



Synthesis and biological activity of fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines [1][☆]

Anton V. Dolzhenko^{a,*}, Bee Jen Tan^a, Anna V. Dolzhenko^{a,b}, Gigi Ngar Chee Chiu^a, Wai Keung Chui^a

^a Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore

^b Perm State Pharmaceutical Academy, 48 Lenin Street, Perm 614990, Russian Federation

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ABSTRACT

In our lead finding program, 12 new fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines were prepared *via* a practical three-step procedure starting from (iso)nicotinic hydrazides. The fluorine substituted aryl fragment was introduced in the last step through cyclocondensation of *N*-(3-pyridyl-1,2,4-triazol-5-yl)guanidines and fluoro/trifluoromethyl substituted benzaldehydes. The structures of the compounds were confirmed by ¹H and ¹³C NMR spectral data. The tautomeric preferences for the compounds were established using 2D NOESY experiments. The antiproliferative activity of the synthesized 1,2,4-triazolo[1,5-*a*][1,3,5]triazines was evaluated against breast, colon and lung cancer cell lines. The highest antiproliferative activity in the series was found for 2-(pyridine-3-yl)-7-(4-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine. The lack of inhibitory activity against bovine dihydrofolate reductase (DHFR) indicated that the antiproliferative activity was realized *via* other mechanisms.

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1. Introduction

The unique character of fluorinated molecules is well known in medicinal chemistry [2–8]. Fluorine substitution has been extensively practiced in the design of new biologically active compounds, including those with potential anticancer properties [8]. A variety of attractive pharmacological effects were attributed to the fluorinated mono- and polycyclic 1,3,5-triazines [9]. A bicyclic system comprising 1,3,5-triazine and 1,2,4-triazole rings, namely 1,2,4-triazolo[1,5-*a*][1,3,5]triazine nucleus, which is 5-aza-bioisoster of purine, has been shown to be a favorable scaffold for the design of biologically active compounds [10]. However, data concerning anticancer properties of these compounds are limited [11]. Recently, we have developed a new method [12] for the synthesis of 5-azapurine derivatives, *viz.* 2-phenyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines *via* ring closure reaction of

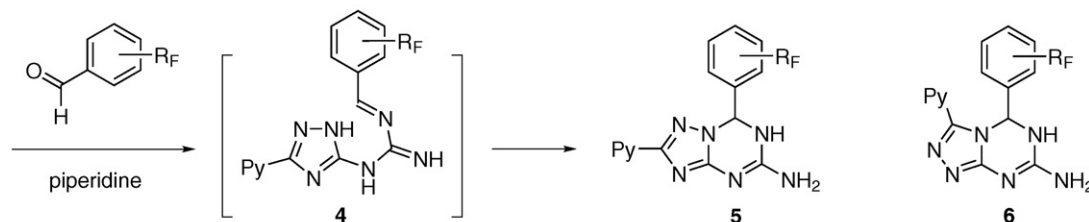
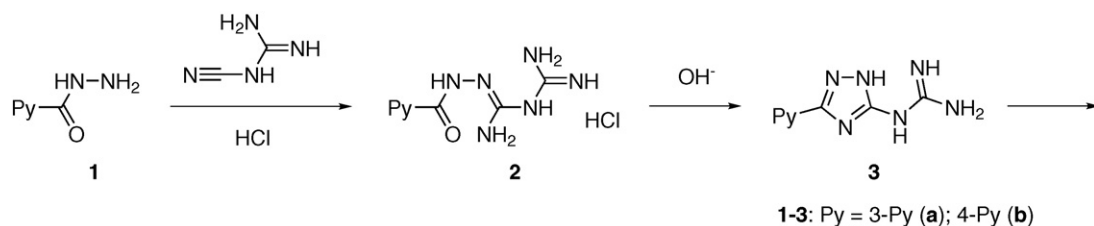
N-(3-phenyl-1,2,4-triazol-5-yl)guanidine using a number of aldehydes and ketones. In continuation of our lead finding program in the area of fused 1,3,5-triazines, particularly fluorine substituted derivatives [13], we applied herein a similar approach for the preparation of new fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines (5). We also aimed to investigate antiproliferative effect of the compounds towards three cancer cell lines. The monocyclic dihydro-1,3,5-triazines (*e.g.* cycloguanil) and some fused dihydro-1,3,5-triazines [14,15] were reported to inhibit the enzyme DHFR, which is a well recognized target in anticancer therapy [16]. Therefore, the potential DHFR inhibitory activity of 5 as one of the possible mechanism of the anticancer effect was evaluated in the present study [1].

We designed the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazines 5 starting from hydrazides of nicotinic and isonicotinic acids (1) which comprised three steps and involved subsequent formation of the 1,2,4-triazole and the 1,3,5-triazine rings (Scheme 1). In order to generate a library of fluorinated 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives for biological screening, fluorine/trifluoromethyl substituted benzaldehydes were used in the step of 1,3,5-triazine ring closure.

[☆] Part 10 in the series "Fused heterocyclic systems with s-triazine ring".

* Corresponding author. Tel.: +65 6516 2657; fax: +65 6779 1554.

E-mail address: phada@nus.edu.sg (A.V. Dolzhenko).



5	Py	R _F	5	Py	R _F
a	3-Py	2-F	g	4-Py	2-F
b	3-Py	3-F	h	4-Py	3-F
c	3-Py	4-F	i	4-Py	4-F
d	3-Py	2-CF ₃	j	4-Py	2-CF ₃
e	3-Py	3-CF ₃	k	4-Py	3-CF ₃
f	3-Py	4-CF ₃	l	4-Py	4-CF ₃

Scheme 1. Synthesis of fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amines (**5a-1**).

2. Results and discussion

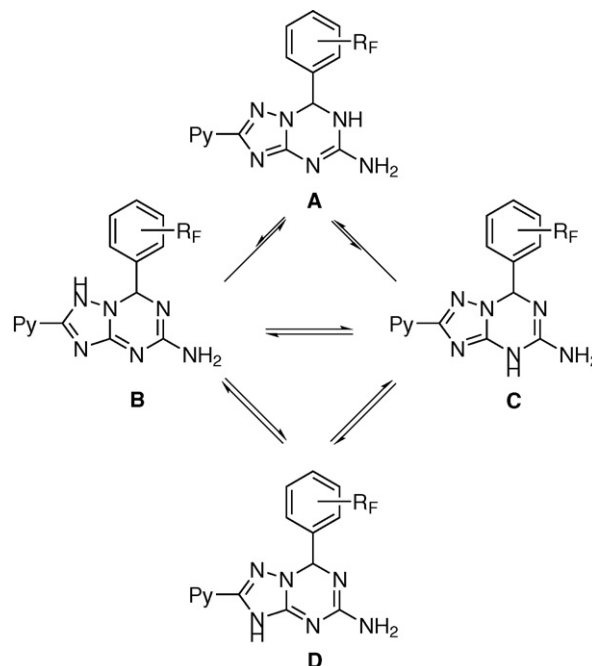
2.1. Chemistry

The reaction of cyanoguanidine with hydrazides of nicotinic and isonicotinic acids (**1**) in the presence of hydrochloric acid provided *N*-(iso)nicotinamidobiguanides (**2**) (Scheme 1). The biguanides **2** were further cyclized under alkaline conditions to afford *N*-(3-pyridyl-1,2,4-triazol-5-yl)guanidines (**3**).

Heating of guanidines **3** with fluorinated benzaldehydes in ethanol in the presence of piperidine gave 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines (**5**) (Scheme 1). The characteristic signal at 64.2–64.8 ppm for the compounds with *ortho*-substituted phenyl group and 67.6–67.8 ppm for the compounds with *meta*- and *para*-substituted phenyl group was found in ¹³C NMR spectra. This indication of the presence of sp³-hybridized carbon atom in the structure supported the triazine ring closure and ruled out the structure of conceivable Schiff base-like intermediates **4**. The heterocyclization was found to proceed regioselectively resulting in the formation of fused triazolotriazine system **5** with the [1,5-*a*] ring junction, exclusively. The theoretically possible regioisomeric 1,2,4-triazolo[4,3-*a*][1,3,5]triazines **6** were not isolated from the reaction mixture. The 2D NOESY experiments were used to differentiate between triazolotriazine structures **5** and **6**. The pyridine ring protons gave no cross-peaks neither with proton at sp³-hybridized carbon nor with protons of fluorinated aryl group. The results are in agreement with those obtained in our previous studies [12] for structurally related compounds.

The annular tautomerism, possible for **5**, might result in the existence of four different forms, viz. 6,7- (**A**), 1,7- (**B**), 4,7- (**C**), and 3,7-dihydro- (**D**) tautomers (Scheme 2). However, form **A** was found to be predominant in Me₂SO solution as indicated by the NMR data. The coupling of proton at sp³-hybridized carbon (H-7)

and proton of endocyclic NH in ¹H NMR spectra was observed for several compounds (*J* = 0–1.1 Hz). Despite *J* value was small and not always detectable, cross-peaks between these signals in 2D NOESY experiments clearly indicated that the NH proton was located in the vicinity of sp³-hybridized carbon.



Scheme 2. Tautomeric forms of fluorinated 7-aryl-2-pyridyl-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amines (**5a-1**).

Table 1Antiproliferative activity of the fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amines (**5a–l**)

Compound	Py	R _F	Concentration (μM)	Cell growth inhibition (%)*		
				MDA-MB-231	HT29	A549
5a	3-Py	2-F	62.5	12 ± 5.9	17 ± 9.4	13 ± 6.7
			125	17 ± 3.8	29 ± 9.3	26 ± 9.6
5b	3-Py	3-F	62.5	21 ± 6.3	28 ± 9.7	12 ± 7.2
5c	3-Py	4-F	62.5	8 ± 7.9	13 ± 9.3	2 ± 5.5
			125	18 ± 6.1	29 ± 7.0	6 ± 6.7
5d	3-Py	2-CF ₃	62.5	3 ± 7.2	1 ± 13.0	1 ± 9.6
			125	12 ± 6.5	7 ± 10.0	6 ± 6.7
5e	3-Py	3-CF ₃	62.5	17 ± 8.0	29 ± 6.1	12 ± 8.3
			125	41 ± 7.7	51 ± 5.1	20 ± 5.5
5f	3-Py	4-CF ₃	62.5	65 ± 12.5	40 ± 16.1	32 ± 11.1
			125	75 ± 6.4	48 ± 17.2	41 ± 9.3
5g	4-Py	2-F	62.5	3 ± 10.4	16 ± 6.4	-3 ± 13.7
			125	11 ± 9.8	25 ± 7.4	-1 ± 10.2
5h	4-Py	3-F	62.5	19 ± 18.8	25 ± 16.1	7 ± 16.4
			125	44 ± 12.7	54 ± 16.9	30 ± 12.5
5i	4-Py	4-F	62.5	21 ± 5.1	13 ± 6.4	-2 ± 5.5
			125	30 ± 6.2	34 ± 5.2	10 ± 6.3
5j	4-Py	2-CF ₃	50	22 ± 6.4	14 ± 6.9	14 ± 6.5
5k	4-Py	3-CF ₃	62.5	2 ± 6.7	14 ± 11.7	0 ± 9.0
			125	19 ± 5.1	42 ± 15.5	15 ± 8.8
5l	4-Py	4-CF ₃	62.5	30 ± 12.5	20 ± 17.1	27 ± 15.1
			125	44 ± 13.3	29 ± 11.1	44 ± 10.2

*±, S.D., n = 5–6.

2.2. Biological activity

The antiproliferative and DHFR inhibitory activities were evaluated for the prepared fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amines (**5a–l**). For both the antiproliferative and DHFR inhibition bioassays the compounds were dissolved in Me₂SO. In order to ensure that the solvent *per se* had no effect on the cell growth and enzymatic activity, negative control tests were performed using Me₂SO at the same concentrations. The antiproliferative activity was performed using MTT assay [17]. The following cancer cell lines were used for the screening: breast cancer MDA-MB-231, colon cancer HT-29 and lung cancer A549. The DHFR inhibition bioassay was carried out using bovine DHFR (Fluka Chemie) according to the previously described method [13]. The concentrations of the compounds for the biological tests were varied according to their solubility in the media. The effect of the compounds was expressed as percentage of cell growth inhibition (antiproliferative assay) and percentage of the enzymatic activity inhibition (DHFR assay). IC₅₀ value was determined for the most active compound.

The results of the antiproliferative tests of compounds **5** are shown in Table 1. In general, the lung cancer cells were more resistant to the treatment with 1,2,4-triazolo[1,5-a][1,3,5]triazin-5, while the colon cancer cells were more susceptible.

Table 2Bovine DHFR inhibitory activity of the fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amines (**5a–l**)

Compound	Py	R _F	Concentration (μM)	Inhibition (%)
5a	3-Py	2-F	500	14
5b	3-Py	3-F	500	12
5c	3-Py	4-F	500	na
5d	3-Py	2-CF ₃	500	32
5e	3-Py	3-CF ₃	250	na
5f	3-Py	4-CF ₃	100	na
5g	4-Py	2-F	500	11
5h	4-Py	3-F	500	20
5i	4-Py	4-F	500	9
5j	4-Py	2-CF ₃	250	22
5k	4-Py	3-CF ₃	500	7
5l	4-Py	4-CF ₃	100	na

na, inactive.

However, the most active compound, namely 2-(pyridine-3-yl)-7-(4-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (**5f**) was more effective against breast cancer cell line MDA-MB-231 (IC₅₀ = 28 μM). The position of fluoro/trifluoromethyl group seemed to play more important role in the anticancer effect of the compounds than position of nitrogen in the pyridine ring. Thus, the active compounds were almost equally distributed within 3- and 4-pyridyl substituted groups (**5a–f** and **5g–l**). At the same time, the pairs of the compounds with pronounced anticancer effect were identified, those included **5f** and **5l** (R = 4-CF₃) as well as **5b** and **5h** (R = 3-F). The pairs showed similar activity and selectivity towards the cell lines used for the evaluation.

None of 1,2,4-triazolo[1,5-a][1,3,5]triazin-5 demonstrated significant inhibition of the bovine DHFR activity in the screening (Table 2). Therefore, the anticancer effect of the compounds appeared to be realized *via* mechanisms other than DHFR inhibition.

3. Conclusions

Twelve new fluorinated 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amines (**5**) were prepared using simple and effective three-step procedure. The biological evaluation showed that compound **5** possessed the anticancer properties not associated with DHFR inhibition. The most active anticancer agents identified in this study was 2-(pyridine-3-yl)-7-(4-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (**5f**). Therefore, it may be used as a lead for further development.

4. Experimental

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. Analytical TLC were carried out on aluminum plates coated with Silica gel 60 F₂₅₄ (Merck) with detection by UV light. ¹H and ¹³C NMR spectra were recorded on a Bruker DPX-300 spectrometer, using Me₂SO-*d*₆ as a solvent and TMS as an internal reference.

4.1. N-Nicotinamidobiguanide hydrochloride (**2a**)

To the solution of nicotinic hydrazide (**1a**, 6.80 g, 50 mmol) in ethanol (50 mL), hydrochloric acid (37%, 5 mL, 50 mmol) and

cyanoguanidine (4.62 g, 55 mmol) were added. The reaction mixture was heated under reflux with stirring for 8 h. After cooling, the product was filtered, washed with cold ethanol and dried. Yield 80%; mp 176–177 °C (aq. EtOH) [lit. [18] mp 178–189 °C]. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.58 (7H, br s, NH-C(=NH)NH-C(=NH)NH₂ HCl), 7.74 (1H, dd, ³J = 7.5 Hz, ³J = 4.9 Hz, H-5), 8.52 (1H, d, ³J = 7.5 Hz, H-4), 8.86 (1H, d, ³J = 4.9 Hz, H-6), 9.21 (1H, s, H-2), 11.16 (1H, s, CONH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 124.3 (C-5), 128.8 (C-3), 138.0 (C-4), 146.8 (C-6), 150.0 (C-2), 157.3, 158.5 (NH-C(=NH)NH-C(=NH)NH₂), 163.6 (C=O).

4.2. *N*-Isonicotinamidobiguanide dihydrochloride (2b)

To the solution of isonicotinic hydrazide (**1b**, 6.80 g, 50 mmol) in methanol (70 mL), hydrochloric acid (37%, 10 mL, 100 mmol) and cyanoguanidine (4.62 g, 55 mmol) were added. The reaction mixture was heated under reflux with stirring for 8 h. After cooling, the product was filtered, washed with cold ethanol and dried. Yield 82%, mp 198 °C (aq EtOH) [lit. [19] mp 200 °C].

¹H NMR (300 MHz, DMSO-*d*₆): δ 7.59 (8H, br s, NH-C(=NH)NH-C(=NH)NH₂ 2HCl), 8.04 (2H, d, ³J = 5.9 Hz, ⁴J = 1.5 Hz, H-3 and H-5), 8.85 (2H, dd, ³J = 5.9 Hz, ⁴J = 1.5 Hz, H-2 and H-6), 11.18 (1H, s, CONH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 122.6 (C-3 and C-5), 141.4 (C-4), 148.0 (C-2 and C-6), 156.7, 158.4 (NH-C(=NH)NH-C(=NH)NH₂), 163.3 (C=O).

4.3. General method for the syntheses of *N*-(3-pyridyl-1*H*-1,2,4-triazol-5-yl)guanidine (3a,b)

N-(Iso)nicotinamidobiguanide (di)hydrochloride (**2**, 20 mmol) was heated at 80 °C in 10% aqueous sodium hydroxide solution (10 mL) for 4–6 h. After cooling, the product was filtered, washed with cold water and dried.

4.3.1. *N*-[3-(Pyridin-3-yl)-1*H*-1,2,4-triazol-5-yl]guanidine (3a)

Yield 86%, mp 269 °C (EtOH) [lit. [18] mp 275–276 °C]; TLC (silica gel, EtOH): *R*_f 0.15. ¹H NMR (300 MHz, DMSO-*d*₆): δ 6.70 (4H, br s, NH-C(=NH)NH₂), 7.45 (1H, dd, ³J = 7.9 Hz, ³J = 4.5 Hz, H-5), 8.28 (1H, dt, ³J = 7.9 Hz, ⁴J = 1.7 Hz, H-4), 8.56 (1H, dd, ³J = 4.5 Hz, ⁴J = 1.5 Hz, H-6), 9.16 (1H, d, ⁴J = 1.9 Hz, H-2), 12.58 (1H, br s, NH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 123.5 (C-5'), 127.9 (C-3'), 132.4 (C-4'), 146.5 (C-6'), 149.0 (C-2'), 154.9 (C-3), 157.9 (NH-C(=NH)NH₂), 160.8 (C-5).

4.3.2. *N*-[3-(Pyridin-4-yl)-1*H*-1,2,4-triazol-5-yl]guanidine (3b)

Yield 92%, mp 289–290 °C (EtOH) [lit. [18] mp 292 °C]; TLC (silica gel, EtOH): *R*_f 0.20. ¹H NMR (300 MHz, DMSO-*d*₆): δ 6.73 (4H, br s, NH-C(=NH)NH₂), 7.88 (2H, dd, ³J = 4.5 Hz, ⁴J = 1.5 Hz, H-3' and H-5'), 8.62 (2H, dd, ³J = 4.5 Hz, ⁴J = 1.5 Hz, H-2' and H-6'), 12.70 (1H, br s, NH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 119.5 (C-3' and C-5'), 139.2 (C-1'), 149.9 (C-2' and C-6'), 155.2 (C-3), 157.9 (NH-C(=NH)NH₂), 160.9 (C-5).

4.4. General method for the syntheses of 7-aryl-2-pyridyl-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (5a–1)

The solution of *N*-(3-pyridyl-1,2,4-triazol-5-yl)guanidine (**3**, 0.50 g, 2.5 mmol), fluorine/trifluoromethyl substituted benzaldehyde (2.5 mmol) and piperidine (0.10 mL, 1.0 mmol) in ethanol (7–10 mL) was heated under reflux for 5–18 h. After cooling, the product was filtered, washed with cold ethanol, dried and recrystallized from ethanol or ethanol–DMF mixture.

4.4.1. 7-(2-Fluorophenyl)-2-(pyridin-3-yl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (5a)

Yield 85%; mp 280 °C (EtOH–DMF); TLC (silica gel, EtOH): *R*_f 0.45. ¹H NMR (300 MHz, Me₂SO-*d*₆): δ 6.48 (2H, s, NH₂), 6.97 (1H, s,

H-7), 7.22–7.34 (3H, m, H-3'', H-5'' and H-6''), 7.42 (1H, dd, ³J = 7.7 Hz, ³J = 4.3 Hz, H-5'), 7.44–7.52 (1H, m, H-4''), 8.03 (1H, s, NH), 8.17 (1H, dt, ³J = 7.9 Hz, ⁴J = 1.9 Hz, H-4'), 8.55 (1H, dd, ³J = 4.7 Hz, ⁴J = 1.7 Hz, H-6'), 9.02 (1H, d, ⁴J = 1.5 Hz, H-2'). ¹³C NMR (75 MHz, Me₂SO-*d*₆): δ 64.2 (d, ³J_{C-F} = 3.5 Hz, C-7), 116.0 (d, ²J_{C-F} = 20.6 Hz, C-3''), 123.4 (C-5'), 124.8 (d, ⁴J_{C-F} = 3.5 Hz, C-5''), 127.0 (d, ²J_{C-F} = 11.8 Hz, C-1''), 127.4 (C-3'), 128.4 (d, ³J_{C-F} = 3.5 Hz, C-6''), 131.4 (d, ³J_{C-F} = 8.2 Hz, C-4''), 132.6 (C-4'), 146.5 (C-6'), 149.5 (C-2'), 155.6, 156.7, 157.3 (C-2, C-3a and C-5), 159.7 (d, ¹J_{C-F} = 248.1 Hz, C-2''). Anal. Calcd. for C₁₅H₁₂FN₇: C, 58.25; H, 3.91; N, 31.70. Found: C, 57.96; H, 4.11; N, 31.58.

4.4.2. 7-(3-Fluorophenyl)-2-(pyridin-3-yl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (5b)

Yield 87%; mp 288 °C (EtOH–DMF); TLC (silica gel, EtOH): *R*_f 0.43. ¹H NMR (300 MHz, Me₂SO-*d*₆): δ 6.60 (2H, s, NH₂), 6.81 (1H, s, H-7), 7.16–7.31 (3H, m, H-2'', H-4'' and H-6''), 7.44 (1H, dd, ³J = 7.9 Hz, ³J = 4.9 Hz, H-5'), 7.51 (1H, td, ³J = 7.6 Hz, ⁴J_{H-F} = 6.4 Hz, H-5''), 8.12 (1H, s, NH), 8.20 (1H, dt, ³J = 7.9 Hz, ⁴J = 1.9 Hz, H-4'), 8.57 (1H, dd, ³J = 4.5 Hz, ⁴J = 1.5 Hz, H-6'), 9.06 (1H, d, ⁴J = 1.5 Hz, H-2'). ¹³C NMR (75 MHz, Me₂SO-*d*₆): δ 67.6 (d, ⁴J_{C-F} = 1.8 Hz, C-7), 113.1 (d, ²J_{C-F} = 21.8 Hz, C-2''), 116.0 (d, ²J_{C-F} = 21.2 Hz, C-4''), 122.3 (d, ⁴J_{C-F} = 2.9 Hz, C-6''), 123.6 (C-5'), 127.4 (C-3'), 131.1 (d, ³J_{C-F} = 8.2 Hz, C-5''), 132.7 (C-4'), 142.9 (d, ³J_{C-F} = 6.5 Hz, C-1''), 146.6 (C-6'), 149.5 (C-2'), 155.6, 156.6, 157.4 (C-2, C-3a and C-5), 162.1 (d, ¹J_{C-F} = 245.2 Hz, C-3''). Anal. Calcd. for C₁₅H₁₂FN₇: C, 58.25; H, 3.91; N, 31.70. Found: C, 57.92; H, 4.07; N, 31.62.

4.4.3. 7-(4-Fluorophenyl)-2-(pyridin-3-yl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (5c)

Yield 91%; mp 281 °C (EtOH–DMF); TLC (silica gel, EtOH): *R*_f 0.40. ¹H NMR (300 MHz, Me₂SO-*d*₆): δ 6.55 (2H, s, NH₂), 6.79 (1H, d, ³J = 1.1 Hz, H-7), 7.28 (2H, dd, ³J = 8.7 Hz, ³J_{H-F} = 9.0 Hz, H-3'' and H-5''), 7.39–7.48 (3H, m, H-5', H-2'' and H-6''), 8.03 (1H, d, ³J = 1.1 Hz, NH), 8.18 (1H, dt, ³J = 7.9 Hz, ⁴J = 1.9 Hz, H-4'), 8.56 (1H, dd, ³J = 4.9 Hz, ⁴J = 1.5 Hz, H-6'), 9.05 (1H, d, ⁴J = 1.9 Hz, H-2'). ¹³C NMR (75 MHz, Me₂SO-*d*₆): δ 67.7 (C-7), 115.7 (d, ²J_{C-F} = 21.8 Hz, C-3' and C-5''), 123.6 (C-5'), 127.4 (C-3'), 128.6 (d, ³J_{C-F} = 8.2 Hz, C-2'' and C-6''), 132.6 (C-4'), 136.5 (d, ⁴J_{C-F} = 2.9 Hz, C-1''), 146.6 (C-6'), 149.5 (C-2'), 155.6, 156.6, 157.3 (C-2, C-3a and C-5), 162.4 (d, ¹J_{C-F} = 245.2 Hz, C-4''). Anal. Calcd. for C₁₅H₁₂FN₇: C, 58.25; H, 3.91; N, 31.70. Found: C, 58.03; H, 3.97; N, 31.62.

4.4.4. 2-(Pyridin-3-yl)-7-(2-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (5d)

Yield 87%; mp 285 °C (EtOH–DMF); TLC (silica gel, EtOH): *R*_f 0.49. ¹H NMR (300 MHz, Me₂SO-*d*₆): δ 6.50 (2H, s, NH₂), 6.99 (1H, s, H-7), 7.34 (1H, d, ³J = 7.9 Hz, H-6''), 7.41 (1H, dd, ³J = 7.9 Hz, ³J = 4.7 Hz, H-5'), 7.64 (1H, t, ³J = 7.5 Hz, H-5''), 7.76 (1H, t, ³J = 7.5 Hz, H-4''), 7.85 (1H, d, ³J = 7.5 Hz, H-3''), 8.00 (1H, s, NH), 8.16 (1H, dt, ³J = 7.9 Hz, ⁴J = 1.9 Hz, H-4'), 8.55 (1H, dd, ³J = 4.9 Hz, ⁴J = 1.5 Hz, H-6'), 9.02 (1H, d, ⁴J = 1.5 Hz, H-2'). ¹³C NMR (75 MHz, Me₂SO-*d*₆): δ 64.7 (q, ⁴J_{C-F} = 2.9 Hz, C-7), 123.6 (C-5'), 127.2 (C-3'), 123.9 (q, ¹J_{C-F} = 274.4 Hz, CF₃), 125.8 (q, ³J_{C-F} = 5.7 Hz, C-3''), 126.0 (q, ²J_{C-F} = 30.6 Hz, C-2''), 128.4 (C-6''), 129.9 (C-4''), 132.7 (C-4'), 133.8 (C-5''), 138.6 (q, ³J_{C-F} = 1.2 Hz, C-1''), 146.6 (C-6'), 149.6 (C-2'), 155.3, 157.1, 157.7 (C-2, C-3a and C-5). Anal. Calcd. for C₁₆H₁₂F₃N₇: C, 53.48; H, 3.37; N, 27.29. Found: C, 53.35; H, 3.56; N, 27.06.

4.4.5. 2-(Pyridin-3-yl)-7-(3-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-amine (5e)

Yield 86%; mp 260 °C (EtOH–DMF); TLC (silica gel, EtOH): *R*_f 0.45. ¹H NMR (300 MHz, Me₂SO-*d*₆): δ 6.63 (2H, s, NH₂), 6.92 (1H, s, H-7), 7.43 (1H, dd, ³J = 7.7 Hz, ³J = 4.7, H-5'), 7.63 (1H, d, ³J = 7.5 Hz, H-4''), 7.71 (1H, t, ³J = 7.9 Hz, C-5''), 7.80 (1H, s, H-2''), 7.81 (1H, d, ³J = 7.9 Hz, H-6''), 8.15 (1H, s, NH), 8.19 (1H, dt, ³J = 7.9 Hz,

$^4J = 1.9$ Hz, H-4'), 8.57 (1H, dd, $^3J = 4.7$ Hz, $^4J = 1.7$ Hz, H-6'), 9.06 (1H, d, $^4J = 1.9$ Hz, H-2'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 67.7 (C-7), 123.0 (q, $^3J_{\text{C-F}} = 3.7$ Hz, H-4'), 123.6 (C-5'), 123.9 (q, $^1J_{\text{C-F}} = 272.4$ Hz, CF_3), 126.0 (q, $^3J_{\text{C-F}} = 3.3$ Hz, C-2''), 127.4 (C-3'), 129.6 (q, $^2J_{\text{C-F}} = 31.9$ Hz, C-3''), 130.2 (C-6''), 130.4 (q, $^4J_{\text{C-F}} = 1.2$ Hz, H-5''), 132.7 (C-4'), 141.5 (C-1''), 146.6 (C-6'), 149.6 (C-2'), 155.6, 156.7, 157.5 (C-2, C-3a and C-5). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_7$: C, 53.48; H, 3.37; N, 27.29. Found: C, 53.16; H, 3.55; N, 26.98.

4.4.6. 2-(Pyridin-3-yl)-7-(4-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5f)

Yield 93%; mp 274 °C (EtOH); TLC (silica gel, EtOH): R_f 0.41. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.58 (2H, s, NH_2), 6.88 (1H, s, H-7), 7.42 (1H, dd, $^3J = 7.9$ Hz, $^2J = 4.5$ Hz, H-5'), 7.58 (2H, d, $^3J = 8.3$ Hz, H-2'' and H-6''), 7.84 (2H, d, $^3J = 8.3$ Hz, H-3'' and H-5''), 8.09 (1H, s, NH), 8.17 (1H, dt, $^3J = 8.3$ Hz, $^4J = 1.8$ Hz, H-4'), 8.55 (1H, dd, $^3J = 4.9$, $^4J = 1.5$ Hz, H-6'), 9.03 (1H, d, $^4J = 1.9$ Hz, H-2'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 67.7 (C-7), 123.6 (C-5'), 123.9 (q, $^1J_{\text{C-F}} = 272.2$ Hz, CF_3), 125.8 (q, $^3J_{\text{C-F}} = 3.7$ Hz, C-3'' and C-5''), 127.2 (C-2'' and C-6''), 127.3 (C-3'), 129.5 (q, $^2J_{\text{C-F}} = 31.8$ Hz, C-4''), 132.7 (C-4'), 144.4 (C-1''), 146.5 (C-6'), 149.5 (C-2'), 155.5, 156.7, 157.5 (C-2, C-3a and C-5). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_7$: C, 53.48; H, 3.37; N, 27.29. Found: C, 53.34; H, 3.62; N, 27.01.

4.4.7. 7-(2-Fluorophenyl)-2-(pyridin-4-yl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5g)

Yield 85%; mp 285 °C (EtOH-DMF); TLC (silica gel, EtOH): R_f 0.46. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.57 (2H, s, NH_2), 7.01 (1H, s, H-7), 7.22–7.39 (3H, m, H-3'', H-5'' and H-6''), 7.44–7.52 (1H, m, H-4''), 7.78 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-3' and H-5'), 8.11 (1H, s, NH), 8.60 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-2' and H-6'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 64.4 (d, $^3J_{\text{C-F}} = 2.9$ Hz, C-7), 116.1 (d, $^2J_{\text{C-F}} = 20.6$ Hz, C-3''), 119.6 (C-3' and C-5'), 124.8 (d, $^4J_{\text{C-F}} = 3.5$ Hz, C-5''), 126.9 (d, $^2J_{\text{C-F}} = 11.8$ Hz, C-1''), 128.5 (d, $^3J_{\text{C-F}} = 3.5$ Hz, C-6''), 131.5 (d, $^3J_{\text{C-F}} = 8.2$ Hz, C-4''), 138.6 (C-1'), 150.0 (C-2' and C-6'), 155.6, 156.8, 157.5 (C-2, C-3a and C-5), 159.7 (d, $^1J_{\text{C-F}} = 248.7$ Hz, C-2''). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{FN}_7$: C, 58.25; H, 3.91; N, 31.70. Found: C, 58.04; H, 4.08; N, 31.52.

4.4.8. 7-(3-Fluorophenyl)-2-(pyridin-4-yl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5h)

Yield 87%; mp 288 °C (EtOH-DMF); TLC (silica gel, EtOH): R_f 0.44. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.61 (2H, s, NH_2), 6.81 (1H, d, $^3J = 1.1$ Hz, H-7), 7.16–7.31 (3H, m, H-2'', H-4'' and H-6''), 7.51 (1H, td, $^3J = 8.1$ Hz, $^4J_{\text{H-F}} = 6.0$ Hz, H-5''), 7.79 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-3' and H-5'), 8.11 (1H, d, $^3J = 1.1$ Hz, NH), 8.60 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-2' and H-6'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 67.6 (d, $^4J_{\text{C-F}} = 1.2$ Hz, C-7), 113.2 (d, $^2J_{\text{C-F}} = 22.3$ Hz, C-2''), 116.0 (d, $^2J_{\text{C-F}} = 20.6$ Hz, C-4''), 119.6 (C-3' and C-5'), 122.3 (d, $^4J_{\text{C-F}} = 2.4$ Hz, C-6''), 131.1 (d, $^3J_{\text{C-F}} = 7.6$ Hz, C-5''), 138.6 (C-1'), 142.7 (d, $^3J_{\text{C-F}} = 6.5$ Hz, C-1''), 150.0 (C-2' and C-6'), 155.6, 156.7, 157.5 (C-2, C-3a and C-5), 162.1 (d, $^1J_{\text{C-F}} = 245.2$ Hz, C-3''). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{FN}_7$: C, 58.25; H, 3.91; N, 31.70. Found: C, 58.14; H, 4.02; N, 31.43.

4.4.9. 7-(4-Fluorophenyl)-2-(pyridin-4-yl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5i)

Yield 92%; mp 288–289 °C (EtOH-DMF); TLC (silica gel, EtOH): R_f 0.46. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.54 (2H, s, NH_2), 6.78 (1H, s, H-7), 7.28 (2H, dd, $^3J = 8.7$ Hz, $^3J_{\text{H-F}} = 9.0$ Hz, H-3'' and H-5''), 7.42 (2H, dd, $^3J = 8.7$ Hz, $^4J_{\text{H-F}} = 5.7$ Hz, H-2'' and H-6''), 7.77 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-3' and H-5'), 8.02 (1H, s, NH), 8.59 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-2' and H-6'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 67.8 (C-7), 115.7 (d, $^2J_{\text{C-F}} = 21.8$ Hz, C-3'' and C-5''), 119.6 (C-3' and C-5'), 128.6 (d, $^3J_{\text{C-F}} = 8.8$ Hz, C-2'' and C-6''), 136.4 (d, $^4J_{\text{C-F}} = 2.9$ Hz, C-1''), 138.7 (C-1'), 150.0 (C-2' and C-6'), 155.7, 156.7, 157.5 (C-2, C-3a and C-5), 162.4 (d, $^1J_{\text{C-F}} = 245.2$ Hz, C-4''). Anal.

Calcd. for $\text{C}_{15}\text{H}_{12}\text{FN}_7$: C, 58.25; H, 3.91; N, 31.70. Found: C, 58.33; H, 3.95; N, 31.54.

4.4.10. 2-(Pyridin-4-yl)-7-(2-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5j)

Yield 86%; mp 278 °C (EtOH-DMF); TLC (silica gel, EtOH): R_f 0.51. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.52 (2H, s, NH_2), 7.01 (1H, s, H-7), 7.36 (1H, d, $^3J = 7.5$ Hz, H-6''), 7.64 (1H, t, $^3J = 7.5$ Hz, H-5''), 7.76 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-3' and H-5'), 7.77 (1H, t, $^3J = 7.5$ Hz, H-4''), 7.85 (1H, d, $^3J = 7.5$ Hz, H-3''), 8.03 (1H, s, NH), 8.58 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-2' and H-6'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 64.8 (q, $^4J_{\text{C-F}} = 2.9$ Hz, C-7), 119.7 (C-3' and C-5'), 123.9 (q, $^1J_{\text{C-F}} = 274.6$ Hz, CF_3), 125.8 (q, $^3J_{\text{C-F}} = 5.3$ Hz, C-3''), 126.0 (q, $^2J_{\text{C-F}} = 30.6$ Hz, C-2''), 128.5 (C-6''), 129.9 (C-4''), 133.8 (C-5''), 138.5 (C-1' and C-1''), 150.0 (C-2' and C-6'), 155.4, 157.2, 157.8 (C-2, C-3a and C-5). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_7$: C, 53.48; H, 3.37; N, 27.29. Found: C, 53.60; H, 3.42; N, 26.02.

4.4.11. 2-(Pyridin-4-yl)-7-(3-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5k)

Yield 90%; mp 258 °C (EtOH-DMF); TLC (silica gel, EtOH): R_f 0.46. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.62 (2H, s, NH_2), 6.92 (1H, s, H-7), 7.62 (1H, d, $^3J = 7.9$ Hz, H-6''), 7.70 (1H, t, $^3J = 7.7$ Hz, H-5''), 7.77 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-3' and H-5'), 7.79 (1H, s, H-2''), 7.81 (1H, d, $^3J = 7.5$ Hz, H-4''), 8.13 (1H, s, NH), 8.60 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-2' and H-6'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 67.7 (C-7), 119.6 (C-3' and C-5'), 123.1 (q, $^3J_{\text{C-F}} = 3.9$ Hz, C-2''), 123.9 (q, $^1J_{\text{C-F}} = 272.2$ Hz, CF_3), 126.0 (q, $^3J_{\text{C-F}} = 3.5$ Hz, C-4''), 129.4 (q, $^2J_{\text{C-F}} = 31.8$ Hz, C-3''), 130.2 (C-6''), 130.4 (q, $^4J_{\text{C-F}} = 1.2$ Hz, C-5''), 138.6 (C-1'), 141.3 (C-1''), 150.0 (C-2' and C-6'), 155.6, 156.8, 157.7 (C-2, C-3a and C-5). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_7$: C, 53.48; H, 3.37; N, 27.29. Found: C, 53.36; H, 3.32; N, 27.40.

4.4.12. 2-(Pyridin-4-yl)-7-(4-trifluoromethylphenyl)-6,7-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-amine (5l)

Yield 88%; mp 297–298 °C (EtOH-DMF); TLC (silica gel, EtOH): R_f 0.46. ^1H NMR (300 MHz, $\text{Me}_2\text{SO}-d_6$): δ 6.61 (2H, s, NH_2), 6.90 (1H, d, $^3J = 1.1$ Hz, H-7), 7.59 (2H, d, $^3J = 8.3$ Hz, H-2'' and H-6''), 7.77 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-3' and H-5'), 7.84 (2H, d, $^3J = 8.3$ Hz, H-3'' and H-5''), 8.11 (1H, d, $^3J = 1.1$ Hz, NH), 8.59 (2H, dd, $^3J = 4.5$ Hz, $^4J = 1.5$ Hz, H-2' and H-6'). ^{13}C NMR (75 MHz, $\text{Me}_2\text{SO}-d_6$): δ 67.8 (C-7), 119.7 (C-3' and C-5'), 123.9 (q, $^1J_{\text{C-F}} = 272.8$ Hz, CF_3), 125.9 (q, $^3J_{\text{C-F}} = 3.5$ Hz, C-3'' and C-5''), 127.3 (C-2'' and C-6''), 129.7 (q, $^2J_{\text{C-F}} = 31.8$ Hz, C-4''), 138.6 (C-1'), 144.3 (C-1''), 150.0 (C-2' and C-6'), 155.7, 156.8, 157.7 (C-2, C-3a and C-5). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_7$: C, 53.48; H, 3.37; N, 27.29. Found: C, 53.23; H, 3.28; N, 27.52.

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