

Article

Fused Heterocyclic Systems with an s-Triazine Ring.

34. Development of a Practical Approach for the Synthesis of 5-Aza-isoguanines

Ahmad Junaid ¹, Felicia Phei Lin Lim ¹ , Yvonne Peijun Zhou ², Wai Keung Chui ² and Anton V. Dolzhenko ^{1,3,*}

¹ School of Pharmacy, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor Darul Ehsan 47500, Malaysia; ahmad.junaid@monash.edu (A.J.); felicia.lim@monash.edu (F.P.L.)

² Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore; marz_vonne@yahoo.com.sg (Y.P.Z.); phacwk@nus.edu.sg (W.K.C.)

³ School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University, GPO Box U1987, Perth, WA 6845, Australia

* Correspondence: anton.dolzhenko@monash.edu; Tel.: +60-3-5514-5867

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Abstract: Purine isosteres present excellent opportunities in drug design and development. Using isosteres of natural purines as scaffolds for the construction of new therapeutic agents has been a valid strategy of medicinal chemistry. Inspired by the similarity to isoguanine, we attempted to develop a practical method for the preparation of 5-aza-isoguanines. Several synthetic approaches were explored to establish a robust general protocol for the preparation of these compounds. The significant difference in the reactivity of the C-5 and C-7 electrophilic centers of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines (5-azapurines) towards nucleophiles was demonstrated. The most practical and general method for the preparation of 5-aza-isoguanines involved a regioselective reaction of ethoxycarbonyl isothiocyanate with a 5-aminotriazole. The intramolecular ring closure of the resulted product followed by the S-methylation afforded 7-methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one, which could be effectively aminated with various amines. The resulted 5-aza-isoguanines resemble a known purine nucleoside phosphorylase inhibitor and could be interesting for further investigations as potential anticancer agents.

Keywords: triazine; triazole; azapurine; purine isostere

1. Introduction

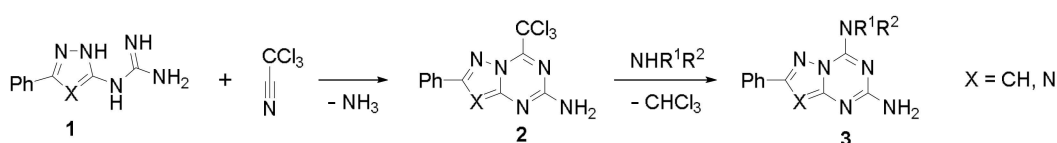
Purine isosteres attract significant attention from medicinal chemists due to their potential for the development of new therapeutic agents. These compounds are known to modulate a complex network of processes, which involves regulatory biogenic purines. The purine-like scaffolds incorporating a 1,3,5-triazine ring became privileged in the construction of bioactive molecules [1]. It was found that pyrazolo[1,5-*a*][1,3,5]triazine (5-aza-9-deazapurine) [2] and 1,2,4-triazolo[1,5-*a*][1,3,5]triazine (5-azapurine) [3,4] were the most promising skeletons in this group of purine isosteres. We developed efficient methods for the synthesis of adenine and xanthine analogues built on these scaffolds [5–15]. The synthesis of 5-aza-guanines has also been reported [16,17].

Isoguanine has been reported as a skeleton for several biologically important alkaloids [18–20]. Some interesting results were obtained on a compound with the 4-aminopyrazolo[1,5-*a*][1,3,5]triazin-2-one skeleton (a triazine-based 5-aza-9-deaza-isostere of isoguanine) [21]. This compound was an efficient inhibitor of purine nucleoside phosphorylase,

which is a valid target for anticancer and antiparasitic therapy [22–25]. This brought our attention to a similar purine-like heterocyclic system, i.e., 5-aza-isoguanine. Herein, we report our attempts to develop an efficient synthesis of 5-aza-isoguanines bearing various substituents on the amino group.

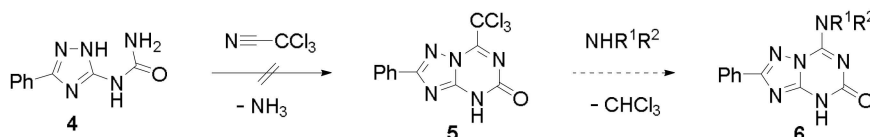
2. Results and Discussion

Earlier we developed convenient syntheses of amino-substituted pyrazolo[1,5-*a*][1,3,5]triazines and 1,2,4-triazolo[1,5-*a*][1,3,5]triazines exploiting good reactivity of highly electrophilic 1,3,5-triazine ring substituted with the trichloromethyl group [26,27]. The key step in this synthetic approach was the ring closure reaction of azolyguanidine **1** using trichloroacetonitrile (Scheme 1). The subsequent nucleophilic substitution of the introduced trichloromethyl leaving group of **2** with variety of amines resulted in the formation of the corresponding pyrazolo[1,5-*a*][1,3,5]triazines and 1,2,4-triazolo[1,5-*a*][1,3,5]triazines **3**.



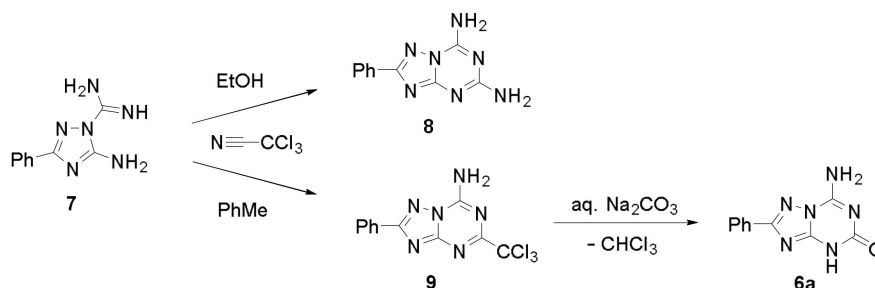
Scheme 1. Synthesis of diamino-substituted azolo[1,5-*a*][1,3,5]triazines **3**.

With our interest leaning towards the synthesis of 5-aza-isoguanines, which are 5-oxo-analogues of compounds **3**, an initial attempt to prepare 1,2,4-triazolo[1,5-*a*][1,3,5]triazine **5** bearing reactive trichloromethyl group was based on the previously developed reaction using triazolylurea **4** in place of guanidine **1** (Scheme 2). However, despite a number of attempts to achieve the triazine ring closure using trichloroacetonitrile under various reaction conditions, we were able to isolate starting urea **4** only.



Scheme 2. An attempt to prepare 5-aza-isoguanines via trichloromethyl-substituted intermediate **5**.

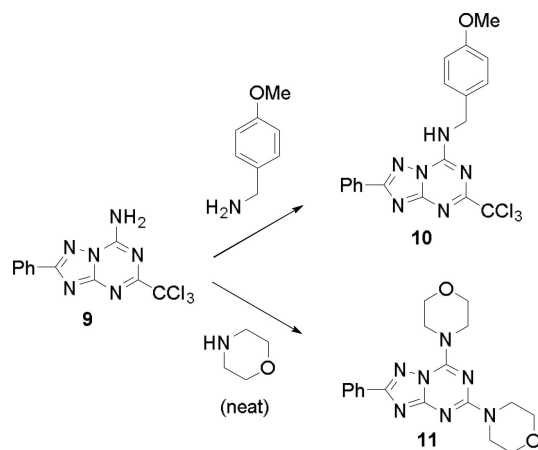
To further explore the trichloromethyl chemistry in the synthesis of 7-amino-substituted 1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-ones **6**, we subjected 1-guanyl-1,2,4-triazole **7**, prepared as reported previously [28], to the reaction with trichloroacetonitrile (Scheme 3). Similar to triazolylguanidine **1** ($X = N$), the reaction pathway of **7** was solvent dependent. In ethanol, diamine **8**, identical to that reported in reference [29], was formed, whereas 1,2,4-triazolo[1,5-*a*][1,3,5]triazine **9** was isolated exclusively when the reaction was carried out in toluene.



Scheme 3. Synthesis of 5-aza-isoguanine **6a**.

In the $^1\text{H-NMR}$ spectrum of **9**, two protons of the amino group gave independent signals appearing at rather low field: 9.22 and 9.65 ppm. These observations can be attributed to the prominent delocalisation of an electron pair on the amino group nitrogen directly attached to the highly electron-deficient 1,3,5-triazine ring. The electron-withdrawing trichloromethyl group further decreased electron density on the triazine ring, enhancing the partial double bond character of the C-NH₂ bond and restricting the rotation around it. The activation energy (ΔG^\ddagger) for the hindered rotation around this bond was estimated using dynamic $^1\text{H-NMR}$ spectroscopy and was equal 68.8 kJ at the coalescence temperature 343 K.

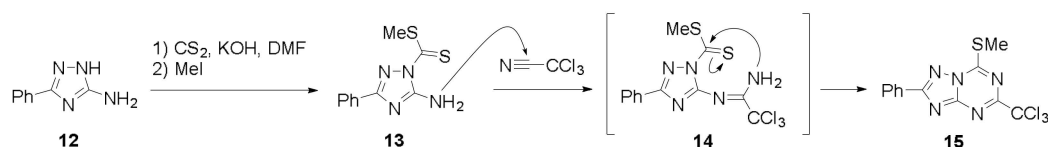
The trichloromethyl group of **9** was hydrolytically removed using aqueous solution of sodium carbonate to afford **6a**. The substitution of trichloromethyl group of **9** with nucleophiles (particularly amines) was more problematic in comparison to the aminolysis of the isomeric structure **2**. No reaction was observed when similar reaction conditions were applied. Moreover, unexpected results were obtained after prolonged heating with excess of amine depending on the reaction conditions and amine structure (Scheme 4). Replacement of the amino group in position 7 of the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine ring was observed instead of the proposed substitution of the trichloromethyl group of **9** (e.g., in the synthesis of **10**) or both the processes took place together (e.g., in the preparation of **11**). It was demonstrated earlier that compounds with identical leaving groups in positions 5 and 7 of the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine ring were suitable for the sequential nucleophilic substitution with amines: first in position 7, then in position 5 [30]. This strategy was successfully applied for the synthesis of bioactive 1,2,4-triazolo[1,5-*a*][1,3,5]triazines [31,32]. However, to the best of our knowledge, no examples on transaminations at the position 7 of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines have been reported. For similar pyrazolo[1,5-*a*][1,3,5]triazines, the replacement of a N-methylanilino substituent in the corresponding position with other amines was effectively employed in drug-discovery programs to prepare a variety of compounds with a diverse substitution pattern [33–40].



Scheme 4. Reaction of **9** with 4-methoxybenzylamine and morpholine.

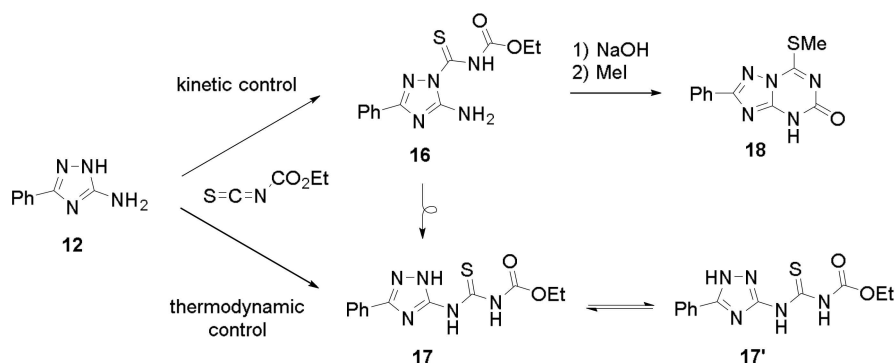
The hydrolysis of 7-amino substituted **10** can be applied to generate **6k**. Nevertheless, difficulties with controlling reaction outcome in the synthesis of analogues of **10** and a long reaction time led us to design an intermediate with a leaving group more accessible for the nucleophilic substitution by amines than **9**. This strategy was realized by the preparation of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**15**) (Scheme 5). Aminotriazole **12** reacted with carbon disulfide in DMF in the presence of potassium hydroxide followed by the selective S-methylation affording **13**. Treatment of **13** with trichloroacetonitrile resulted in the formation of **15**. Trichloroacetonitrile provided a C–N fragment for the construction of the triazine ring. We propose that the reaction involved an initial addition to the triple bond of trichloroacetonitrile followed by the intramolecular ring closure of intermediate **14** via nucleophilic substitution at the methyl dithiocarboxylate moiety. Surprisingly, hydrogen sulfide instead of methylthiol played the role

of a leaving group in this reaction. The methylthio group of **15** can be readily replaced by stoichiometric quantity of amines, e.g., 4-methoxybenzylamine, providing **10**. However, a long reaction time at the hydrolysis step arising from the low aqueous solubility of **10** and the necessity of chromatographic purification limited the practicality of this approach.



Scheme 5. Synthesis of 5-trichloromethyl-7-methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**15**).

Considering the smooth replacement of the methylthio group at position 7 of the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine with amines, we implemented an alternative synthetic approach for the preparation of **6**. The key intermediate in this protocol was **16**, prepared from 5-amino-3-phenyl-1,2,4-triazole (**12**) (Scheme 6). The selective addition of **12** to ethoxycarbonyl isothiocyanate was challenging due to the presence of several competing nucleophilic centers on **12**. It was found that regioselective addition occurs only at the endocyclic N-1 atom of **16** when the reaction was carried out under kinetic control in cold acetone for not more than 15 min. Extension of the reaction time or increasing temperature led to the formation of more thermodynamically stable *N*-carbethoxy-*N'*-(1,2,4-triazol-5-yl)thiourea, which existed in solution as a mixture of two tautomers **17** and **17'** ($K_T = 0.74$) crystallizing in the form of the predominant tautomer **17** (Figure 1) [41].



Scheme 6. Synthesis of 7-methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one (**18**).

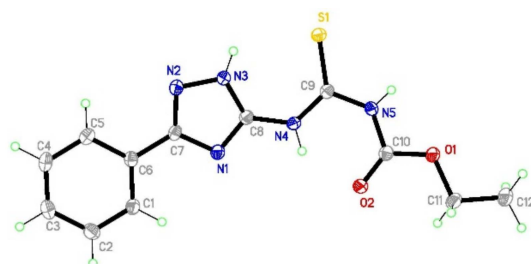
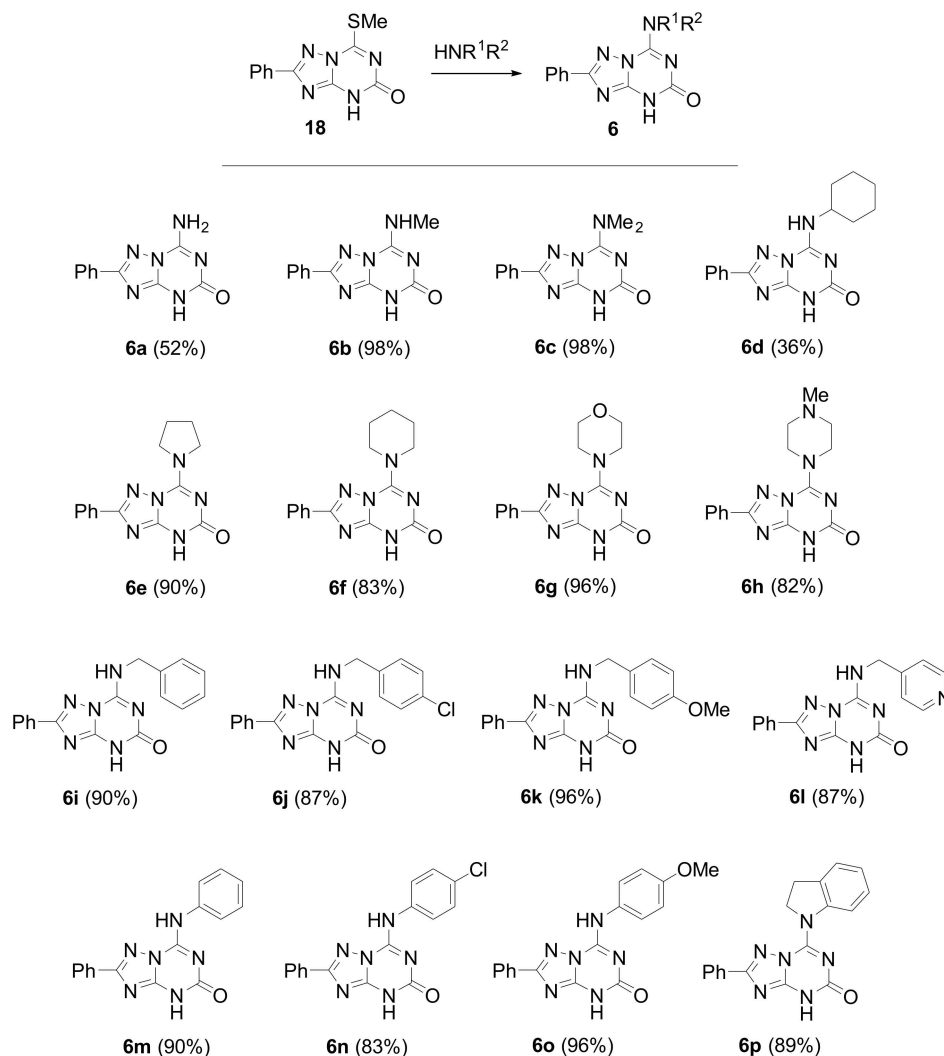


Figure 1. X-ray structure of **17**.

In solutions, **16** readily underwent rearrangement to **17** even at room temperature. Therefore, instant preparation of sufficiently pure **16** was critical, as changes in the product structure during purification were unavoidable. The alkali-induced cyclocondensation of **16** followed by *S*-methylation afforded 7-methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one (**18**).

The nucleophilic replacement of the methylthio group of **18** proceeded smoothly, with a variety of amines affording **6** with reasonable yields and high purity (Scheme 7). The reaction remained

chemoselective even when aqueous solutions of fairly basic amines (synthesis of **6a–c**) were used. The structure of the prepared compounds **6** was confirmed by NMR spectroscopy, clearly showing that the signal of methylthio group of **18** was replaced by signals of the corresponding amines in spectra of **6**. The X-ray data for a representative example **6l** also confirmed the proposed structure (Figure 2) [42].



Scheme 7. Synthesis of 5-aza-isoguanines **6**.

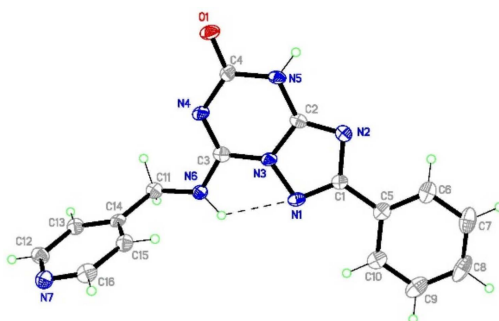


Figure 2. X-ray structure of **6l**.

The synthetic route described in Schemes 6 and 7 was not placed on the top priority at the initial design of the synthetic routes due to the relatively low yield of the intermediate **16** and the uncertainty involved in the preparation of a pure regioisomer **16** as a result of the addition reaction between

ethoxycarbonyl isothiocyanate to 5-amino-3-phenyl-1,2,4-triazole (**12**). However, this synthetic route unexpectedly became the most practical and versatile method that could be applied to the synthesis of a library of 7-amino substituted 2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-ones (**6a–p**).

3. Conclusions

In conclusion, several synthetic approaches to the synthesis of 5-aza-isoguanines **6** were explored to develop a practical method for their preparation. The significant difference in the reactivity of the C-5 and C-7 electrophilic centers of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines towards nucleophiles was demonstrated. The most practical and general method for the preparation of 5-aza-isoguanines **6** involved a regioselective addition of 5-amino-3-phenyl-1,2,4-triazole (**12**) to ethoxycarbonyl isothiocyanate followed by an intramolecular ring closure and S-methylation to afford **18**, which could be effectively aminated with various amines. The prepared compound **6** resemble a known purine nucleoside phosphorylase inhibitor and could be interesting for further investigations as potential anticancer agents.

4. Materials and Methods

4.1. General

The melting points (uncorrected) were determined via the use of the Gallenkamp melting point apparatus. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer (Fällanden, Switzerland), using DMSO- d_6 as a solvent and TMS as the internal reference. All the reactions were monitored with the use of the analytical TLC carried out on aluminum plates coated on Silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany) with detection by UV light.

4.2. Synthesis

4.2.1. Synthesis of 2-Phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5,7-diamine **8**

1-Guanyl-3-phenyl-1,2,4-triazol-5-amine (**7**, 0.3 g, 1.5 mmol) and trichloroacetonitrile (0.45 mL, 4.5 mmol) were heated in EtOH (10 mL) under reflux for 6 h. After cooling, the precipitated colorless product was filtered and washed with cold EtOH to give the product, which was found to be identical to compound **8** prepared earlier via an alternative method [29]. Yield 210 mg, 62%.

4.2.2. Synthesis of 7-Amino-2-phenyl-5-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine **9**

1-Guanyl-3-phenyl-1,2,4-triazol-5-amine (**7**, 0.3 g, 1.5 mmol) and trichloroacetonitrile (0.45 mL, 4.5 mmol) were refluxed in toluene (10 mL) for 6 h. After cooling, the colorless product was filtered and recrystallized from EtOH. Yield 310 mg, 63%; mp > 300 °C (EtOH). ^1H -NMR (300 MHz, DMSO- d_6): δ 7.53–7.65 (3H, m, H-3', H-4' and H-5'), 8.19–8.28 (2H, m, H-2' and H-6'), 9.22 (1H, s, NH), 9.65 (1H, s, NH). ^{13}C -NMR (75 MHz, DMSO- d_6): δ 96.2, 126.9 (2C), 128.9 (2C), 129.7, 130.9, 151.6, 157.2, 164.1, 164.6. Combustion elemental analysis calculated for C₁₁H₇Cl₃N₆: C, 40.09; H, 2.14; N, 25.50. Found: C, 39.96; H, 2.35; N, 25.32.

4.2.3. Synthesis of 7-Amino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-ones **6a** from **9**

To 10% aqueous Na₂CO₃ (10 mL), 7-amino-2-phenyl-5-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine (**9**, 0.33 g, 1 mmol) was added, and the mixture was heated under reflux for 72 h. The reaction mixture was allowed to cool and HCl (1 N) was added dropwise to pH 4. The precipitate formed was filtered and washed with water to give colorless crystalline powder. Yield 36 mg, 16%; mp > 300 °C (MeOCH₂CH₂OH). ^1H -NMR (300 MHz, DMSO- d_6): δ 7.48–7.58 (3H, m, H-3', H-4' and H-5'), 8.01–8.11 (2H, m, H-2' and H-6'), 8.26 (1H, s, NH), 8.64 (1H, brs, NH), 12.17 (1H, brs, N(4)H). ^{13}C -NMR (75 MHz, DMSO- d_6): δ 126.5 (2C), 128.8 (2C), 129.6, 130.4, 149.8, 153.1, 153.6 (2C), 161.4. Combustion elemental analysis for C₁₀H₈N₆O: C, 52.63; H, 3.53; N, 36.83. Found: C, 52.42; H, 3.71; N, 36.66.

4.2.4. Synthesis of *N*-(4-Methoxybenzyl)-2-phenyl-5-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-7-amine **10**

To the suspension of **9** (0.33 g, 1 mmol) in EtOH (5 mL), 4-methoxybenzylamine (0.36 mL, 3 mmol) was added and the mixture was heated under reflux for 24 h. After cooling, the resulting precipitate was filtered, washed with cold EtOH, dried and recrystallized from EtOH. Yield 212 mg, 47%; mp 174–175 °C (EtOH). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.73 (3H, s, OMe), 4.73 (2H, d, *J* = 5.8 Hz, CH₂), 6.91 (2H, d, *J* = 8.7 Hz, C-3'' and C-5''), 7.47 (2H, d, *J* = 8.7 Hz, C-2'' and C-6''), 7.52–7.61 (3H, m, H-3', H-4' and H-5'), 8.16–8.28 (2H, m, H-2' and H-6'), 10.20 (1H, t, *J* = 5.8 Hz, NH). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 43.7, 55.0, 96.1, 113.7 (2C'), 126.9 (2C), 128.9 (2C), 129.3, 129.7, 129.8 (2C), 130.9, 149.6, 157.1, 158.7, 163.9, 164.5. Combustion elemental analysis calculated for C₁₉H₁₅Cl₃N₆O: C, 50.74; H, 3.36; N, 18.69. Found: C, 50.55; H, 3.52; N, 18.42.

4.2.5. Synthesis of 5,7-Bis(morpholino)-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine **11**

To 5 mL of morpholine, **9** (0.33 g, 1 mmol) was added, and the mixture was heated under reflux for 5 h. After cooling, the resulting precipitate was filtered, washed with cold EtOH, dried and recrystallized from EtOH. Yield 180 mg, 49%; mp 278–279 °C (EtOH). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.61–3.85 (12H, m), 4.27 (4H, br. s, *W*_{1/2} = 24.9 Hz), 7.46–7.55 (3H, m, H-3', H-4' and H-5'), 8.05–8.15 (2H, m, H-2' and H-6'). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 44.0 (br. s), 46.7, 65.7, 65.8, 126.6 (2C), 128.6 (2C), 130.2 (2C), 148.5, 158.8, 161.1, 162.1. Combustion elemental analysis calculated for C₁₈H₂₁N₇O₂: C, 58.84; H, 5.76; N, 26.69. Found: C, 58.61; H, 6.02; N, 26.41.

4.2.6. Synthesis of Methyl 5-Amino-3-phenyl-1,2,4-triazoyl-1-dithiocarbonate **13**

3-Phenyl-1,2,4-triazol-5-amine (**12**, 3.20 g, 20 mmol) was dissolved in cold DMF (8 mL). Carbon disulphide (1.2 mL, 20 mmol) was added drop-wise, followed by 10 N KOH solution (2 mL, 20 mmol). The reaction mixture was stirred for 1 h on an ice-bath. Iodomethane (1.25 mL, 20 mmol) was added, and stirring was continued for 10 min. Subsequently, the ice-bath was removed and the reaction mixture was stirred for 2 h. Cold water (15 mL) was added to the mixture, the yellow precipitate was filtered, washed with water, dried and recrystallized from EtOH. Yield 80 mg, 32%; mp 207–209 °C (EtOH). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 2.63 (3H, s, SMe), 7.47–7.57 (3H, m, H-3', H-4' and H-5'), 8.03 (2H, dd, *J* = 6.8, 3.0 Hz, H-2' and H-6'), 8.64 (2H, s, NH₂). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 18.8, 126.6 (2C), 128.8 (2C), 129.1, 130.6, 157.5, 158.8, 199.5. Combustion elemental analysis calculated for C₁₀H₁₀N₄S₂: C, 47.98; H, 4.03; N, 22.38. Found: C, 48.06; H, 4.12; N, 22.14.

4.2.7. Synthesis of 7-Methylthio-2-phenyl-5-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine **15**

Methyl 5-amino-3-phenyl-1,2,4-triazoyl-1-dithiocarbonate (**13**, 0.75 g, 3 mmol) and trichloroacetonitrile (0.75 mL, 7.5 mmol) were heated under reflux in toluene (10 mL) for 12 h. Solvent was evaporated under reduced pressure, the residue was triturated with diethyl ether and solid was collected by filtration. Yield 245 mg, 68%. ¹H-NMR (300 MHz, DMSO-*d*₆): δ 2.85 (3H, s, SMe), 7.56–7.68 (3H, m, H-3', H-4' and H-5'), 8.26 (2H, dd, *J* = 6.6, 2.8 Hz, H-2' and H-6'). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 13.1, 95.2, 127.2 (2C), 129.0, 129.1 (2C), 131.6, 155.2, 161.2, 164.4, 166.0. Combustion elemental analysis calculated for C₁₂H₈Cl₃N₅S: C, 39.97; H, 2.24; N, 19.42. Found: C, 40.12; H, 2.33; N, 19.11.

4.2.8. Reactions of 3-Phenyl-1,2,4-triazol-5-amine (**12**) with Ethoxycarbonyl Isothiocyanate

Synthesis of 5-amino-1-carbethoxythiocarbamoyl-1,2,4-triazole (**16**). To a solution of acetone (30 mL), 3-phenyl-1,2,4-triazol-5-amine (**12**, 3.20 g, 20 mmol) and ethoxycarbonyl isothiocyanate (2.62 mL, 20 mmol) were added. The reaction mixture was stirred on an ice-bath for 15 min. The yellow precipitate formed was filtered immediately. Yield 2.16 g, 37%; mp 207–209 °C. ¹H-NMR (300 MHz, DMSO-*d*₆): δ 2.63 (3H, s, SMe), 7.47–7.57 (3H, m, H-3', H-4' and H-5'), 8.03 (2H, dd, *J* = 6.8, 3.0 Hz,

H-2' and H-6'), 8.64 (2H, s, NH₂). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 18.8, 126.6 (2C), 128.8 (2C), 129.1, 130.6, 157.5, 158.8, 199.5. Combustion elemental analysis calculated for C₁₂H₁₃N₅O₂S: C, 49.47; H, 4.50; N, 24.04. Found: C, 49.20; H, 4.67; N, 23.88.

Synthesis of *N*-carbethoxy-*N'*-(3-phenyl-1*H*-1,2,4-triazol-5-yl)thiourea (**17**). To the solution of 3-phenyl-1,2,4-triazol-5-amine (**12**, 0.48 g, 3 mmol) in anhydrous DMF (4 mL), ethoxycarbonyl isothiocyanate (0.43 mL, 3.3 mmol) was added. After stirring the mixture for 5 h at room temperature, cold water (60 mL) was added. The precipitated product was filtered, washed with cold water and recrystallized from EtOH. Yield 0.74 g, 85%; mp 180–181 °C (EtOH). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 1.28 (3H, t, *J* = 7.1 Hz, CH₃), 4.25 (2H, q, *J* = 7.1 Hz, CH₂), 7.37–7.66 (3H, m, H-3', H-4' and H-5'), 8.01 (2H, d, *J* = 7.2 Hz, H-2' and H-6'), 11.56* (12H, brs, 2NH), 11.87 (1H, brs, NH), 12.16 (1H, brs, NH), 13.93 (1H, brs, N(1)H), 14.47 (1H, brs, N(1)H)*. *—signals of the minor tautomer. Combustion elemental analysis calculated for C₁₂H₁₃N₅O₂S: C, 49.47; H, 4.50; N, 24.04. Found: C, 49.33; H, 4.62; N, 23.95.

4.2.9. Synthesis of 7-Methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one **18**

5-Amino-1-carbethoxythiocarbamoyl-1,2,4-triazole (**16**, 2.9 g, 10 mmol) was heated under reflux in aqueous EtOH (80%, 20 mL) for 20 min and then cooled. The creamy product was filtered, dissolved in 5 mL of water and then stirred with iodomethane (0.62 mL, 10 mmol) for 30 min at 10 °C with a slow warming to room temperature. The colorless precipitate formed was filtered and dissolved in water (30 mL). A solution of HCl (1 N) was then added drop-wise to pH 3. The colorless precipitate formed was filtered, washed with water and dried. Yield 1.68 g, 65%; mp 275–276 °C. ¹H-NMR (300 MHz, DMSO-*d*₆): δ 2.62 (3H, s, SMe), 7.49–7.59 (3H, m, H-3', H-4' and H-5'), 8.00–8.11 (2H, m, H-2' and H-6'), 13.07 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 12.8, 126.7 (2C), 128.9 (2C), 130.0, 130.8, 151.2, 152.1 (2C), 162.2, 162.6. Combustion elemental analysis calculated for C₁₁H₉N₅OS: C, 50.96; H, 3.50; N, 27.01. Found: C, 50.77; H, 3.62; N, 26.90.

4.2.10. Synthesis of 7-Amino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one **6a** from **18**

7-Methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one (**18**, 0.52 g, 2 mmol) was added to a solution of aqueous NH₃ (28%, 20 mL), and the mixture was stirred on a water bath at 55 °C for 18 h. The colorless precipitate formed was filtered and recrystallized from MeOCH₂CH₂OH. Yield 238 mg, 52%. Analytical data obtained were identical to those of compound **6a** obtained from the method described under Section 4.2.3.

4.2.11. Synthesis of 7-Amino-substituted-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-ones **6b** and **6c**

7-Methylthio-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-one (**18**, 0.52 g, 2 mmol) was added to an aqueous solution of methylamine (40%) or dimethylamine (40%) (10 mL) and the mixture was stirred at room temperature for 12 h. Solvent was evaporated under reduced pressure and recrystallized from a suitable solvent.

*7-Methylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine-5-one (6b)*. Colorless crystalline powder; yield 474 mg, 98%; mp 298–299 °C (EtOH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 2.94 (3H, d, *J* = 3.8 Hz, NHMe), 7.48–7.58 (3H, m, H-3', H-4' and H-5'), 8.02–8.11 (2H, m, H-2' and H-6'), 8.71 (1H, q, *J* = 3.8 Hz, NH), 12.18 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 27.3, 126.5 (2C), 128.8 (2C), 129.6, 130.4, 148.3, 152.8, 153.4 (brs), 161.3. Combustion elemental analysis calculated for C₁₁H₁₀N₆O: C, 54.54; H, 4.16; N, 34.69. Found: C, 54.46; H, 4.21; N, 34.60.

*7-Dimethylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazine-5-one (6c)*. Colorless crystalline powder; yield 500 mg, 98%; mp > 300 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.47 (6H, brs, NMe₂), 7.49–7.57 (3H, m, H-3', H-4' and H-5'), 8.00–8.10 (2H, m, H-2' and H-6'), 12.24 (1H, s, N(4)H). Combustion elemental analysis calculated for C₁₂H₁₂N₆O: C, 56.24; H, 4.72; N, 32.79. Found: C, 56.11; H, 4.89; N, 32.62.

4.2.12. Synthesis of 7-Amino-substituted-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-ones **6d–6p**.

7-Methylthio-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (18, 0.52 g, 2 mmol) was added to a solution of appropriate amine (3 mmol) in DMF (5 mL), and the mixture was heated at 70–80 °C with stirring for 4–14 h. After cooling, ice cold water (40 mL) was added and the product was filtered and recrystallized from a suitable solvent.

7-Cyclohexylamino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (6d). Colorless crystalline powder; yield 245 mg, 36%; mp > 300 °C (EtOH). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 1.03–1.21 (1H, m, H_{c-Hex}), 1.23–1.42 (2H, m, H_{c-Hex}), 1.44–1.69 (3H, m, H_{c-Hex}), 1.71–1.92 (4H, m, H_{c-Hex}), 3.85–4.01 (1H, m, H-1''), 7.47–7.59 (3H, m, H-3', H-4' and H-5'), 8.02–8.16 (2H, m, H-2' and H-6'), 8.56 (1H, d, *J* = 8.3 Hz, NH), 12.17 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 24.85 (2C), 24.89, 31.5 (2C), 50.0, 126.6 (2C), 128.7 (2C), 129.6, 130.4, 147.1, 152.9, 153.5 (2C), 161.3. Combustion elemental analysis calculated for C₁₆H₁₈N₆O: C, 61.92; H, 5.85; N, 27.08. Found: C, 61.86; H, 6.02; N, 26.89.

2-Phenyl-7-pyrrolidino-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (6e). Colorless crystalline powder; yield 508 mg, 90%; mp > 300 °C (DMSO); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 1.82–2.08 (4H, m, C(3'')H₂C(4'')H₂), 3.64 (1H, t, *J* = 6.0 Hz, C(5'')H₂), 4.25 (1H, t, *J* = 6.0 Hz, C(2'')H₂), 7.48–7.57 (3H, m, H-3', H-4' and H-5'), 8.00–8.09 (2H, m, H-2' and H-6'), 12.15 (1H, s, N(4)H). Combustion elemental analysis calculated for C₁₄H₁₄N₆O: C, 59.56; H, 5.00; N, 29.77. Found: C, 59.43; H, 5.24; N, 29.51.

7-Piperidino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (6f). Colorless crystalline powder; yield 492 mg, 83%; mp 286 °C (MeOCH₂CH₂OH). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 1.57–1.78 (6H, m, C(3'')H₂, C(4'')H₂, C(5'')H₂), 3.80–4.50 (4H, m, C(2'')H₂, C(6'')H₂), 7.47–7.58 (3H, m, H-3', H-4' and H-5'), 7.98–8.10 (2H, m, H-2' and H-6'), 12.29 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 23.7, 25.5, 47.9, 126.5 (2C), 128.8 (2C), 129.3, 130.5, 147.4, 152.8, 154.9 (2C), 160.4. Combustion elemental analysis calculated for C₁₅H₁₆N₆O: C, 60.80; H, 5.44; N, 28.36. Found: C, 60.68; H, 5.63; N, 28.21.

7-Morpholino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (6g). Colorless crystalline powder; yield 573 mg, 96%; mp > 300 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.77 (4H, t, *J* = 4.5 Hz, CH₂OCH₂), 4.23 (4H, brs, CH₂NCH₂), 7.47–7.57 (3H, m, H-3', H-4' and H-5'), 8.00–8.10 (2H, m, H-2' and H-6'), 12.39 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 47.2 (brs), 65.7, 126.6 (2C), 128.8 (2C), 129.1, 130.6, 147.7, 152.7, 154.9 (2C), 160.5. Combustion elemental analysis calculated for C₁₄H₁₄N₆O₂: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.22; H, 4.87; N, 28.02.

7-N-methylpiperazino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (6h). Colorless crystalline powder; yield 510 mg, 82%; mp 266–268 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 2.24 (3H, s, NMe), 2.44–2.55 (4H, m, CH₂N(Me)CH₂), 4.24 (4H, brs, CH₂NCH₂), 7.47–7.59 (3H, m, H-3', H-4' and H-5'), 7.99–8.10 (2H, m, H-2' and H-6'), 12.11 (1H, brs, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 45.3, 46.6 (brs), 54.1, 126.5 (2C), 128.8 (2C), 129.2, 130.6, 147.6, 152.8, 154.9 (2C), 160.5. Combustion elemental analysis calculated for C₁₅H₁₇N₇O: C, 57.87; H, 5.50; N, 31.49. Found: C, 57.64; H, 5.62; N, 31.35.

7-Benzylamino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (6i). Colorless crystalline powder; yield 572 mg, 90%; mp > 300 °C (DMF); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 4.63 (2H, d, *J* = 6.0 Hz, CH₂), 7.27 (1H, t, *J* = 7.0 Hz, H-4''), 7.35 (2H, t, *J* = 7.3 Hz, H-3'' and H-5''), 7.41 (2H, d, *J* = 7.9 Hz, H-2'' and H-6''), 7.48–7.59 (3H, m, H-3', H-4' and H-5'), 8.03–8.13 (2H, m, H-2' and H-6'), 9.31 (1H, t, *J* = 6.0 Hz, NH), 12.24 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 43.4, 126.5 (2C), 127.0, 127.4 (2C), 128.2 (2C), 128.8 (2C), 129.6, 130.5, 138.0, 148.2, 152.9, 153.5 (2C), 161.4. Combustion elemental analysis calculated for C₁₇H₁₄N₆O: C, 64.14; H, 4.43; N, 26.40. Found: C, 63.89; H, 4.64; N, 26.19.

7-(4-Chlorobenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6j**). Colorless crystalline powder; yield 614 mg, 87%; mp > 300 °C (DMF); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 4.61 (2H, d, *J* = 5.7 Hz, CH₂), 7.40 (2H, d, *J* = 8.7 Hz, H-3'' and H-5''), 7.44 (2H, d, *J* = 8.7 Hz, H-2'' and H-6''), 7.48–7.59 (3H, m, H-3', H-4' and H-5'), 8.03–8.13 (2H, m, H-2' and H-6'), 9.32 (1H, t, *J* = 5.7 Hz, NH), 12.25 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 42.8, 126.5 (2C), 128.1 (2C), 128.8 (2C), 129.3 (2C), 129.5, 130.5, 131.6, 137.0, 148.2, 152.9, 153.4 (2C), 161.5. Combustion elemental analysis calculated for C₁₇H₁₃ClN₆O: C, 57.88; H, 3.71; N, 23.82. Found: C, 57.71; H, 4.00; N, 23.69.

7-(4-Methoxybenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6k**). Colorless crystalline powder; yield 670 mg, 96%; mp > 300 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.73 (3H, s, OMe), 4.56 (2H, d, *J* = 6.0 Hz, CH₂), 6.90 (2H, d, *J* = 8.7 Hz, H-3'' and H-5''), 7.35 (2H, d, *J* = 8.7 Hz, H-2'' and H-6''), 7.48–7.58 (3H, m, H-3', H-4' and H-5'), 8.03–8.12 (2H, m, H-2' and H-6'), 9.25 (1H, t, *J* = 6.0 Hz, NH), 12.25 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 42.9, 55.0, 113.6 (2C), 126.5 (2C), 128.8 (2C), 128.9 (2C), 129.5, 129.9, 130.4, 148.0, 152.9, 153.5 (brs), 158.4, 161.4. Combustion elemental analysis calculated for C₁₈H₁₆N₆O₂: C, 62.06; H, 4.63; N, 24.12. Found: C, 61.96; H, 4.88; N, 23.93.

2-Phenyl-7-[(pyridin-4-ylmethyl)amino]-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6l**). Colorless crystalline powder; yield 556 mg, 87%; mp 293–294 °C (EtOH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 4.66 (2H, d, *J* = 5.7 Hz, CH₂), 7.40 (2H, d, *J* = 4.9 Hz, H-3'' and H-5''), 7.48–7.61 (3H, m, H-3', H-4' and H-5'), 8.02–8.15 (2H, m, H-2' and H-6'), 8.52 (2H, d, *J* = 4.9 Hz, H-2'' and H-6''), 9.36 (1H, t, *J* = 5.7 Hz, NH), 12.29 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 42.5, 122.1 (2C), 126.5 (2C), 128.8 (2C), 129.5, 130.5, 146.9, 148.4, 149.4 (2C), 153.0, 153.4 (2C), 161.5. Combustion elemental analysis calculated for C₁₆H₁₃N₇O: C, 60.18; H, 4.10; N, 30.70. Found: C, 59.97; H, 4.34; N, 30.51.

2-Phenyl-7-phenylamino-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6m**). Colorless crystalline powder; yield 545 mg, 90%; mp 298–299 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 7.23 (1H, t, *J* = 7.9 Hz, H-4''), 7.43 (2H, t, *J* = 7.9 Hz, H-3'' and H-5''), 7.49–7.61 (3H, m, H-3', H-4' and H-5'), 7.80 (2H, d, *J* = 7.9 Hz, H-2'' and H-4''), 8.09–8.21 (2H, m, H-2' and H-6'), 10.44 (1H, s, NH), 12.45 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 123.3 (2C), 125.2, 126.7 (2C), 128.4 (2C), 128.8 (2C), 129.5, 130.5, 136.4, 146.5, 153.1, 153.3 (2C), 161.4. Combustion elemental analysis calculated for C₁₆H₁₂N₆O: C, 63.15; H, 3.97; N, 27.62. Found: C, 62.99; H, 4.24; N, 27.41.

7-(4-Chlorophenylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6n**). Colorless crystalline powder; yield 562 mg, 83%; mp > 300 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 7.48 (2H, d, *J* = 8.7 Hz, H-3'' and H-5''), 7.52–7.61 (3H, m, H-3', H-4' and H-5'), 7.87 (2H, d, *J* = 8.7 Hz, H-2'' and H-4''), 8.08–8.20 (2H, m, H-2' and H-6'), 10.55 (1H, s, NH), 12.49 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 124.7 (2C), 126.7 (2C), 128.4 (2C), 128.8 (2C), 129.0, 129.5, 130.6, 135.5, 146.4, 153.1 (2C), 161.5. Combustion elemental analysis calculated for C₁₆H₁₁ClN₆O: C, 56.73; H, 3.27; N, 24.81. Found: C, 56.62; H, 3.36; N, 24.70.

7-(4-Methoxyphenylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6o**). Colorless crystalline powder; yield 650 mg, 96%; mp 296–297 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.78 (3H, s, OMe), 6.99 (2H, d, *J* = 8.7 Hz, H-3'' and H-5''), 7.49–7.60 (3H, m, H-3', H-4' and H-5'), 7.65 (2H, d, *J* = 8.7 Hz, H-2'' and H-4''), 8.08–8.19 (2H, m, H-2' and H-6'), 10.35 (1H, s, NH), 12.37 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 55.2, 113.6 (2C), 125.0 (2C), 126.7 (2C), 128.8 (2C), 129.1, 129.5, 130.6, 146.6, 153.1, 153.3 (2C), 156.8, 161.4. Combustion elemental analysis calculated for C₁₇H₁₄N₆O₂: C, 61.07; H, 4.22; N, 25.14. Found: C, 60.87; H, 4.49; N, 24.98.

7-Indolino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-one (**6p**). Colorless crystalline powder; yield 588 mg, 89%; mp 297 °C (MeOCH₂CH₂OH); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.27 (2H, t, *J* = 8.3 Hz,

C(3'')H₂), 4.91 (2H, d, *J* = 8.3 Hz, C(2'')H₂), 7.13 (1H, t, *J* = 7.3 Hz, H-5''), 7.27 (1H, t, *J* = 7.7 Hz, H-6''), 7.34 (1H, d, *J* = 7.2 Hz, H-5''), 7.44–7.60 (3H, m, H-3', H-4' and H-5'), 8.00–8.15 (2H, m, H-2' and H-6'), 8.40 (1H, d, *J* = 7.9 Hz, H-7''), 12.48 (1H, s, N(4)H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 27.9, 52.3, 118.9, 124.7, 124.8, 126.5 (2C), 126.7, 128.8 (2C), 129.3, 130.5, 133.1, 141.7, 146.1, 153.0, 154.6 (2C), 160.5. Combustion elemental analysis calculated for C₁₈H₁₄N₆O: C, 65.44; H, 4.27; N, 25.44. Found: C, 65.23; H, 4.54; N, 25.19.

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Sample Availability: Samples of compounds **6a–p** are available from the authors.



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