (2C), 129.6, 130.4, 142.4, 144.2, 154.9, 155.5, 159.6.

#### 4.12.7. 2-Phenyl-5-(4-pyridyl)-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (3g).

Yellowish crystalline powder; yield 68%, mp 333-335 °C. TLC (silica gel, EtOH):  $R_f$  0.38. LC-MS (APCI)  $m/z$  292 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{13}\text{N}_7$ : C 61.84; H 4.50; N 33.66. Found: C 61.63; H 4.76; N 33.52%. IR (KBr):  $\nu$  3442, 3230, 3126, 1705, 1697, 1625, 1533, 1496, 1453, 1436, 1416, 1340, 1036, 771, 735, 685  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  5.96 (s, 1H, H-5), 6.72 (s, 2H,  $\text{NH}_2$ ), 7.39-7.54 (m, 5H, H-3', H-4', H-5', H-2'' and H-6''), 8.00 (dd, 2H,  $^3J=7.0$ ,  $^4J=2.4$  Hz, H-2' and H-6'), 8.59 (dd, 2H,  $^3J=4.7$ ,  $^4J=1.3$  Hz, H-3'' and H-5''), 8.81 (s, 1H, NH).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  67.1, 121.3 (2C), 126.2 (2C), 128.5 (2C), 129.7, 130.4, 143.8, 149.7 (2C), 151.1, 155.9, 159.9.

#### 4.13. 5,5-Dimethyl-2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (3h).

The mixture of 1-guanyl-3-phenyl-1,2,4-triazol-5-amine (**6**, 0.50 g, 2.5 mmol) and piperidine (0.10 ml, 1.0 mmol) in acetone (7 ml) was heated under reflux for 12 h. After cooling, the precipitated product was filtered, washed with acetone, dried and recrystallized from EtOH. White crystalline powder; yield 65%, mp 210 °C. TLC (silica gel, EtOH):  $R_f$  0.55. LC-MS (APCI)  $m/z$  243 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{N}_6$ : C 59.49; H 5.82; N 34.69. Found: C 59.31; H 6.03; N 34.56%. IR (KBr):  $\nu$  3414, 3388, 3072, 2965, 1705, 1698, 1624, 1522, 1495, 1448, 1431, 1380 (d, *gem*- $\text{Me}_2$ ), 1340, 913, 740, 705, 694  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  1.37 (s, 6H,  $\text{Me}_2$ ), 6.30 (s, 2H,  $\text{NH}_2$ ), 7.42-7.51 (m, 3H, H-3', H-4' and H-5'), 7.98 (dd, 2H,  $^3J=7.2$ ,  $^4J=1.9$  Hz, H-2' and H-6'), 8.25 (s, 1H, NH).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  30.5 (2C), 68.8, 126.1 (2C), 128.5 (2C), 129.5, 130.7, 141.5, 155.4, 159.5.

#### 4.14. General method for preparation of the spiro derivatives of 2-phenyl-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amines (3i,j).

The solution of 1-guanyl-3-phenyl-1,2,4-triazol-5-amine (**6**, 0.50 g, 2.5 mmol), appropriate cyclic ketone (**7i,j**, 5.0 mmol) and piperidine (0.10 ml, 1.0 mmol) in EtOH (8 ml) was heated under reflux for 10-15 h. After cooling, the product was filtered, washed with cold EtOH, dried and recrystallized from EtOH.

##### 4.14.1. 2'-Phenyl-4'*H*-spiro[cyclopentane-1,5'-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin]-7'-amine (3i).

White crystalline powder; yield 62%, mp 206-207 °C. TLC (silica gel, EtOH):  $R_f$  0.60. LC-MS (APCI)

$m/z$  269 ( $MH^+$ ). Anal. Calcd for  $C_{14}H_{16}N_6$ : C 62.67; H 6.01; N 31.32. Found: C 62.50; H 6.22; N 31.16%. IR (KBr):  $\nu$  3389, 3246, 3074, 2959, 1702, 1698, 1628, 1527, 1494, 1452, 1436, 1374, 1344, 743, 690  $cm^{-1}$ .  $^1H$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  1.62-1.87 (m, 8H,  $(CH_2)_4$ ), 6.30 (s, 2H,  $NH_2$ ), 7.39-7.52 (m, 3H, H-3', H-4' and H-5'), 7.98 (dd, 2H,  $^3J=7.5$ ,  $^4J=1.9$  Hz, H-2' and H-6'), 8.33 (s, 1H, NH).  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  22.2 (2C), 41.0 (2C), 78.9, 126.1 (2C), 128.5 (2C), 129.5, 130.7, 141.7, 155.9, 159.6.

#### 4.14.2. 2'-Phenyl-4'H-spiro[cyclohexane-1,5'-[1,2,4]triazolo[1,5-a][1,3,5]triazin]-7'-amine (3j).

White crystalline powder; yield 73%, mp 216-217 °C. TLC (silica gel, EtOH):  $R_f$  0.59. LC-MS (APCI)  $m/z$  283 ( $MH^+$ ). Anal. Calcd for  $C_{15}H_{18}N_6$ : C 63.81; H 6.43; N 29.77. Found: C 63.57; H 6.53; N 29.64%. IR (KBr):  $\nu$  3374, 3287, 3072, 2936, 2922, 1702, 1698, 1635, 1527, 1491, 1451, 1430, 1354, 746, 692  $cm^{-1}$ .  $^1H$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  1.25-1.81 (m, 10H,  $(CH_2)_5$ ), 6.31 (s, 2H,  $NH_2$ ), 7.39-7.54 (m, 3H, H-3', H-4' and H-5'), 7.99 (dd, 2H,  $^3J=7.3$ ,  $^4J=1.7$  Hz, H-2' and H-6'), 8.20 (s, 1H, NH).  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  21.3 (2C), 25.0, 38.9 (2C), 70.3, 126.1 (2C), 128.5 (2C), 129.5, 130.7, 141.2, 155.6, 159.6.

### Acknowledgments

This work is supported by the Academic Research Fund from the National University of Singapore. The authors thank Tan Geok Kheng and Koh Lip Lin for the X-ray crystallography study.

### References and notes

1. Dolzhenko, A. V.; Tan, G. K.; Koh, L. L.; Dolzhenko, A. V.; Chui, W. K. *Acta Crystallogr.* **2007**, *E63*, o2797.
2. (a) Chan, D. C. M.; Anderson, A. C. *Curr. Med. Chem.* **2006**, *13*, 377-398; (b) Kompis, I. M.; Islam, K.; Then, R. L. *Chem. Rev.* **2005**, *105*, 593-620; (c) Gangjee, A.; Jain, H. D.; Kupur, S. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 524-542.
3. (a) Dolzhenko, A. V.; Chui, W. K. *J. Heterocycl. Chem.* **2006**, *43*, 95-100. (b) Toyoda, T.; Brobey, R. K. B.; Sano, G.-I.; Horii, T.; Tomioka, N.; Itai, A. *Biochem. Biophys. Res. Commun.* **1997**, *235*, 515-519.
4. Dolzhenko, A. V.; Chui, W. K.; Dolzhenko, A. V.; Chan, L. W. *J. Fluorine Chem.* **2005**, *126*, 759-763.



5. For review see: Dolzhenko, A. V.; Dolzhenko, A. V.; Chui, W. K. *Heterocycles* **2006**, *68*, 1723-1759.
6. Dolzhenko, A. V.; Tan, G. K.; Koh, L. L.; Dolzhenko, A. V.; Chui, W. K. *Acta Crystallogr.* **2007**, *E63*, o2796.
7. Hoggarth, E. *J. Chem. Soc.* **1950**, 612-614.
8. For recent reviews see: (a) Katritzky, A. R.; Manju, K.; Singh, S. K.; Meher, N. K. *Tetrahedron* **2005**, *61*, 2555-2581; (b) Katritzky, A. R.; Rogovoy, B. V. *Chem. Eur. J.* **2003**, *9*, 4586-4593.
9. For applications of 1-guanylbenzotriazole hydrochloride as a guanylation agent see (a) Zahariev, S.; Guarnaccia, C.; Lamba, D.; Cemazar, M.; Pongor, S. *Tetrahedron Lett.* **2004**, *45*, 9423-9426; (b) Bokaldere, R. P.; Grinsteins, V. Ya. *Khim. Geterotsikl. Soedin.* **1970**, 563-564.
10. For recent review on guanylation agents see: Katritzky, A. R.; Rogovoy, B. V. *ARKIVOC* **2005**, *4*, 49-87.
11. Mamalis, P.; Green, J.; McHale, D. *J. Chem. Soc.* **1960**, 229-238.
12. Logemann, W.; Artini, D.; Canavesi, L.; Tosolini, G. *Chem. Ber.* **1963**, *96*, 2909-2913.
13. Dolzhenko, A. V.; Dolzhenko, A. V.; Chui, W. K. *Heterocycles* **2007**, *71*, 429-436.
14. Grinsteins, V. Ya.; Veveris, A. P. *USSR Pat. 215225*, **1968** (*Chem. Abstr.* 69:59251).

**Table 1.** 5-Amino-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**)

<b>2</b>	R <sup>1</sup>	R <sup>2</sup>	Yields (%)
<b>a</b>	H	Ph	92
<b>b</b>	H	4-MeO-C <sub>6</sub> H <sub>4</sub>	91
<b>c</b>	H	4-Me-C <sub>6</sub> H <sub>4</sub>	90
<b>d</b>	H	4-Cl-C <sub>6</sub> H <sub>4</sub>	73
<b>e</b>	H	4-F-C <sub>6</sub> H <sub>4</sub>	76
<b>f</b>	H	2-Furyl	72
<b>g</b>	H	4-Py	78
<b>h</b>	Me	Me	98
<b>i</b>		-(CH <sub>2</sub> ) <sub>4</sub> -	96
<b>j</b>		-(CH <sub>2</sub> ) <sub>5</sub> -	72

**Table 2.** 7-Amino-4,5-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**3**)

<b>3</b>	R <sup>1</sup>	R <sup>2</sup>	Yield (%)
<b>a</b>	H	Ph	78
<b>b</b>	H	4-MeO-C <sub>6</sub> H <sub>4</sub>	87
<b>c</b>	H	4-Me-C <sub>6</sub> H <sub>4</sub>	72
<b>d</b>	H	4-Cl-C <sub>6</sub> H <sub>4</sub>	70
<b>e</b>	H	4-F-C <sub>6</sub> H <sub>4</sub>	78
<b>f</b>	H	2-Furyl	70
<b>g</b>	H	4-Py	68
<b>h</b>	Me	Me	65
<b>i</b>		-(CH <sub>2</sub> ) <sub>4</sub> -	62
<b>j</b>		-(CH <sub>2</sub> ) <sub>5</sub> -	73

## Figure and Scheme Legends

**Figure 1.** Selected antifolate 1,3,5-triazines and the compounds of interest

**Figure 2.** X-ray crystal structure of **2h**

**Figure 3.** X-ray crystal structure of **3h**

**Scheme 1.** Design of the 5(7)-amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines synthesis

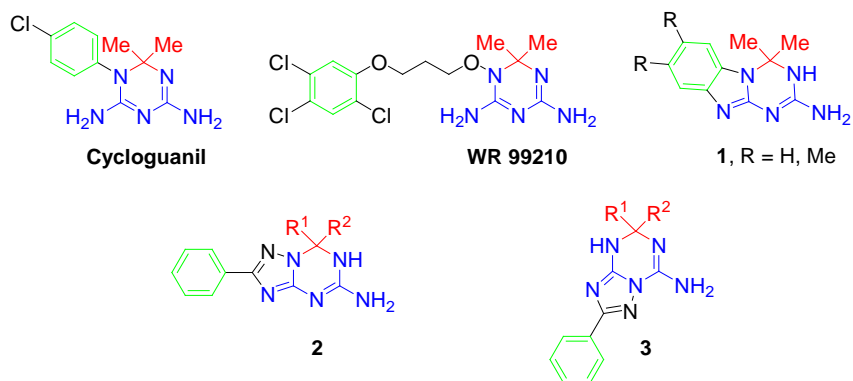
**Scheme 2.** Reagents and conditions: (i) cyanoguanidine (1.1 equiv), HCl (1 equiv), EtOH, 4 h, reflux (80%); (ii) 10% NaOH, 6 h, 80 °C (68%)

**Scheme 3.** Reagents and conditions: (i) **7a-g** (1 equiv) or **7i,j** (2 equiv), EtOH, piperidine (0.4 equiv), 3-24 h, reflux (72-96%); for **2h** acetone, piperidine (0.4 equiv), 18 h, reflux (98%)

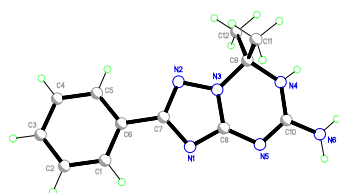
**Scheme 4.** Reagents and conditions: (i) *S*-methylisothiuronium sulfate (0.5 equiv), NaOH (1 equiv), H<sub>2</sub>O, 72 h, rt, 3 h, 50 °C (86%); (ii) H<sub>2</sub>O, 4 h, reflux (97%)

**Scheme 5.** Reagents and conditions: (i) guanylbenzotriazole hydrochloride (1 equiv), EtOH, 30 min, reflux 62%; (ii) 10% Na<sub>2</sub>CO<sub>3</sub>, 25 °C (92%); (iii) **7a-g** (1 equiv) or **7i,j** (2 equiv), EtOH, piperidine (0.4 equiv), 2-15 h, reflux (62-87%); for **3h** acetone, piperidine (0.4 equiv), 12 h, reflux (65%)

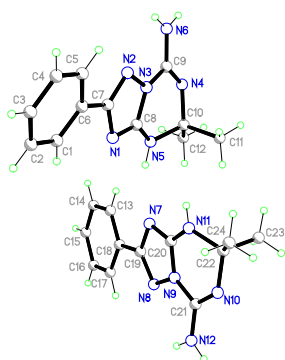
**Scheme 6.** Tautomerism in 5(7)-amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines



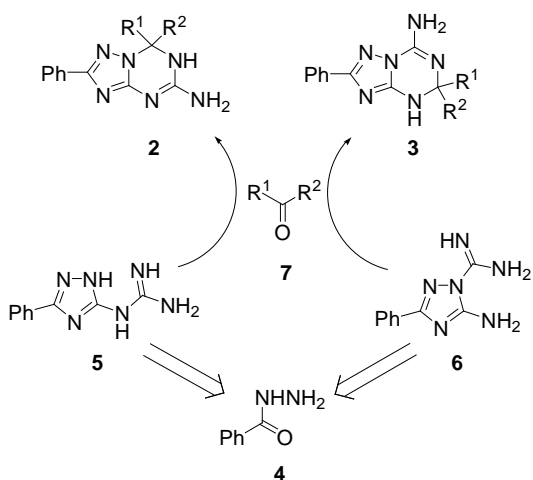
**Figure 1.** Selected antifolate 1,3,5-triazines and the compounds of interest



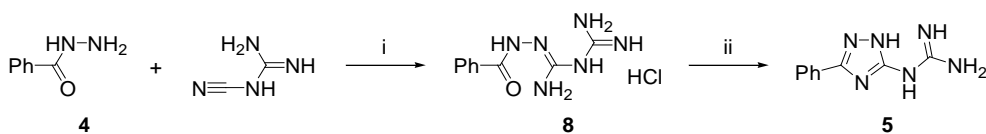
**Figure 2.** X-ray crystal structure of **2h**



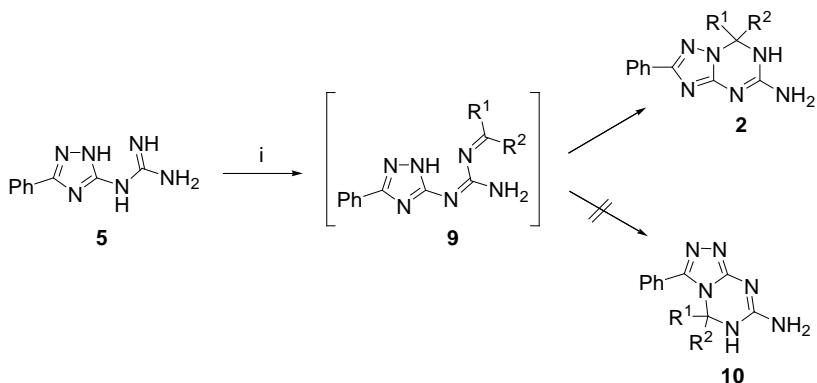
**Figure 3.** X-ray crystal structure of **3h**



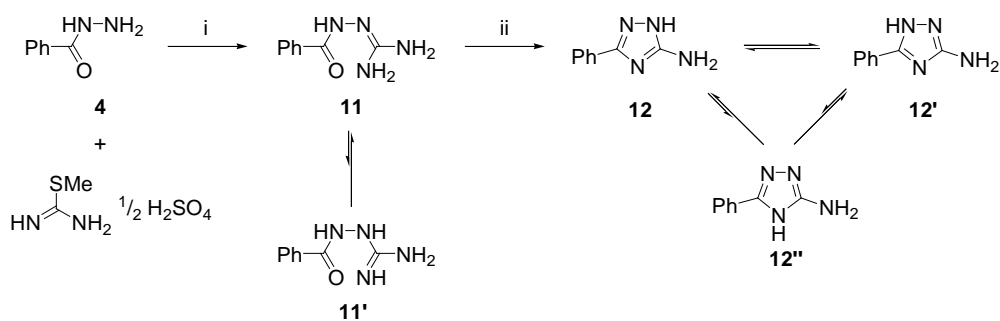
**Scheme 1.** Design of the 5(7)-amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines synthesis



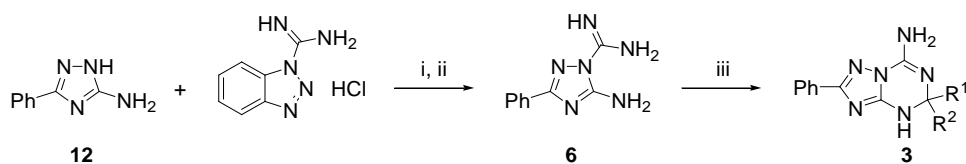
**Scheme 2.** Reagents and conditions: (i) cyanoguanidine (1.1 equiv), HCl (1 equiv), EtOH, 4 h, reflux (80%); (ii) 10% NaOH, 6 h, 80 °C (68%)



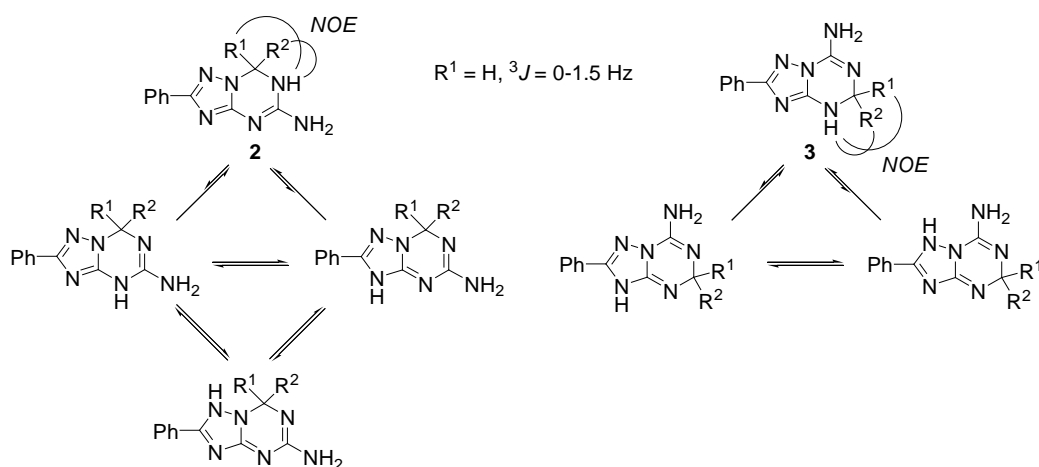
**Scheme 3.** Reagents and conditions: (i) **7a-g** (1 equiv) or **7i,j** (2 equiv), EtOH, piperidine (0.4 equiv), 3-24 h, reflux (72-96%); for **2h** acetone, piperidine (0.4 equiv), 18 h, reflux (98%)



**Scheme 4.** Reagents and conditions: (i) *S*-methylisothiuronium sulfate (0.5 equiv), NaOH (1 equiv), H<sub>2</sub>O, 72 h, rt, 3 h, 50 °C (86%); (ii) H<sub>2</sub>O, 4 h, reflux (97%)



**Scheme 5.** Reagents and conditions: (i) guanylbenzotriazole hydrochloride (1 equiv), EtOH, 30 min, reflux (62%); (ii) 10% Na<sub>2</sub>CO<sub>3</sub>, 25 °C (92%); (iii) **7a-g** (1 equiv) or **7i,j** (2 equiv), EtOH, piperidine (0.4 equiv), 2-15 h, reflux (62-87%); for **3h** acetone, piperidine (0.4 equiv), 12 h, reflux (65%)



**Scheme 6.** Tautomerism in 5(7)-amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazines