Eur. J. Med. Chem. 34 (1999) 867–875

Short communication

New amino derivatives of 1,2,3-triazolo[4,5-d]pyrimidines and their affinity towards A_1 and A_{2A} adenosine receptors

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(Received 19 December 1998; revised 7 April 1999; accepted 21 April 1999)

Abstract – Starting from the appropriate azides (4-chlorobenzyl-, 2-thiophenemethyl-, 2-fluorobenzyl-, and 4-fluorobenzylazides) in which the variation of the substituent is at the basis of the four series of derivatives (**a–d**), the 7-aminosubstituted 1,2,3-triazolo[4,5-d]pyrimidines **4** were prepared by a well known synthetic route. The biological activity of compounds **4** was expected on the basis of the presence of particular substituents on N(7), and these substituents were introduced by the reaction of the 7 lactamic carbonyl function, present on precursors **3**, with cycloalkyl-, aralkyl- and arylamines. Radioligand binding assays at bovine brain adenosine A_1 and A_{2A} receptors showed that some compounds possessed a high affinity and selectivity for the A_1 receptor subtype. Furthermore, biological results indicated that the *p*-chlorobenzyl substituent lowered receptor binding, compared with the previously prepared benzyl and 2-chlorobenzyl derivatives, suggesting certain particular steric requirements of the lipophilic region which interacts with the benzyl substituent. The thiophenemethyl substituent conferred more activity than the benzyl one. The presence of a fluorine atom on the benzyl group determined a high affinity, especially when it was in the *ortho* position. Compounds **4c.1** (R = 2-fluorobenzyl, R′ = cyclopentyl, Ki = 10.5 nM), **4c.2** (R = 2-fluorobenzyl, R′ = cyclohexyl, Ki = 19.5 nM) and **4d.1** (R = 4-fluorobenzyl, R′ = cyclopentyl, Ki = 26 nM) were the most active for A_1 receptors. © 1999 Éditions scientifiques et médicales Elsevier SAS

1,2,3-triazoles / 1,2,3-triazolo[4,5-d]pyrimidines / A_1-adenosine and A_{2A}-adenosine receptor antagonists

1. Introduction

Our previous studies on 7-aminosubstituted 1,2,3-triazolopyrimidines [1] indicated that certain compounds, bearing a benzyl or 2-chlorobenzyl as the lipophilic substituent in the 3 position, showed a high affinity and selectivity towards the A_1 receptor subtype.

These and other studies of ours on 1,2,3-triazolopyrimidines [2–6], in accordance with the receptor binding site models proposed for adenosine agonists and antagonists [7], revealed three lipophilic binding regions (corresponding to the N-6, C-2 and N-9 positions of the azapurine ring) in the A_1 adenosine receptors, arranged as a fan-shape with respect to the NH function, which was engaged as a hydrogen bond donor.

On the basis of our previous results, the investigations into these structures were continued in order to study the mode of binding of these compounds with A_1-receptors by comparative SAR analysis. We report here the synthesis and biological evaluation of a series of 1,2,3-triazolo[4,5-d]pyrimidines (**4**) characterized by new benzyl-type substituents (R = 4-chlorobenzyl, 2-thenyl, 2-fluorobenzyl and 4-fluorobenzyl). In the 7 position, amino substituents which conferred a high biological activity to previously prepared molecules (cyclopentyl- and cyclohexylamino, *meta*- and *para*-toluidino, α-methylbenzylamino, amphetamino) as well as other amino substituents (isopropylamino, 2-butylamino, 2-pentylamino, 3-pentylamino, *p*-bromoanilino, furfurylamino, 2-pyridylamino and 2-hydroxy-cyclohexylamino) were introduced.

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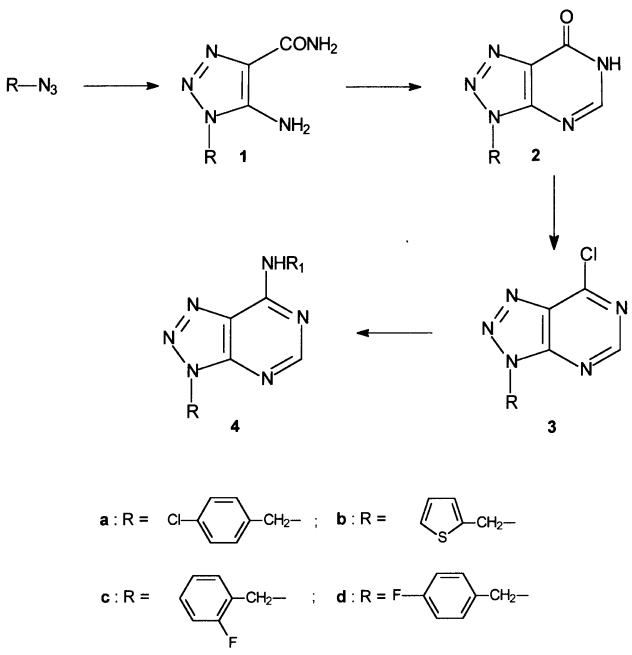


Figure 1. Synthetic route for compounds **4**.

2. Chemistry

The scheme for the preparation of compounds **4** started from the appropriate azide, which determined the R substituent (series **a–d**) and followed a well-experimented synthetic route (*figure 1*). All the azides have been reported in the literature (4-chlorobenzylazide, [8]; 2-thiophenemethylazide, [9]; 2-fluorobenzylazide [10]; and 4-fluorobenzylazide [11]) but preparation of 2-thiophenemethylazide has not been described.

The appropriate azide was reacted with cyanoacetamide in the presence of sodium ethoxide in refluxing ethanol to give the 1-substituted-4-carboxamido-5-amino-1H-1,2,3-triazoles **1a–d** in good yields. These compounds, by heating with formamide, easily cyclized to the corresponding 7-hydroxy-triazolopyrimidines **2a–d** which, by reaction with thionyl chloride in chloroform, provided the expected chloroderivatives **3a–d**. Compounds **1a, 2a** and **3a** have been described in the literature [8]. The chlorine atom in the 7 position was reactive enough to undergo a nucleophilic displacement by primary aliphatic or aromatic amines (consequently, several triazolopyrimidine derivatives **4** were prepared, corresponding to the four series characterized by the lipophilic substituent in the 3 position: **a** (4-chlorobenzyl), **b** (2-thiophenemethyl), **c** (2-fluorobenzyl) and **d** (4-fluorobenzyl) (*table I*).

The structures of all the new compounds were assigned on the basis of the well-known reaction mechanisms already regularly carried out in our laboratory: 1,3-dipolar cycloaddition of azides to activated methylenic compounds, formation of the pyrimidine ring, chlorination and nucleophilic displacement of the halogen by amines. The structures were also confirmed by analytical and spectroscopic data.

3. Biochemistry

The 7-(aminosubstituted)-1,2,3-triazolo[4,5-d]pyrimidines were tested in radioligand binding assays for affinity at A_1 and A_{2A} adenosine receptors in bovine brain cortical membranes and in bovine brain striatal membranes respectively. [^{3}H]R-(-)-N^6-cyclohexyl-adenosine (CHA) was used as the A_1 radio-ligand and [^{3}H]-2-{[[p-(2-carboxyethyl)phenyl]ethyl]amino}-5′-(N-ethylcarbamoyl)adenosine (CGS 21680) as the A_{2A} radioligand. The experimental details of the receptor binding assays were reported in a previous paper [1].

4. Results and discussion

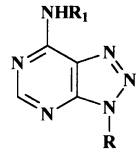
The results of the A_1 and A_{2A} adenosine receptor binding assays, expressed as inhibition constants (K_i, nM) in *table II* show that introduction of the *p*-chlorobenzyl substituent on the triazole ring in the 3 position (compounds **4a.1–7**) reduces A_1 and A_{2A} adenosine receptor binding compared with the 2-chlorobenzyl substituent [1]. The azapurine compound **4a.1**, a cyclopentylamino derivative, is an exception, with an A_1 affinity constant of K_i = 128 nM, followed by the cyclohexylamino derivative **4a.2** with a K_i = 1 360 nM. Both compounds showed a high receptor selectivity (*table II*); the inhibition percentages of the A_{2A} adenosine receptor binding assays were so low that the corresponding K_i values were not calculated. The shift of the chlorine from the *ortho* to the *para* position suggested a moderate and well-defined extent of the lipophilic region which receives the benzyl substituent. Whereas the *ortho*-chlorine atom on the benzyl group increased the affinity towards the A_1 receptor [1], the results of the **4a** series indicated that the chlorine atom in the *para* position decreased the capacity to bind with the receptor site. This fact could mean that the *para*-chloro substitution generally caused a steric repulsion within the receptor, owing to the limited depth of the lipophilic site.

Table I. Physicochemical data of triazolopyrimidine derivatives and intermediates.

Compound	Yield%	crystall. solvent	M.p. °C	Molecular formula (molecular weight)	MS M^+	m/z base peak
1b	56	EtOH	197–198	$C_8H_9N_5OS$ (223.25)	223	97
1c	73	EtOH	186–188	$C_{10}H_{10}N_5OF$ (235.22)	235	109
1d	87	EtOH	221–223	$C_{10}H_{10}N_5OF$ (235.22)	235	109
2b	59	EtOH	225–230	$C_9H_7N_5OS$ (233.25)	233	204
2c	56	EtOH	221–224	$C_{11}H_8N_5OF$ (245.22)	216 (M^+-29)	109
2d	52	EtOH	225–228	$C_{11}H_8N_5OF$ (245.22)	245	109
3b	27	60–80 °C petr. ether	79–80	$C_9H_6N_5SCl$ (251.69)	251	97
3c	63	60–80 °C petr. ether	85–88	$C_{11}H_7N_5FCl$ (263.66)	263	109
3d	61	60–80 °C petr. ether	74–76	$C_{11}H_7N_5FCl$ (263.66)	263	109
4a.1	18	MeOH	115–116	$C_{16}H_{17}N_6Cl$ (328.80)	328	125
4a.2	36	60–80 °C petr. ether	109–111	$C_{17}H_{19}N_6Cl$ (342.83)	342	125
4a.3	65	EtOH	110–112	$C_{19}H_{17}N_6Cl$ (364.84)	364	125
4a.4	50[a]	EtOH	158–161	$C_{20}H_{19}N_6Cl$-HCl (415.33)	379	125
4a.5	83	EtOH	175–176	$C_{18}H_{15}N_6Cl$ (350.81)	350	125
4a.6	84	EtOH	163–164	$C_{18}H_{15}N_6Cl$ (350.81)	350	125
4a.7	16	MeOH	115–117	$C_{17}H_{12}N_7O_2Cl$ (381.78)	316 (M^+-65)	125
4b.1	49	MeOH	120–122	$C_{14}H_{16}N_6S$ (300.38)	300	203
4b.2	42	MeOH	132–135	$C_{15}H_{18}N_6S$ (314.41)	314	97
4b.3	10[a]	EtOH	148–152	$C_{18}H_{18}N_6S$-HCl (386.90)	259 (M^+-91)	97
4b.4	18[a]	MeOH	139–142	$C_{17}H_{16}N_6S$-HCl (372.88)	336	97
4b.5	56	EtOH	149–151	$C_{16}H_{14}N_6S$ (322.39)	322	97
4b.6	73	EtOH	170–172	$C_{16}H_{14}N_6S$ (322.39)	322	97
4c.1	46	MeOH	109–112	$C_{16}H_{17}N_6F$ (312.35)	312	109
4c.2	71	MeOH	107–109	$C_{17}H_{19}N_6F$ (326.38)	326	109
4c.3	27[a]	EtOH	159–162	$C_{20}H_{19}N_6F$-HCl (398.87)	362	109
4c.4 [b]	42[a]	EtOH	181–185	$C_{20}H_{19}N_6F$-HCl (398.87)	271 (M^+-91)	109
4c.5	54	EtOH	139–142	$C_{18}H_{15}N_6F$ (334.36)	334	109
4c.6	71	MeOH	139–141	$C_{18}H_{15}N_6F$ (334.36)	334	109
4c.7	44	EtOH	153–156	$C_{17}H_{19}N_6OF$ (342.38)	342	109
4c.8	84	EtOH	170–173	$C_{16}H_{13}N_6OF$ (324.32)	324	109
4c.9	52	EtOH	196–198	$C_{17}H_{12}N_6BrF$ (399.22)	399	109
4c.10	45[a]	EtOH	182–185	$C_{16}H_{12}N_7F$-HCl (357.78)	321	109
4c.11	68	MeOH	127–129	$C_{14}H_{15}N_6F$ (286.31)	286	109
4c.12	41[a]	EtOH	160–165	$C_{15}H_{17}N_6F$-HCl (336.80)	300	109
4c.13	23[a]	EtOH	167–170	$C_{16}H_{19}N_6F$-HCl (350.83)	314	109
4c.14	42	MeOH	107–109	$C_{16}H_{19}N_6F$ (314.37)	314	109
4d.1	59	MeOH	121–124	$C_{16}H_{17}N_6F$ (312.35)	312	109
4d.2	31	60–80 °C petr. ether	115–118	$C_{17}H_{19}N_6F$ (326.38)	326	109
4d.3	67[a]	EtOH	154–157	$C_{20}H_{19}N_6F$-HCl (398.87)	362	109
4d.4	78	EtOH	184–186	$C_{18}H_{15}N_6F$ (334.36)	334	109
4d.5	54	EtOH	139–141	$C_{18}H_{15}N_6F$ (334.36)	334	109
4d.6	45	100–140 °C petr. ether	142–144	$C_{17}H_{19}N_6OF$ (342.38)	342	109
4d.7	88	EtOH	195–198	$C_{16}H_{13}N_6OF$ (324.32)	324	109
4d.8	84	EtOH	205–208	$C_{17}H_{12}N_6BrF$ (399.22)	399	109
4d.9	71	Toluene	214–217	$C_{16}H_{12}N_7F$ (321.32)	321	109
4d.10	66	EtOH/H_2O	99–101	$C_{15}H_{17}N_6F$ (300.34)	300	109
4d.11	73	60–80 °C petr. ether	82–84	$C_{16}H_{19}N_6F$ (314.37)	314	109
4d.12	65	60–80 °C petr. ether	90–92	$C_{16}H_{19}N_6F$ (314.37)	314	109

[a]Isolated as a hydrochloride; [b]Specific rotation of the free base $[\alpha]_D^{28} = -42.3°$ (c = 1.30, $CHCl_3$).

Table II. A_1 and A_{2A} adenosine receptor binding of triazolopyrimidines **4a–d**.



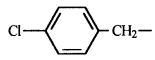
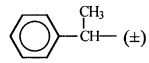
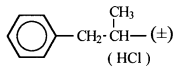
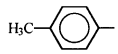
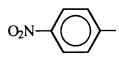
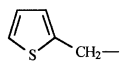
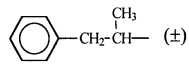
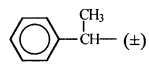
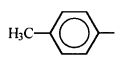
Compound	R	R_1	A_1 K_i nM	A_{2A} K_i nM	K_i A_{2A}/K_i A_1
4a.1			128 ± 12	> 10 000	> 78
4a.2	″		1 360 ± 82	> 10 000	> 7.3
4a.3	″		> 10 000	> 10 000	–
4a.4	″		> 10 000	> 10 000	–
4a.5	″		> 10 000	> 10 000	–
4a.6	″		> 10 000	> 10 000	–
4a.7	″		> 10 000	> 10 000	–
4b.1			37 ± 3	1 460 ± 131	39.4
4b.2	″		75 ± 5	1 740 ± 156	23.2
4b.3	″		39 ± 3	> 10 000	> 256
4b.4	″		68 ± 4	798 ± 56	11.7
4b.5	″		42 ± 3	616 ± 43	14.6
4b.6	″		172 ± 7	1 305 ± 104	7.5

Table II. continued.

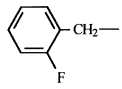
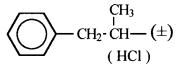
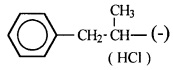
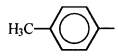
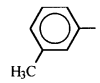
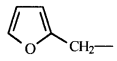
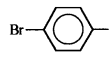
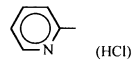
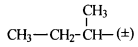
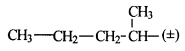
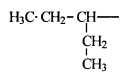
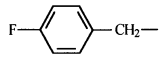
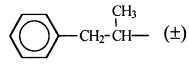
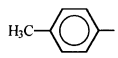
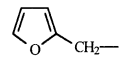
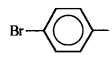
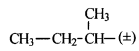
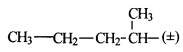
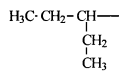
Compound	R	R_1	A_1 K_i nM	A_{2A} K_i nM	K_i A_{2A}/K_i A_1
4c.1			10.5 ± 0.7	3 422 ± 205	325.9
4c.2	″		19.5 ± 1.2	3 973 ± 198	203.7
4c.3	″		73.4 ± 6.6	2 181 ± 153	29.7
4c.4	″		91 ± 6	932 ± 83	10.2
4c.5	″		56 ± 4	1 906 ± 172	34.0
4c.6	″		402 ± 32	> 10 000	> 24.8
4c.7	″		1 188 ± 95	> 10 000	> 8.4
4c.8	″		1 650 ± 149	9 800 ± 686	5.9
4c.9	″		112 ± 7	> 10 000	> 89
4c.10	″		45 ± 3	2 613 ± 183	58.0
4c.11	″		204 ± 18	> 10 000	> 49
4c.12	″		81 ± 6	> 10 000	> 123
4c.13	″		262 ± 23	> 10 000	> 38
4c.14	″		55 ± 4	4 000 ± 240	72.7

Table II. continued.

Compound	R	R_1	A_1 K_i nM	A_{2A} K_i nM	K_i A_{2A}/K_i A_1
4d.1			26 ± 2	> 10 000	> 384
4d.2	″		72 ± 6	> 10 000	> 138
4d.3	″		287 ± 26	> 10 000	> 34
4d.4	″		254 ± 18	> 10 000	> 39
4d.5	″		1 190 ± 83	> 10 000	> 8
4d.6	″		1 137 ± 102	> 10 000	> 8
4d.7	″		> 10 000	> 10 000	–
4d.8	″		377 ± 26	> 10 000	> 26
4d.9	″		429 ± 30	> 10 000	> 23
4d.10	″		119.5 ± 9.5	> 10 000	> 83
4d.11	″		268 ± 24	> 10 000	> 37
4d.12	″		84 ± 6	7 586 ± 683	90.3

The 2-thiophenemethyl (2-thenyl) substituent (series **b**) was chosen because it could partially imitate the 2-chlorobenzyl substituent in view of its steric and electronic characteristics. This substituent appeared to be actively involved in A_1 adenosine receptor binding: the triazolopyrimidines **4b.1–5** (*table II*) showed a high A_1 adenosine affinity (K_i < 100 nM) except for the *m*-toluidino derivative **4b.6** (K_i = 172 nM). The most active compound of this series was again the cyclopentylamino derivative **4b.1** (K_i = 37 nM) but its affinity was lower than those of the 2-chlorobenzyl (K_i = 21 nM) [1] and 2-fluorobenzyl **4c.1** (K_i = 10.5 nM) derivatives. These results further confirmed the ability of the A_1 receptor to interact with lipophilic substituents bearing a

sulphur or a chlorine atom in the 2 position. The amphetamino **4b.3** and the *p*-toluidino **4b.5** derivatives possessed equivalent affinities (K_i = 39 and 42 nM respectively), confirming the effectiveness of the amphetamino substituent and of the *para*-methyl position, compared with the *meta*-methyl one (**4b.6**, Ki = 173 nM). This effect was also recorded in the *para*-fluorobenzyl series (**4d.4**, Ki = 254 nM; **4d.5**, Ki = 1 190 nM). Among compounds of the **b** series, **4b.3** showed the best selectivity (K_i A_{2A}/K_i A_1 > 256) and the most active compound **4b–1** followed with a ratio K_i A_{2A} / K_i A_1 = 39.5.

The selection of the fluorobenzyl substituents, to compare with the corresponding chlorobenzyl substituents, was based upon the consideration that the strong electronegativity of the fluorine, which is capable of accepting a hydrogen bond, allowed us to evaluate the effect of possible electronic, as well as steric factors on receptor binding. As regards the 2-fluorobenzyl substituent, used for the preparation of the triazolopyrimidines **4c.1–14** (*table II*), a generalized increase in affinity was observed towards the A_1 adenosine receptors, compared with the 2-chlorobenzyl derivatives [1], excluding the amphetamino derivatives **4c.3** and **4c.4**. The most active compounds were once again the cyclopentylamino **4c.1** (K_i = 10.5 nM) and the cyclohexylamino **4c.2** (K_i = 19.5 nM) derivatives, which also showed the best selectivity towards the A_1 adenosine receptors (K_i A_{2A}/K_i A_1 = 326 and 203, respectively); the introduction of an alcoholic OH function on the cyclohexyl ring (compound **4c.7**) (see GR-79326, GlaxoWellcome A_1 adenosine agonist) markedly decreased binding affinity. The amphetamino substituent maintained a good affinity (K_i < 100 nM), but it is worth noting that the racemic derivative **4c.3** was found to be slightly more effective than the levorotatory isomer **4c.4**. The new furfurylamino substituent (**4c.8**) markedly lowered receptor binding. As regards the known 7-arylamino substituents, the binding affinity was confirmed to be high with the *para*-toluidino derivative **4c.5** (K_i = 56 nM), while it decreased with the *meta*-toluidino derivative **4c.6**. Also the new pyridylamino substituent of **4c.10** induced a high receptor affinity (K_i = 45 nM) and a good selectivity. As for the four derivatives bearing branched aliphatic amines not previously tested by us, coming from the simplification or opening of the cyclopentyl ring, it is interesting to point out the high affinity of **4c.14** (3-pentylamino derivative, K_i = 55 nM) and **4c.12** (2-butylamino derivative, K_i = 81 nM).

Finally, as regards the 4-fluorobenzyl substituent used for the preparation of the triazolopyrimidine derivatives **4d.1–12**, the results reported in *table II* show that inhibition constants K_i < 100 nM were obtained for **4d.1** (cyclopentyl derivative, K_i = 26 nM), **4d.2** (cyclohexyl derivative, K_i = 72 nM) and **4d.12** (3-pentyl derivative, K_i = 84 nM). Thus in this series, a high affinity towards A_1 adenosine receptors was conferred by aliphatic aminosubstituents, which also showed a high receptor selectivity. The other aminosubstituents induced a noticeable decrease in binding affinity compared with the corresponding derivatives of the 2-chlorobenzyl [1] or 2-fluorobenzyl series.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler hot-stage and are uncorrected. IR spectra in nujol mulls were recorded on a Perkin-Elmer Mod. 1310 spectrometer. Mass spectra were performed with a Hewlett Packard MS/System 5988. Elemental analyses (C, H, N) were within ± 0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1106 apparatus. Optical rotations were measured with a Violet AA-5 polarimeter.

5.2. 2-Azidomethyl-thiophene

NaN_3 (7.68 g, 118 mmol) was added to a solution of 2-chloromethyl-thiophene (5.24 g, 39.5 mmol) in 25 mL of MeOH and 4 mL of H_2O, and the suspension was stirred at room temperature for 24 h. The inorganic precipitate was removed by filtration and the filtrate was concentrated, treated with H_2O and extracted with $CHCl_3$. The chloroform extract was dried and evaporated in vacuo to give the title compound as a yellow oil which was used without purification: 5.19 g, 94.5% yield. The azide was short distilled in a tubular oven at 40–45 °C, 1.8 mm Hg; IR (cm^{-1}): 2 105 (N_3).

5.2.1. 1-Substituted-4-carboxamido-5-amino-1H-1,2,3-triazoles **1b–d**

0.934 g (11 mmol) of cyanacetamide was added to a stirred solution of sodium ethoxide (0.253 g, 0.011 g atom of Na) in 10 mL of absolute EtOH, and stirring was continued for 30 min. A solution of 10.0 mmol of the suitable azide (2-thenyl-, 2-fluorobenzyl- or 4-fluorobenzylazide) in 10 mL of absolute EtOH was slowly added to the suspension, and then the mixture was heated under reflux for 1.5–2 h. The reaction mixture was concentrated in vacuo and treated with H_2O, and the insoluble material, consisting of the title compounds, was collected and purified by crystallization (*table I*).

5.2.2. 3-Substituted-7-hydroxy-1,2,3-triazolo[4,5-d]pyrimidines **2b–d**

A solution of 10.0 mmol of the suitable triazole derivative (**1b**, **1c** or **1d**) in 8 mL of formamide was refluxed for 2 h. After cooling the reaction mixture was diluted with H_2O and the solid precipitate was collected by filtration and recrystallized (*table I*).

5.2.3. 3-Substituted-7-chloro-1,2,3-triazolo[4,5-d]pyrimidines **3b–d**

0.8 ml of DMF and 4.5 mL of $SOCl_2$ were added to a suspension of 5.0 mmol of the suitable triazolopyrimidine (**2b**, **2c** or **2d**) in 22 mL of boiling anhydrous $CHCl_3$. The reaction mixture was refluxed for 2 h, the solvent was evaporated in vacuo (temperature ≤ 35 °C), and the residue, after cooling at 0 °C, was triturated with crushed ice. The solid formed was collected by filtration, dried and extracted repeatedly with boiling 60–80 °C petroleum ether. The combined extracts were evaporated in vacuo to give the title compounds as white solids (*table I*).

5.2.4. 3-(4-Chlorobenzyl)-7-(substituted amino)-1,2,3-triazolo[4,5-d]pyrimidines **4a.1–7**

A mixture of 3-(4-chlorobenzyl)-7-chloro-1,2,3-triazolo[4,5-d]pyrimidine **3a** (0.40 g, 1.43 mmol), triethylamine (0.24 mL, 1.70 mmol) and the suitable amine (1.70 mmol of cyclopentyl-, cyclohexyl-, (±)-α-methylphenethylamine, 3.70 mmol of (±)-α-methylbenzylamine, 5.0 mmol of *para*- and *meta*-toluidine or 1.0 mmol of *para*-nitroaniline) in 10 mL of absolute EtOH was refluxed for 2.5 h. For the isolation of compounds **4a.1** and **4a.2**, the reaction mixture was evaporated in vacuo, the residue was treated with H_2O and 5% HCl (pH ≅ 3) and the insoluble material was collected and purified by crystallization (**4a.1**) or by extraction with 60–80 °C petroleum ether (**4a.2**) respectively. For the isolation of compounds **4a.3**, **4a.5**, **4a.6**, and **4a.7**, the reaction mixture was allowed to cool and the crystallized precipitate was collected by filtration. Isolation of **4a.7** required further treatment of the precipitate with 10% HCl to remove unreacted *p*-nitroaniline. For the isolation of **4a.4**, the reaction mixture was evaporated in vacuo, the residue was dissolved in MeOH and the derivative was precipitated as a hydrochloride by addition of Et_2O-HCl (*table I*).

5.2.5. 3-(2-Thenyl)-7-(substituted amino)-1,2,3-triazolo[4,5-d]pyrimidines **4b.1–6**

A mixture of **3b** (0.25 g, 1.0 mmol), triethylamine (0.17 mL, 1.2 mmol) and the suitable amine (1.2 mmol of cyclopentyl-, cyclohexyl-, (±)-α-methylphenethyl-, (±)-α-methylbenzylamine, *para*- or *meta*-toluidine) in 10 mL of absolute EtOH was heated under reflux for 2.5 h. The reaction mixture was evaporated in vacuo and the residue was treated with H_2O and purified by crystallization to give compounds **4b.1**, **4b.2**, **4b.5**, and **4b.6**. For the isolation of **4b.3** and **4b.4**, the residue was extracted with Et_2O, and then Et_2O-HCl was added to the ether solution to precipitate the derivatives as hydrochlorides which were collected and crystallized (*table I*).

5.2.6. 3-(2-Fluorobenzyl)-7-(substituted amino)-1,2,3-triazolo[4,5-d]pyrimidines **4c.1–14**

A mixture of **3c** (0.30 g, 1.14 mmol), triethylamine (0.16 mL, 1.15 mmol) and the suitable amine (1.40 mmol of cyclopentyl-, cyclohexyl-, (±)-α-methylphenethyl- or (–)-α-methylphenethylamine; 4.0 mmol of *para* or *meta*-toluidine; 2.2 mmol of *trans*-2-cyclohexanol-, furfuryl-, 4-bromophenyl-, 2-pyridyl-, 2-butyl-, 2-pentyl- or 3-pentylamine) in 10 mL of absolute EtOH (10 mL of toluene for the pyridylamino derivative **4c.10**) was heated under reflux for 2.5 h. For the isolation of **4c.5**, **4c.6**, **4c.8**, **4c.9** and **4c.14**, the reaction mixture was allowed to cool and the crystallized precipitate was collected by filtration (*table I*). For the isolation of **4c.1**, **4c.2**, **4c.7** and **4c.10**, the reaction mixture was evaporated in vacuo, the residue was treated with H_2O and 10% HCl and the insoluble material was collected by filtration and crystallized (*table I*). For the isolation of **4c.3**, **4c.4**, **4c.12** and **4c.13**, the reaction mixture was evaporated in vacuo, the residue was washed with H_2O and 5% HCl and dissolved in Et_2O or absolute EtOH or MeOH. Addition of Et_2O-HCl to the solution precipitated the compounds as hydrochlorides which were purified by crystallization (*table I*). The isopropylamino derivative **4c.11** was obtained by heating the 7-chloro-triazolopyrimidine **3c** (0.39 g, 1.48 mmol) in 2 mL of isopropylamine at 100–110 °C in a closed tube for 2.5 h and treating the reaction mixture with H_2O to precipitate the expected derivative (*table I*).

5.2.7. 3-(4-Fluorobenzyl)-7-(substituted amino)-1,2,3-triazolo[4,5-d]pyrimidines **4d.1–12**

A mixture of **3d** (0.30 g, 1.14 mmol), triethylamine (0.16 mL, 1.15 mmol) and the suitable amine (1.40 mmol of cyclopentyl-, cyclohexyl-, (±)-α-methylphenethyl- or furfurylamine; 4.0 mmol of *para* or *meta*-toluidine; 2.3 mmol of *trans*-2-cyclohexanol-, 4-bromophenyl-, 2-pyridyl-, 2-butyl-, 2-pentyl- or 3-pentylamine) in 10 mL of absolute EtOH (10 mL of toluene for the pyridylamino derivative **4d.9**) was heated under reflux for 2.5 h. The reaction mixture was evaporated in vacuo,

the residue was washed with H_2O and 5% HCl (pH ≅ 3) and the insoluble material was collected and purified by crystallization (**4d.1**, **4d.6**, **4d.10**, **4d.11** and **4d.12**) or by extraction with 60–80 °C petroleum ether (**4d.2**) (*table I*). For the isolation of **4d.3**, the liquid residue obtained after the rinses was dissolved in Et_2O or MeOH, and Et_2O-HCl was added to the solution to precipitate the derivative as a hydrochloride (*table I*). For the isolation of **4d.4**, **4d.5**, **4d.7**, **4d.8** and **4d.9** the reaction mixture was allowed to cool and the crystallized precipitate was collected by filtration (*table I*).

Acknowledgements

We wish to thank the Consiglio Nazionale delle Ricerche (C.N.R.) for financial support.

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