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Synthesis and biological activity of fluorinated 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines☆

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ABSTRACT

New fluorinated 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines were designed as potential anticancer agents and a practical method for their preparation was developed. The reaction of benzhydrazide with cyanoguanidine followed by intramolecular cyclocondensation resulted in the formation of triazolylguanidine, which upon condensation with trichloroacetonitrile afforded a key intermediate – 2-phenyl-7-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amine. In mild conditions, this intermediate underwent nucleophilic displacement of the trichloromethyl group with a series of fluorinated benzylamines providing the target compounds. Antiproliferative activity of the prepared compounds against the lung and breast cancer cells was explored. Together with anticancer effect, some compounds demonstrated anti-angiogenic properties.

1. Introduction

In the drug discovery programs utilizing purine isosteres as scaffolds, 1,3,5-triazine-based purine analogs are known to possess a broad range of biological activities [2]. The 5-azapurine (1,2,4-triazolo[1,5-*a*][1,3,5]triazine) skeleton [3] has been recognized as one of the most promising scaffolds among this type of molecules, particularly for development of new potent bioactive compounds. Our studies on this heterocyclic system resulted in the development of a series of practical synthetic methods [4–10] and identification of several compounds with potent anticancer properties [11]. Moreover, some of the identified compounds were found to inhibit expression of several angiogenic factors, which are associated with the tumor growth and metastasis [12–15].

The unique character of fluorinated compounds has been translated into several successful achievements in drug development [16–19] and many advancements in the field of anticancer agents [20,21].

Previously, in our program dedicated to the search of new antiproliferative agents, we identified fluorinated 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives **1** (Fig. 1), which were active against several cancer cell lines [11]. Further development of structure **1** faced two important medicinal chemistry problems associated with the dihydro-1,2,4-triazolo[1,5-*a*][1,3,5]triazine core structure: (i) existence of the compounds in the form of racemate due to chirality at C-7 and (ii) potential metabolic (hydrolytic) instability of the partially saturated dihydrotriazine ring. The oxidation-aromatization of the heterocyclic system seemed to be a potential solution for both problems. However, the aromatization of the structure would significantly change the geometry of the molecule **1** by eliminating the NH hydrogen bond donor and by making the fluorinated phenyl ring constrained in the plane of the 1,2,4-triazolo[1,5-*a*][1,3,5]triazine system. To overcome these drawbacks we designed structures **2** (Fig. 1) with the NH hydrogen donor in close vicinity of the N-6 atom. This modification aims to retain ability of **2** to interact with the biological target *via* a hydrogen bonding pattern, which is similar to the one proposed for compounds **1**. The introduction of the flexible methylenic bridge should allow the fluorinated phenyl ring

☆ Part 28 in the series "Fused heterocyclic systems with an *s*-triazine ring", for part 27, see [1].

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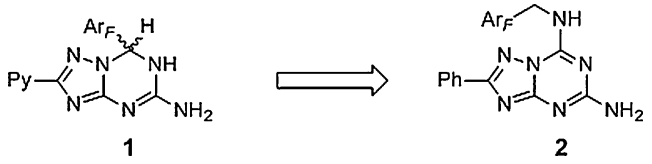


Fig. 1. Design of fluorinated 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines with potential antiproliferative activity.

of **2** to enter into the same pocket as the fluorinated phenyl ring of the prototype **1**. To test this hypothesis, a small library of fluorinated 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines (**2**) was synthesized and their antiproliferative activity against cancer cell lines was explored. In our previous evaluation of compounds **1**, no significant difference in the biological activity was observed between compounds substituted with 3- or 4-pyridyl groups [11]. Therefore, we assumed that removal of the pyridine nitrogen atom can minimize potential undesirable interaction without altering binding pattern with the target responsible for the anticipated antiproliferative effect.

Herein, we report synthesis of new fluorinated 1,2,4-triazolo[1,5-*a*][1,3,5]triazines **2** and their biological activity *viz.* antiproliferative activity against lung cancer A549 and breast cancer MDA-MB-231 cell lines and anti-angiogenic properties.

2. Results and discussion

2.1. Chemistry

The synthesis of the designed compounds **2** was performed using a practical method based on the introduction of fluorinated benzylamino moieties by the replacement of a trichloromethyl group on the electrophilic center [10,22,23].

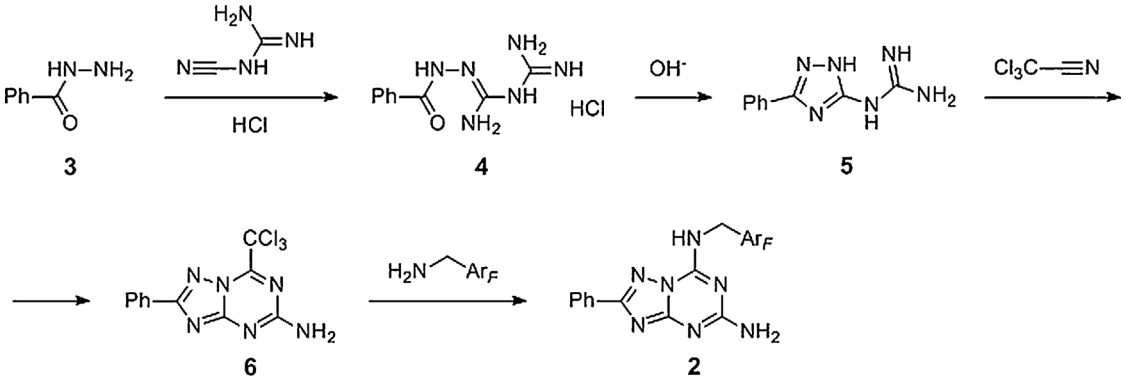
The reaction of benzhydrazide (**3**) with cyanoguanidine in the presence of hydrochloric acid provided *N*-benzamidobiguanide (**4**) (Scheme 1). The biguanide **4** was further cyclized under alkaline conditions according to the previously reported method [4] to afford *N*-(3-phenyl-1,2,4-triazol-5-yl)guanidine (**5**). Heating guanidine **5** with trichloroacetonitrile in toluene resulted in the chemo- and regioselective triazine ring closure affording 2-phenyl-7-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amine (**6**). This compound (**6**) has been recognized as an excellent building block for the synthesis of 5-azapurine derivatives [10]. Heating **6** with benzylamines in DMF at 70–80 °C resulted in the formation of **2** (Scheme 1). The fluorine/trifluoromethyl substituted benzylamines were used for nucleophilic displacement of the trichloromethyl group to generate a small library of fluorinated 1,2,4-triazolo[1,5-*a*][1,3,5]triazines **2** for biological screening. The neutral character of the leaving group and formation of chemically inert and volatile byproduct (chloroform) as well as mild reaction conditions and straightforward isolation of the product make this clean and practical procedure a useful tool for the construction of new bioactive substances.

The structure of the synthesized compounds was supported by the NMR spectral data compared with structurally related amino substituted 1,2,4-triazolo[1,5-*a*][1,3,5]triazines [5–7,10] and further confirmed by X-ray crystallographic study (Fig. 2). The downfield chemical shift of the benzylamino NH triplet in the ^{1}H NMR spectra can be attributed to the anisotropic effect of the heterocyclic ring and indicates a high degree of delocalization of the electron pair of this exocyclic nitrogen. This geometry facilitates a potential intramolecular hydrogen bonding of the NH proton with the triazole ring nitrogen. The molecular structure of **2c**, established using X-ray crystallography data, supported the NMR spectroscopy findings.

2.2. Biological activity

The antiproliferative activity of the prepared compounds **2** against lung cancer A549 and breast cancer MDA-MB-231 cell lines was evaluated *in vitro* using MTT assay and results were expressed as IC_{50} values [25]. The results of the anticancer activity tests are presented in Table 1. In general, compounds **2** were more active than their prototypes **1** in the growth inhibition of selected cancer cell lines. Moreover, despite the fact that the actual target for both sets of compounds remains unknown, our design appeared to be reliable. The most active compounds of series **1** and **2** had the same substitution pattern on the phenyl ring *i.e.* identical types and positions of fluorinated groups. Particularly, the highest activity was observed for 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines with 3-fluoro (**2b**), 3-trifluoromethyl (**2e**) and 4-trifluoromethyl (**2f**) substituents on the benzyl moiety. In the antiproliferative activity tests against the A549 cell line, the IC_{50} value of **2b** was lower than that of other compounds in the series. However, activity of **2b** was rather selective and to achieve an antiproliferative effect against the MDA-MB-231 cell line, **2b** should be applied at higher concentrations than other compounds reported here.

It should be noted that at higher concentrations, **2f** was considerably more active than other compounds. The concentration-response curve for this compound was found to be distinct



2: Ar_F = 2-FC_6H_4 (**a**), 3-FC_6H_4 (**b**), 4-FC_6H_4 (**c**), 2-$CF_3C_6H_4$ (**d**), 3-$CF_3C_6H_4$ (**e**), 4-$CF_3C_6H_4$ (**f**), 3,5-$(CF_3)_2C_6H_3$ (**g**)

Scheme 1. Synthesis of fluorinated 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines (**2a–g**).

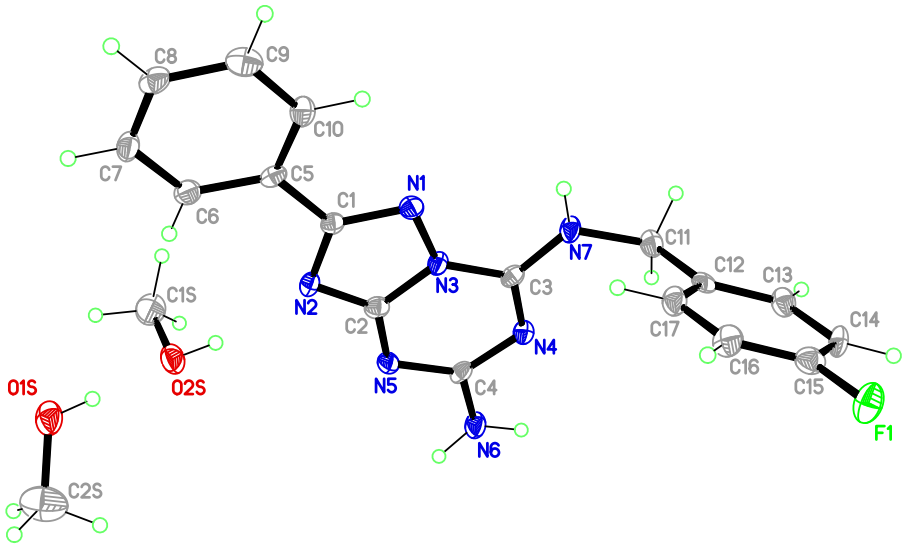


Fig. 2. Molecular structure of 7-(4-fluorobenzylamino)-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amine (**2c**) crystallized with two methanol molecules in the asymmetric unit [24]. Displacement ellipsoids are drawn at the 50% probability level.

Table 1
Antiproliferative activity of 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines (**2a–g**).

Compound	Ar_F	IC_{50}, μM	
		A549	MDA-MB-231
2a	2-FC_6H_4	63 ± 8.0	41 ± 0.7
2b	3-FC_6H_4	19 ± 5.4	99 ± 21.2
2c	4-FC_6H_4	70 ± 10.2	60 ± 3.2
2d	2-$CF_3C_6H_4$	64 ± 19.4	71 ± 13.3
2e	3-$CF_3C_6H_4$	30 ± 10.3	56 ± 9.7
2f	4-$CF_3C_6H_4$	29 ± 6.7	27 ± 6.4
2g	3,5-$(CF_3)_2C_6H_3$	50 ± 11.6	48 ± 11.1

from those of other compounds in this series. This characteristic behavior of **2f** is illustrated in Fig. 3, which compares response of A549 cells to various concentrations of **2f** after 72 h with effects of **2b** (lowest IC_{50} value in the series) and **2e** (similar to **2f** IC_{50} value). The antiproliferetive effect of **2f** expanded in relatively narrow range of concentrations and culminated with a nearly complete cessation of the cell growth well before a similar effect could be observed for other active compounds. In order to get a more meaningful picture of the actual antiproliferative effect of the synthesized compounds, together with their IC_{50} values, the IC_{90} values were also determined using CalcuSyn 2.0. For the most active (from the IC_{50} value prospective) against A549 cells compounds (**2b**, **2e**, and **2f**), their IC_{90} values indicated that **2f** could be more than 5 times more effective than **2b** and **2e** (Table 2). Moreover, the both type of cancer cells were more responsive to the treatment with **2f** at higher concentrations. Thus, the IC_{90} value for **2f** against the MDA-MB-231 cell line was 58 ± 6.2 μM.

The evaluation of anti-angiogenenic properties of the synthesized compounds was performed by Eli Lilly and Co. using the tube formation assay in co-cultured human clonal ECFC cells with human adipose ADSC cells under the PD2/OIDD scheme [26]. The compounds with the benzylamino moiety bearing trifluoromethyl group(s) demonstrated concentration-dependent anti-angiogenic effect (Table 3). The most active in the antiproliferative experiments against both cancer cell lines used in the study, compound **2f** also exhibited highest in the series activity in the anti-angiogenic screening at the concentration 2 μM. However, at higher concentrations, the most active compound was **2g**, which possessed EC_{50} value of 9.4 μM in this assay.

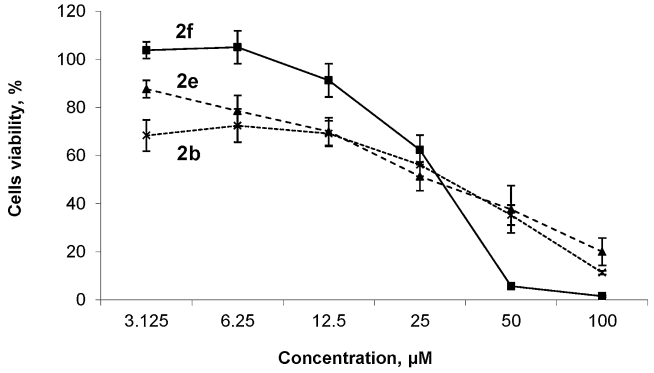


Fig. 3. Concentration-dependent antiproliferative effect of the most active fluorinated 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines (**2b, 2e** and **2f**) against A549 lung cancer cells lines after 72 h.

Table 2
IC_{90} values for the most active 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines (**2b, 2e** and **2f**) against A549 cell line.

Compound	Ar_F	IC_{90}, μM
2b	3-FC_6H_4	272 ± 14.7
2e	3-$CF_3C_6H_4$	272 ± 93.9
2f	4-$CF_3C_6H_4$	54 ± 6.8

Table 3
Anti-angiogenenic activity of trifluoromethyl-substituted 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amines (**2d–g**) in the anti-angiogenesis tube formation assay (tube area).

Compound	Ar_F	Inhibition, %	
		2 μM	10 μM
2d	2-$CF_3C_6H_4$	11	28
2e	3-$CF_3C_6H_4$	6	16
2f	4-$CF_3C_6H_4$	26	49
2g	3,5-$(CF_3)_2C_6H_3$	12	55

3. Conclusion

We successfully designed new anticancer fluorinated 5-azapurine derivatives *via* rational structural modifications of the previously identified series of active compounds. Anticancer and anti-angiogenic properties of the most active compounds **2f** and **2g** make them promising leads for further development of new anticancer agents.

4. Experimental

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. The reaction progress was monitored using TLC, which was carried out on aluminum plates coated with Silica gel 60 F_{254} (Merck) with detection by UV light. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer, using Me_2SO-d_6 as a solvent and TMS as an internal reference.

*4.1. Synthesis of 2-phenyl-7-trichloromethyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**6**)*

Guanidine **5** (4.04 g, 20 mmol) and trichloroacetonitrile (2.0 mL, 40 mmol) were heated in toluene (50 mL) with stirring for 7 h. After cooling, the product formed was filtered and washed with toluene (10 mL) and cold EtOH (10 mL). Yield = 94%, mp 262–263 °C.

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 7.48–7.66 (m, 3H, H-3′, H-4′ and H-5′), 8.09–8.22 (m, 2H, H-2′ and H-6′), 8.24 (s, 2H, NH_2).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 89.2 (CCl_3), 127.1 (C-3′ and C-5′), 128.8 (C-2′ and C-6′), 129.7 (C-1′), 130.9 (C-4′), 150.2 (C-7), 160.3 (C-2), 160.7 (C-3a), 164.4 (C-5).

Anal. Calcd. for $C_{11}H_7Cl_3N_6$: C, 40.09; H, 2.14; N, 25.50%. Found: C, 39.85; H, 2.23; N, 25.36%.

*4.2. General method for the preparation of 7-benzylamino-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amines (**2a–g**)*

2-Phenyl-7-trichloromethyl-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-amine (**6**, 0.66 g, 2.0 mmol) was added to a solution of the appropriate fluorinated benzylamine (2.5 mmol) in DMF (5 mL) and the mixture was heated at 70–80 °C with stirring for 3 h. After cooling, ice-cold water (40 mL) was added and the product was filtered and recrystallized from MeOH or EtOH.

*4.2.1. 7-(2-Fluorobenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2a**)*

Yield = 85%; mp 250–252 °C (EtOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.74 (2H, d, ^3J = 5.7 Hz, CH_2), 7.08 (2H, s, NH_2), 7.14–7.26 (2H, m, H-3″ and H-5″), 7.34 (1H, td, ^3J = 7.9 Hz, $^4J_{H-F}$ = 5.7 Hz, H-4″), 7.42–7.57 (3H, m, H-3′, H-4′, H-5′ and H-6″), 8.09–8.19 (2H, m, H-2′ and H-6′), 9.01 (1H, t, ^3J = 5.7 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 37.2 (d, $^3J_{C-F}$ = 4.7 Hz, CH_2), 115.0 (d, $^2J_{C-F}$ = 21.2 Hz, C-3″), 124.3 (d, $^4J_{C-F}$ = 3.5 Hz, C-5″), 125.0 (d, $^2J_{C-F}$ = 14.1 Hz, C-1″), 126.6 (C-3′ and C-5′), 128.6 (C-2′ and C-6′), 129.0 (d, $^3J_{C-F}$ = 8.2 Hz, C-4″), 129.2 (d, $^3J_{C-F}$ = 4.7 Hz, C-6″), 130.0 (C-4′), 130.7 (C-1′), 149.2 (C-7), 159.4 (C-2), 159.8 (d, $^1J_{C-F}$ = 244.6 Hz, C-1″), 162.3 (C-3a), 162.8 (C-5).

Anal. Calcd. for $C_{17}H_{14}FN_7$: C, 60.89; H, 4.21; N, 29.24%. Found: C, 60.75; H, 4.23; N, 28.98%.

*4.2.2. 7-(3-Fluorobenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2b**)*

Yield = 87%; mp 239–240 °C (MeOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.68 (2H, d, ^3J = 6.0 Hz, CH_2), 7.02–7.16 (3H, m, NH_2 and H-4″), 7.22–7.31 (2H, m, H-2″ and H-6″), 7.38 (1H, td, ^3J = 7.8 Hz, $^4J_{H-F}$ = 6.3 Hz, H-5″), 7.46–7.59 (3H, m, H-3′, H-4′ and H-5′), 8.08–8.19 (2H, m, H-2′ and H-6′), 9.05 (1H, t, ^3J = 6.0 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 42.7 (CH_2), 113.8 (d, $^2J_{C-F}$ = 21.2 Hz, C-4″), 114.2 (d, $^2J_{C-F}$ = 21.8 Hz, C-2″), 123.4 (d, $^4J_{C-F}$ = 2.9 Hz, C-6″), 126.6 (C-3′ and C-5′), 128.6 (C-2′ and C-6′), 130.0 (C-4′), 130.2 (d, $^3J_{C-F}$ = 8.2 Hz, C-5″), 130.8 (C-1′), 141.4 (d, $^3J_{C-F}$ = 7.1 Hz, C-1″), 149.1 (C-7), 159.4 (C-2), 162.1 (d, $^1J_{C-F}$ = 243.4 Hz, C-3″), 162.3 (C-3a), 162.8 (C-5).

Anal. Calcd. for $C_{17}H_{14}FN_7$: C, 60.89; H, 4.21; N, 29.24%. Found: C, 60.77; H, 4.30; N, 29.06%.

*4.2.3. 7-(4-Fluorobenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2c**)*

Yield = 88%; mp 249–250 °C (EtOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.64 (2H, d, ^3J = 6.0 Hz, CH_2), 7.08 (2H, s, NH_2), 7.17 (2H, dd, ^3J = 8.7 Hz, $^3J_{H-F}$ = 9.0 Hz, H-3″ and H-5″), 7.47 (2H, dd, ^3J = 8.3 Hz, $^4J_{H-F}$ = 5.7 Hz, H-2″ and H-6″), 7.48–7.57 (3H, m, H-3′, H-4′ and H-5′), 8.07–8.18 (2H, m, H-2′ and H-6′), 9.03 (1H, t, ^3J = 6.0 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 42.5 (CH_2), 115.0 (d, $^2J_{C-F}$ = 21.8 Hz, C-3″ and C-5″), 126.6 (C-3′ and C-5′), 128.6 (C-2′ and C-6′), 129.5 (d, $^3J_{C-F}$ = 8.2 Hz, C-2″ and C-6″), 130.0 (C-4′), 130.7 (C-1′), 134.6 (d, $^4J_{C-F}$ = 2.9 Hz, C-1″), 149.0 (C-7), 159.4 (C-2), 161.3 (d, $^1J_{C-F}$ = 242.8 Hz, C-4″), 162.3 (C-3a), 162.8 (C-5).

Anal. Calcd. for $C_{17}H_{14}FN_7$: C, 60.89; H, 4.21; N, 29.24%. Found: C, 60.69; H, 4.40; N, 29.18%.

*4.2.4. 7-(2-Trifluoromethylbenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2d**)*

Yield = 90%; mp 242–243 °C (EtOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.90 (2H, d, ^3J = 5.7 Hz, CH_2), 7.04 (2H, s, NH_2), 7.45–7.57 (4H, m, H-3′, H-4′, H-5′ and H-4″), 7.59 (1H, d, ^3J = 7.5 Hz, H-6″), 7.66 (1H, t, ^3J = 7.3 Hz, H-5″), 7.76 (1H, d, ^3J = 7.9 Hz, H-3″), 8.10–8.21 (2H, m, H-2′ and H-6′), 9.04 (1H, t, ^3J = 5.7 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 39.9 (CH_2), 124.4 (q, $^1J_{C-F}$ = 274.0 Hz, CF_3), 125.8 (q, $^2J_{C-F}$ = 30.2 Hz, C-2″), 125.8 (q, $^3J_{C-F}$ = 5.9 Hz, C-3″), 126.6 (C-3′ and C-5′), 127.4 (C-4″), 127.8 (C-6″), 128.6 (C-2′ and C-6′), 130.0 (C-4′), 130.8 (C-1′), 132.7 (C-5″), 136.4 (q, $^3J_{C-F}$ = 1.2 Hz, C-1″), 149.4 (C-7), 159.4 (C-2), 162.3 (C-3a), 162.9 (C-5).

Anal. Calcd. for $C_{18}H_{14}F_3N_7$: C, 56.10; H, 3.66; N, 25.44%. Found: C, 55.89; H, 3.83; N, 25.34%.

*4.2.5. 7-(3-Trifluoromethylbenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2e**)*

Yield = 92%; mp 245–246 °C (MeOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.77 (2H, d, ^3J = 6.0 Hz, CH_2), 7.12 (2H, s, NH_2), 7.46–7.56 (3H, m, H-3′, H-4′ and H-5′), 7.60 (1H, t, ^3J = 7.7 Hz, H-5″), 7.66 (1H, d, ^3J = 7.5 Hz, H-4″), 7.76 (1H, d, ^3J = 7.5 Hz, H-6″), 7.80 (1H, s, H-5″), 8.10–8.21 (2H, m, H-2′ and H-6′), 9.12 (1H, t, ^3J = 6.0 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 42.9 (CH_2), 123.8 (q, $^3J_{C-F}$ = 3.7 Hz, C-4″), 124.1 (q, $^3J_{C-F}$ = 3.7 Hz, C-2″), 124.2 (q, $^1J_{C-F}$ = 272.4 Hz, CF_3), 126.7 (C-3′ and C-5′), 128.6 (C-2′ and C-6′), 129.0 (q, $^2J_{C-F}$ = 31.6 Hz, C-3″), 129.4 (C-5″), 130.0 (C-4′), 130.8 (C-1′), 131.7 (q, $^4J_{C-F}$ = 1.2 Hz, C-6″), 139.9 (C-1″), 149.1 (C-7), 159.4 (C-2), 162.4 (C-3a), 162.9 (C-5).

Anal. Calcd. for $C_{18}H_{14}F_3N_7$: C, 56.10; H, 3.66; N, 25.44%. Found: C, 56.01; H, 3.80; N, 25.25%.

*4.2.6. 7-(4-Trifluoromethylbenzylamino)-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2f**)*

Yield = 94%; mp 230–231 °C (MeOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.75 (2H, d, ^3J = 6.2 Hz, CH_2), 7.07 (2H, s, NH_2), 7.46–7.57 (3H, m, H-3′, H-4′ and H-5′), 7.63 (2H, d, ^3J = 8.3 Hz, H-2″ and H-6″), 7.71 (2H, d, ^3J = 8.3 Hz, H-3″ and H-5″), 8.09–8.18 (2H, m, H-2′ and H-6′), 9.11 (1H, t, ^3J = 6.2 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 42.8 (CH_2), 124.2 (q, $^1J_{C-F}$ = 272.1 Hz, CF_3), 125.1 (q, $^3J_{C-F}$ = 3.7 Hz, C-3″ and C-5″), 126.6 (C-3′ and C-5′), 127.6 (q, $^2J_{C-F}$ = 31.6 Hz, C-4″), 128.0 (C-2″ and C-6″), 128.6 (C-2′ and C-6′), 130.0 (C-4′), 130.7 (C-1′), 143.2 (q, $^5J_{C-F}$ = 1.2 Hz, C-1″), 149.1 (C-7), 159.4 (C-2), 162.3 (C-3a), 162.9 (C-5).

Anal. Calcd. for $C_{18}H_{14}F_3N_7$: C, 56.10; H, 3.66; N, 25.44%. Found: C, 55.92; H, 3.76; N, 25.32%.

*4.2.7. 7-[3,5-bis-(Trifluoromethyl)benzylamino]-2-phenyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-amine (**2g**)*

Yield = 93%; mp 261–262 °C (MeOH).

^{1}H NMR (300 MHz, Me_2SO-d_6): δ 4.84 (2H, d, ^3J = 6.0 Hz, CH_2), 7.10 (2H, s, NH_2), 7.46–7.58 (3H, m, H-3′, H-4′ and H-5′), 8.03 (1H, s, H-4″), 8.09–8.20 (4H, m, H-2′, H-6′, H-2″ and H-6″), 9.11 (1H, t, ^3J = 6.0 Hz, NH).

^{13}C NMR (75 MHz, Me_2SO-d_6): δ 42.6 (CH_2), 120.9 (m, $^3J_{C-F}$ = 3.2 Hz, C-4″), 123.3 (q, $^1J_{C-F}$ = 272.8 Hz, $2CF_3$), 126.6 (C-3′ and C-5′), 128.5 (q, $^3J_{C-F}$ = 4.3 Hz, C-2″ and C-6″), 128.6 (C-2′ and C-6′), 130.0 (C-4′), 130.1 (q, $^2J_{C-F}$ = 32.7 Hz, C-3″ and C-5″), 130.8 (C-1′), 141.9 (C-1″), 149.1 (C-7), 159.4 (C-2), 162.3 (C-3a), 162.9 (C-5).

Anal. Calcd. for $C_{19}H_{13}F_6N_7$: C, 50.34; H, 2.89; N, 21.63%. Found: C, 50.18; H, 2.74; N, 21.45%.

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