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1,2,3-Triazolo[4,5-d]pyridazines Part VI. New 1-substituted-4-amino derivatives and their affinity towards A_1 and A_{2A} adenosine receptors

Giuliana Biagi [a], Irene Giorgi [a], Oreste Livi [a,*], Clementina Manera [a], Valerio Scartoni [a], Laura Betti [b], Gino Giannaccini [b], Antonio Lucacchini [b]

[a] *Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Pisa, via Bonanno 6, I-56126 Pisa, Italy*
[b] *Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, via Bonanno 6, I-56126 Pisa, Italy*

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Abstract

Starting from the appropriate azides (4-chlorobenzyl-, 2-thiophenemethyl-, 2-fluorobenzyl-, and 4-fluorobenzylazides) agreeing with the substituent determining four series of derivatives (**a–d**), some 4-amino-substituted 1,2,3-triazolo[4,5-d]pyridazines (**4a–d**) corresponding to previously prepared derivatives were obtained by a well experimented synthetic route. Other new derivatives (**6c**,**e**) which were different from **4a–d** because a chlorine atom had substituted the hydroxyl or the tautomeric oxamido group in the 7 position of the triazolopyridazine ring, were prepared from the suitable azides (2-fluorobenzyl and 2-chlorobenzyl), which similarly determine the series **c** and **e**, respectively, via the 4,7-dichloro derivatives **5**. The radioligand binding assays at bovine brain adenosine A_1 and A_{2A} receptors showed that some compounds **4** possessed high affinity and selectivity for the A_1 receptor subtype whilst binding affinity decreased in compounds **6** indicating the importance of a hydrogen bond donor in the 7 position of the triazolopyridazine ring. It is worth noting that compounds bearing the new lipophilic substituents 2-fluorobenzyl and 2-thiophenemethyl in the 1 position of the triazolopyridazine ring were the most active in the series.

Keywords: 1,2,3-Triazolo[4,5-d]pyridazines; Adenosine receptor binding

1. Introduction

Our previous studies on 4-amino-substituted 1,2,3-triazolopyridazines [1–4] reported that some compounds, bearing a benzyl or 2-chlorobenzyl group as a lipophilic substituent on the 1 position, showed high affinity and selectivity toward the A_1 receptor subtype.

These and our other studies on 1,2,3-triazolopyrimidines [5–10], according to the actual receptor binding site models for adenosine agonists and antagonists [11], showed 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 the A_1-receptors by comparison with the SAR analysis. Therefore we report the synthesis and biological evaluation of some series of 1,2,3-triazolo[4,5-d]pyridazines (**4a–d**), amino-substituted analogues of derivatives previously reported [1–4] characterized by new benzyl-type substituents (**a**: R = 4-chlorobenzyl; **b**: R = 2-thenyl; **c**: R = 2-fluorobenzyl; **d**: R = 4-fluorobenzyl).

In addition, in order to examine the eventual involvement of the hydroxylic and/or of the tautomeric oxamido group in the 7 position of the triazolopyridazine ring in the binding toward the A_1 adenosine receptors, two series of 4-amino-substituted triazolopyridazines (**6c**: R = 2-fluorobenzyl; and **6e**: R = 2-chlorobenzyl), bearing a chlorine atom in the 7 position, were prepared and tested.

* Corresponding author. Fax: +39-050 40517.

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The binding affinities of these compounds have been compared to those of the corresponding analogous derivatives **4c** and 1-(2-chlorobenzyl)-substituted previously prepared and tested [4], as well as to those of the corresponding 8-azapurine (or triazolopyrimidine) derivatives [5] bearing a nuclear nitrogen atom in that position of the ring.

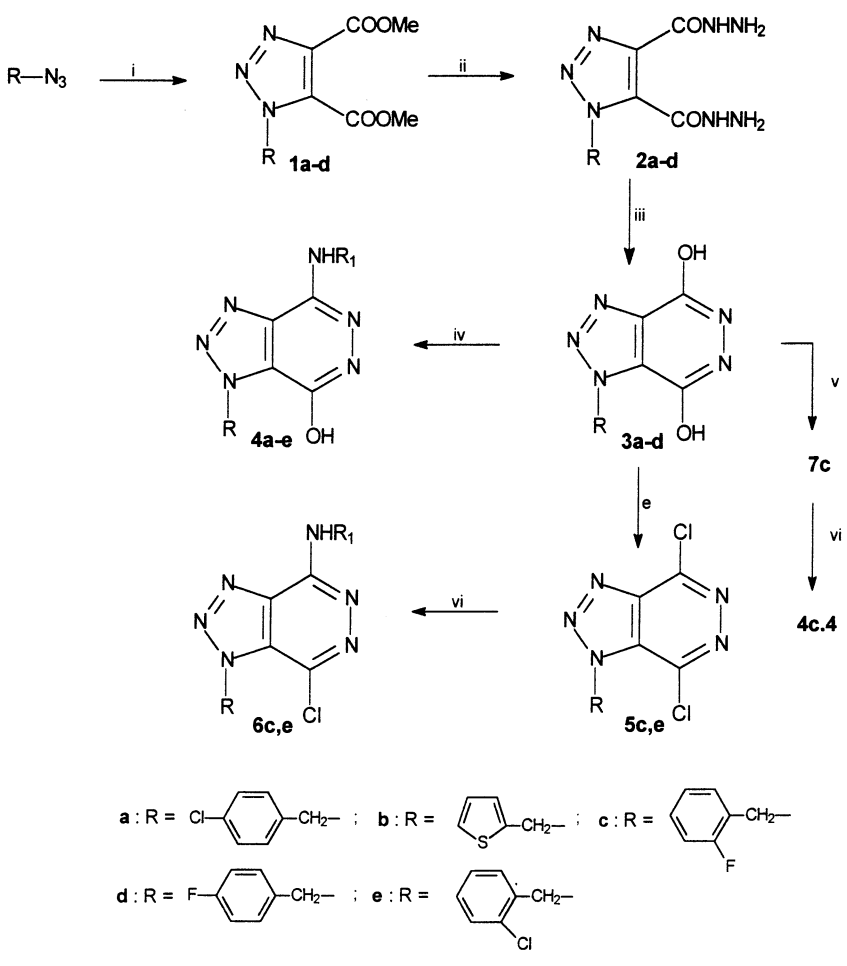
In the 4 position of the triazolopyridazine ring the amino substituents which conferred biological activity to previously prepared molecules (cyclopentyl- and cyclohexylamino, *meta*- and *para*-toluidino, (±)-α-methylbenzylamino, (±)-α-methylphenethylamino or amphetamino) and the 3-pentylamino, a substituent corresponding to the opening of the cyclopentane ring , have been introduced.

2. Chemistry

Compounds **4** were prepared starting from the appropriate azide, which determined the R substituent (series **a**–**d**) and following a well experimented synthetic route (see Scheme 1). All the azides are reported in the literature (4-chlorobenzylazide [12], 2-thenylazide [13], 2-fluorobenzylazide [14] and 4-fluorobenzylazide [15]) but preparation of 2-thenylazide is not described.

For the preparation of the triazolopyridazine derivatives **4a**–**d** (Scheme 1), the suitable azide was reacted with methylacetylenedicarboxylate to give the triazole diesters **1a**–**d** which were converted into the corresponding dicarboxyhydrazides **2a**–**d** by treatment with excess of 99% hydrazine hydrate. Compounds **2a**,**c**,**d**, by heating with 10% HCl, underwent an intramolecular cyclization with the elimination of hydrazine to give the expected 1-substituted-4,7-dihydroxy-1,2,3-triazolo-[4,5-*d*]pyridazines (**3a**,**c**,**d**) in good yield; cyclization of **2b** to **3b** was accomplished by heating in boiling Dowtherm A to avoid polymerization of thiophene in acid medium.

Some derivatives bearing cycloalkylamino or aralkylamino substituents on the 4 position were prepared from **3a**–**d** by the procedure based upon the HMDS/$(NH_4)_2SO_4$ reagent [16]. On the contrary, with the less basic primary aromatic amines, the P_2O_5/TEA · HCl



Scheme 1. (i) MeOOCC≡CCOOMe; (ii) $N_2H_4H_2O$; (iii) 10% HCl; (iv) R_1NH_2, HMDS; (v) $POCl_3$; (vi) R_1NH_2.

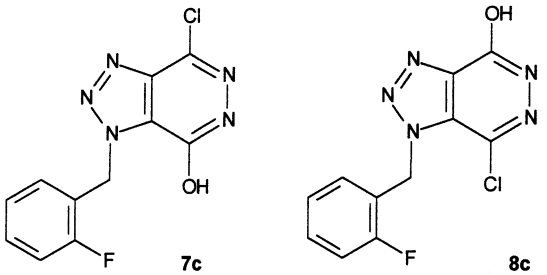


Fig. 1. Monochloro isomers.

reagent had to be employed [17], but this reagent gave unsatisfactory results with **3a** and **3c**. However, the *meta*-toluidino derivative **4c.4** (Scheme 1) was prepared via the 4-monochloro derivative **7c**, in its turn obtained together with the 7-monochloro derivative isomer **8c** (Fig. 1) by chlorination of **3c** and isolated by column chromatography. The tedious separation of the **7c** and **8c** isomers led us to prepare only the **4c.4** as an example of *N*(4)-aryl-substituted compound. The physico-chemical data of synthesized derivatives **4**, corresponding to the four series **a**, **b**, **c**, **d** (**a**: R = 4-chlorobenzyl; **b**: R = 2-thenyl; **c**: R = 2-fluorobenzyl; **d**: R = 4-fluorobenzyl) are reported in Table 1.

For the preparation of the two series of the 7-chloro-4-amino derivatives **6**, the 2-fluorobenzyl azide [14] (series **c**) and the 2-chlorobenzyl azide [12] (series **e**) were employed by the reaction sequence described in Scheme 1 to give the corresponding 4,7-dichloro-1,2,3-triazolo[4,5-d]pyridazines **5c** and **5e** [4] via chlorination with phosphorus oxychloride of **3c** and **3e**, respectively. The intermediates **1e**, **2e** and **3e** of the 2-chlorobenzyl series have been described in the literature [4]. The desired derivatives **6c** and **6e** (Table 2) were obtained by reaction in a closed tube at 120°C in toluene and 160°C in DMF, respectively, of the dichloro derivatives **5c** and **5e** with an equimolar amount of the suitable primary amine (cyclopentyl, cyclohexyl, (±)-α-methylbenzyl, (±)-α-methylphenetyl or amphetamine, *m*-toluidine, *p*-toluidine or 3-pentyl amine) in the presence of triethylamine. The physico-chemical data of compounds **6** corresponding to the series **c** (R = 2-fluorobenzyl) and **e** (R = 2-chlorobenzyl) are reported in Table 1.

The structures of all the new compounds were assigned on the basis of the well-known reaction mechanisms, already largely experimented in our laboratory [1–4,18]: 1,3-dipolar cycloaddition of azides to alkynes, formation of a pyridazine ring, chlorination reaction and nucleophilic displacement of the halogen by amines or *O*-silylation–amination reaction of amides. The structures were also confirmed by analytical and spectroscopic data.

3. Biological evaluation

The 4-(amino-substituted)-1,2,3-triazolo[4,5-d]pyridazines 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-cyclohexyladenosine (CHA) was used as the A_1 radioligand 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 have been reported in a previous paper [5].

4. Results and discussion

The results of the A_1 and A_{2A} adenosine receptor binding assays, expressed as inhibition constants (K_i, nM) reported in Table 2, show that introduction of the *p*-chlorobenzyl substituent on the triazole ring in the 1 position (compounds **4a.1**,**2**) reduces the A_1 adenosine receptor binding compared to the 2-chlorobenzyl substituent (corresponding compounds have $K_i = 70$ and 200 nM, respectively) [4]. Only the triazolopyridazine **4a.2** (racemic α-methylbenzylamino derivative) showed a moderate affinity toward A_1 ($K_i = 800$ nM) as well as A_{2A} ($K_i = 900$ nM) receptors, therefore it was lacking in selectivity. Thus, moving the chlorine atom 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 to A_1 receptors [4]. This fact could signify that the *para*-chloro substitution generally caused a steric repulsion within the receptor, owing to the limited depth of the lipophilic site.

The 2-thiophenemethyl (2-thenyl) substituent (series **4b**) had been chosen because it could partially imitate the 2-chlorobenzyl substituent for its steric and electronic characteristics. In fact, this substituent appeared actively involved in the A_1 adenosine receptor binding: the triazolopyridazine derivatives **4b** showed high A_1 adenosine receptor affinity ($K_i < 100$ nM): **4b.1** (cyclopentylamino, $K_i = 47$ nM), **4b.3** (racemic amphetamino, $K_i = 56$ nM) and **4b.2** (cyclohexylamino, $K_i = 75$ nM).

The selection of the fluorobenzyl substituents to be compared with the corresponding chlorobenzyl substituents [4] was based upon the consideration that the strong electronegativity of the fluorine able to accept a hydrogen bond, could allow the evaluation of the effect of the eventual electronic factors, as opposed to steric factors, on the receptor binding.

Table 1
Physico-chemical data of triazolopyridazines **4, 6** and intermediates

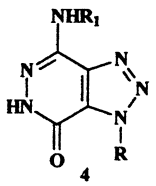
Comp.	Yield (%)	Crystall. solvent	M.p. (°C)	Analyses (C, H, N)	MS m/z	
					M^+	Base peak
1a	65	EtOH	83–84	$C_{13}H_{12}N_3O_4Cl$	278 (M^+-31)	125
1b	85	[a]	[a]			
1c	92	[a]	[a]			
1d	59	EtOH	45–48	$C_{13}H_{12}N_3O_4F$	262 (M^+-31)	109
2a	93	EtOH	185–186	$C_{11}H_{12}N_7O_2Cl$	309	125
2b	85	EtOH	158–159	$C_9H_{11}N_7O_2S$	281	97
2c	84	EtOH	167–170	$C_{11}H_{12}N_7O_2F$	293	109
2d	90	EtOH	175–178	$C_{11}H_{12}N_7O_2F$	293	109
3a	75	H_2O	300–301	$C_{11}H_8N_5O_2S$	248 (M^+-29)	125
3b	40	EtOH	232–234	$C_9H_7N_5O_2Cl$	249	97
3c	88	EtOH	269–271	$C_{11}H_8N_5O_2F$	261	109
3d	94	H_2O	300–303	$C_{11}H_8N_5O_2F$	232 (M^+-29)	109
4a.1	10	EtOH	199–201	$C_{16}H_{17}N_6OCl$	344	125
4a.2	29	EtOH	195–197	$C_{19}H_{17}N_6OCl$	380	125
4b.1	12	[b]	190–192	$C_{14}H_{16}N_6OS$	316	97
4b.2	18	[b]	186–188	$C_{15}H_{18}N_6OS$	330	97
4b.3	14	[b]	174–176	$C_{18}H_{18}N_6OS$	366	97
4c.1	8	[b]	194–195	$C_{16}H_{17}N_6OF$	328	109
4c.2	12	MeOH	179–181	$C_{17}H_{19}N_6OF$	342	109
4c.3	77	EtOH	166–169	$C_{20}H_{19}N_6OF$	378	109
4c.4	20	[b]	251–254	$C_{18}H_{15}N_6OF$	350	109
4d.1	10	[b]	176–180	$C_{16}H_{17}N_6OF$	328	109
4d.2	15	EtOH	207–209	$C_{17}H_{19}N_6OF$	342	109
4e.1	13	MeOH	202–206	$C_{16}H_{19}N_6OCl$	346	125
6c.1	48	EtOH	132–136	$C_{16}H_{16}N_6ClF$	346	109
6c.2	39	EtOH–H_2O	68–72	$C_{17}H_{18}N_6ClF$	361	109
6c.3	51	MeOH	144–146	$C_{19}H_{16}N_6ClF$	382	109
6c.4	32	MeOH	126–130	$C_{20}H_{18}N_6ClF$	396	305
6c.5	50	EtOH	172–175	$C_{18}H_{14}N_6ClF$	367	109
6c.6	15	EtOH–H_2O	90–93	$C_{18}H_{14}N_6ClF$	367	109
6e.1	33	EtOH	167–170	$C_{16}H_{16}N_6Cl_2$	363	125
6e.2	28	EtOH	144–146	$C_{17}H_{18}N_6Cl_2$	377	125
6e.3	36	MeOH	151–155	$C_{19}H_{16}N_6Cl_2$	398	125
6e.4	37	EtOH	147–150	$C_{20}H_{18}N_6Cl_2$	412	321
6e.5	11	[b]	107–110	$C_{18}H_{14}N_6Cl_2$	383	125
6e.6	10	[b]	148–152	$C_{18}H_{14}N_6Cl_2$	383	125
6e.7	23	[b]	112–116	$C_{16}H_{18}N_6Cl_2$	365	125

[a] Liquid compound, uncharacterized.
[b] Isolated by flash-chromatography.

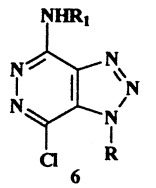
With regard to the 2-fluorobenzyltriazolopyridazine derivatives **4c.1**–**4**, the most effective compound and the only one with a K_i value < 100 nM was the *meta*-toluidino derivative **4c.4** ($K_i = 31.5$ nM, and high selectivity). The results of the **4c** series agree with those of previous analogous triazolopyridazines, either for the aromatic *meta* substitution [3,4] or for the lower receptor affinity of the cycloaliphatic amino derivatives [1,2]. Contrary to what occurred with the triazolopyrimidine derivatives [5], the results of the A_1 adenosine receptor binding in the triazolopyridazine derivatives indicated that the 2-fluorobenzyl substituent is less effective than the 2-chlorobenzyl one [4]. Similarly, the two compounds (**4d.1** and **4d.2)** bearing the 4-fluorobenzyl substituent showed a moderate affinity, the cyclohexylamino derivative **4d.2** being more effective than the corresponding cyclopentylamino **4d.1**.

A molecular modelling study based upon a model of the trans-membrane portion of the human A_1 adenosine receptor [19] confirmed that the high affinity shown by the 2-methylthiophene derivatives might be supported by a stacking interaction with the 278-histidine present in

Table 2
A_1 and A_{2A} adenosine receptor binding of derivatives **4** and **6**



4



6

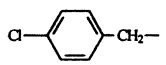
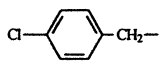
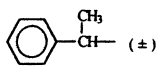
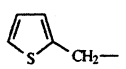
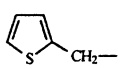
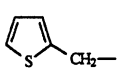
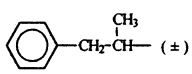
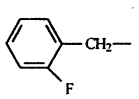
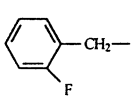
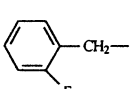
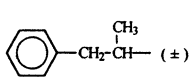
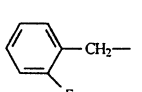
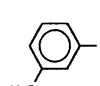
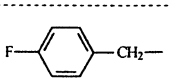
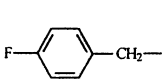
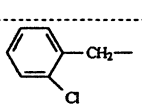
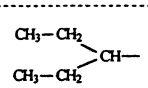
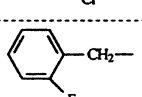
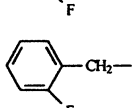
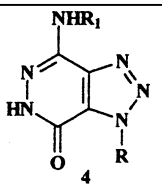
Comp.	R	R_1	A_1 K_i nM	A_{2A} K_i nM	K_iA_{2A}/K_iA_1
4a.1			> 10000	> 10000	——
4a.2			800 ± 56	900 ± 72	1.1
4b.1			46.7 ± 3.2	895 ± 63	19.1
4b.2			74.8 ± 3.8	1608 ± 145	21.5
4b.3			56.3 ± 5.1	497 ± 48	8.8
4c.1			153 ± 10	4114 ± 370	26.8
4c.2			220 ± 15	4214 ± 422	19.1
4c.3			118 ± 10	1224 ± 98	10.3
4c.4			31.5 ± 2.5	> 10000	>300
4d.1			408 ± 29	> 10000	>24
4d.2			121 ± 8	> 10000	>82
4e-1			59 ± 5	> 10000	> 169
6c.1			135 ± 12	3225 ± 310	24
6c.2			295 ± 17	> 10000	> 34

Table 2 (continued)



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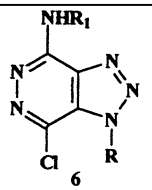
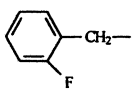
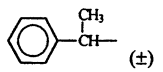
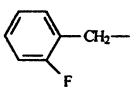
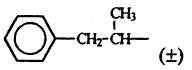
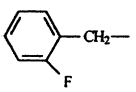
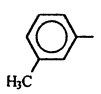
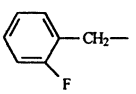
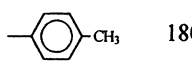
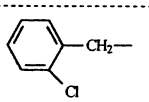
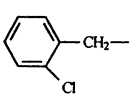
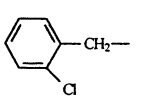
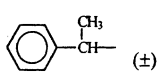
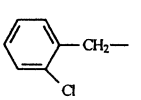
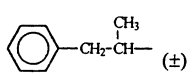
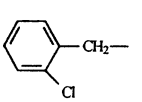
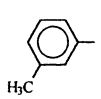
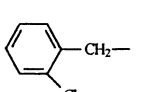
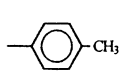
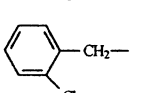
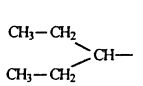


6

Comp.	R	R_1	A_1 K_i nM	A_{2A} K_i nM	K_iA_{2A}/K_iA_1
6c.3			1000 ± 98	4570 ± 450	4.5
6c.4			305 ± 29	5210 ± 505	17
6c.5			899 ± 87	> 10000	>11
6c.6			1800 ± 175	> 10000	>5.5
6e.1			195 ± 17	> 10000	>51
6e.2			195 ± 16	> 10000	>51
6e.3			312 ± 30	> 10000	>32
6e.4			307 ± 28	> 10000	>32
6e.5			413 ± 45	> 10000	>24
6e.6			246 ± 22	> 10000	>40
6e.7			91 ± 8	534 ± 51	5.8

the VII trans-membrane domain analogously to that previously observed for the benzyl group in triazolopyrimidines [19].

The triazolopyridazino derivatives **6** (Table 2), bearing a chlorine atom in the 7 position, show a decrease of the receptor binding ($K_i > 100$ nM). The comparison of the binding values of the 2-fluorobenzyl derivatives **6c.1**, **6c.2**, **6c.4** and **6c.6** with the corresponding derivatives **4c** shows a gradual and increasing reduction of the binding affinity, starting from the cyclopentyl derivative **6c.1** (K_i value similar to **4c.1**), the cyclohexylamino derivative **6c.2** and the (±)-amphetamino derivative **6c.4** up to the *m*-toluidino derivative **6c.5** (K_i value ≈ 30-fold higher than **4c.4**).

The comparison of the binding values of the **6e.1–6** derivatives with the corresponding 2-chlorobenzyl-triazolopyridazines previously prepared [4] shows a much more marked affinity decrease. The 3-pentylamino derivative **6e.7** with a good affinity value ($K_i = 91$ nM) is an exception. The corresponding 7-hydroxy derivative **4e.1** was prepared and it showed a binding affinity ($K_i = 59$ nM) higher than the chloro derivative **6e.7**. Therefore this result confirms that the 7 or 6 position of the triazolopyridazine ring can be involved in the receptor binding as a hydrogen bond donor and that the aliphatic 3-pentylamino substituent in the 4 position is biologically effective.

5. Experimental

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 Model 1310 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AC 200 spectrometer in δ units from TMS as an internal standard. Mass spectra were performed with a Hewlett Packard MS/System 5988. The TLC data were obtained with a Riedel de Haen, 37360 DC-Karten F_{254}, 0.2 mm, eluting with ethyl acetate/40–60°C petroleum ether, 1:3 mixture. Elemental analyses (C, H, N) were within $\pm$0.4% of the theoretical values and were performed on a Carlo Erba Elemental Analyzer Model 1106 apparatus.

5.1.1. 2-Azidomethyl thiophene

To a solution of 2-chloromethyl thiophene (5.24 g, 39.5 mmol) in 25 ml of MeOH and 4 ml of H_2O, NaN_3 (7.68 g, 118 mmol) was added 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 distilled in a tubular oven at 40–45°C/1.8 mmHg; IR (μ): 4.75 (N_3).

5.1.2. 1-Substituted-4,5-dicarbomethoxy-1H-1,2,3-triazoles (**1a–d**)

A solution of the suitable azide (13.15 mmol) and dimethylacetylenedicarboxylate (2.75 ml, 22.4 mmol) in 20 ml of anhydrous benzene was heated under reflux for 22 h. The reaction mixture was evaporated in vacuo to give a liquid residue consisting of the title compounds. The residues of **1a** and **1d** triturated with EtOH and MeOH, respectively, converted to the corresponding solid diester; **1b** and **1c** diesters were used without purification (Table 1).

5.1.3. 1-Substituted-4,5-dicarboxyhydrazide-1H-1,2,3-triazoles (**2a–d**)

To a solution of 13 mmol of the appropriate triazole diester (**1a**, **1b**, **1c** or **1d**) in 25 ml of EtOH, 99% hydrazine hydrate (6.4 ml, $\cong$ 130 mmol) was added and the mixture was refluxed for 1 h. The title compounds were crystallized by cooling the reaction mixture and were collected by filtration (Table 1).

5.1.4. 1-Substituted-4,7-dihydroxy-1,2,3-triazolo[4,5-d]-pyridazines (**3a**,**c**,**d**)

A mixture of 10 mmol of the appropriate dicarboxyhydrazide (**2a**, **2c** or **2d**) in 30 ml of 10% HCl was heated under reflux for 2 h. The solid precipitated after cooling was collected and recrystallized (Table 1).

5.1.5. 1-(2-Thenyl)-4,7-dihydroxy-1,2,3-triazolo[4,5-d]-pyridazine (**3b**)

A solution of **2b** (0.500 g, 1.77 mmol) in 10 ml of Dowtherm was refluxed ($\cong$ 220°C) for 1 h. After cooling, 40–60°C petroleum ether was added to the suspension and the solid material was collected by filtration and washed with petroleum ether. The solid was stirred with 10% HCl for 30 min, collected and washed with H_2O (Table 1).

5.1.6. 1-Substituted-4-cyclopentylamino-7-hydroxy-1,2,3-triazolo[4,5-d]pyridazine derivatives (**4a.1**, **4c.1** and **4d.1**)

A mixture of the suitable dihydroxytriazolopyridazine (3.0 mmol of **3a**, **3c** or **3d**), HMDS (5 ml, $\cong$ 24 mmol), ammonium sulfate (0.132 g, 1.0 mmol) and cyclopentylamine (0.5 ml, 5.0 mmol) was heated at 120°C for 24 h. The reaction mixture was dissolved in $CHCl_3$ and the solution was washed with 10% HCl, 10% NaOH and H_2O, dried and evaporated. The crude residue obtained underwent a flash-chromatography on silica gel (eluting mixture AcOEt/40–60°C petroleum ether, 1:2) to give the title compounds generally in very poor yields (Table 1).

5.1.7. 1-Substituted-4-amino-substituted-7-hydroxy-1,2,3-triazolo[4,5-d]pyridazine derivatives (**4a.2**, **4b.1–3**, **4c.2**,**3** and **4d.2**)

A mixture of the suitable dihydroxytriazolopyridazine (3.0 mmol of **3a**, **3b**, **3c** or **3d**), in HMDS (5 ml, $\cong$ 24 mmol) was heated at 140°C for 3 h. After cooling, ammonium sulfate (0.132 g, 1.0 mmol) and 5 mmol of the appropriate amine (cyclohexyl-, ($\pm$)-α-methylbenzyl-, ($\pm$)-α-methylphenethyl-, or cyclopentyl-amine) were added and heating at 140°C was continued for 20 h. The reaction mixture was dissolved in $CHCl_3$ and the chloroform extract was washed with 10% HCl, 10% NaOH and H_2O, dried and evaporated. The crude residue of **4a.2** and **4d.2** was purified by crystallization (Table 1), all other derivatives underwent a flash-chromatography on silica gel (eluting mixture AcOEt/40–60°C petroleum ether, 1:2) to give the title compounds (Table 1).

*5.1.8. 1-(2-Fluorobenzyl)-4,7-dichloro-1,2,3-triazolo-[4,5-d]pyridazine (**5c**)*

A solution of **3c** (1.0 g, 3.80 mmol) in 5 ml of $POCl_3$ and 1 ml of *N*,*N*-diethylaniline was refluxed for 8 h. The reaction mixture poured into crushed ice and the precipitated solid was collected by filtration and treated with 10% NaOH. The insoluble material collected and washed with H_2O consisted of **5c**: 0.876 g, 77%; m.p. 118–120°C (MeOH); MS 298 (M^+), 109 (base peak). *Anal.* (C, H, N) for $C_{11}H_6N_5Cl_2F$.

*5.1.9. 1-(2-Fluorobenzyl)-4-chloro-7-hydroxy-1,2,3-triazolo[4,5-d]pyridazine (**7c**) and 1-(2-fluorobenzyl)-4-hydroxy-7-chloro-1,2,3-triazolo[4,5-d]pyridazine (**8c**)*

A solution of the dihydroxytriazolopyridazine (**3c**) (2.80 g, 10.6 mmol) in 20 ml of $POCl_3$ was refluxed for 4 h. The reaction mixture was poured into crushed ice and the solid precipitated was collected by filtration, washed (H_2O) and stirred in 5% $NaHCO_3$ to solubilize the starting material. The solid residue collected and dried (1.450 g) was extracted with $CHCl_3$ and the soluble material ($\cong$0.950 g) underwent a flash-chromatography on silica gel (eluting mixture AcOEt/40–60°C petroleum ether, 1:3). After elution of a small amount of the 4,7-dichloro derivative **5c** (R_f 0.70), the 4-monochloro derivative **7c** (R_f 0.36) and its isomer 7-monochloro **8c** (R_f 0.11) were eluted.

7c: 0.490 g, 16.5%; m.p. 203–207°C; MS 279 (M^+), 109 (base peak). ^{1}H NMR (DMSO) δ: 6.09 (s, 2H, CH_2); 7.16–7.45 (m, 4H, Ar); 13.30 (brs, 1H, NH). ^{13}C NMR (DMSO) δ: 47.02 J_{CF} 3.5 Hz (CH_2); 115.63 J_{CF} 20.8 Hz (C-3′); 121.87 J_{CF} 14.4 Hz (C-1′); 124.85 J_{CF} 3.2 Hz (C-5′); 128.64 (C-3a); 130.74 J_{CF} 3.0 Hz (C-4′); 131.00 J_{CF} 8.3 Hz (C-6′); 131.10 (C-7a); 141.42 (C-4); 153.51 (C-7); 160.05 J_{CF} 247.4 Hz (C-2′). *Anal.* (C, H, N) for $C_{11}H_7N_5OClF$.

8c: 0.250 g, 8.5%; m.p. 220–222°C; MS 279 (M^+), 109 (base peak). ^{1}H NMR (DMSO) δ: 6.13 (s, 2H, CH_2); 7.16–7.46 (m, 4H, Ar); 13.26 (brs, 1H, NH). ^{13}C NMR (DMSO) δ: 46.78 J_{CF} 3.5 Hz (CH_2); 115.63 J_{CF} 20.8 Hz (C-3′); 122.10 J_{CF} 14.3 Hz (C-1′); 124.67 (C-3a); 124.94 J_{CF} 3.0 Hz (C-5′); 129.70 J_{CF} 2.9 Hz (C-4′); 130.77 J_{CF} 8.2 Hz (C-6′); 131.41 (C-7a); 139.64 (C-7); 155.67 (C-4). 159.64 J_{CF} 246.4 Hz (C-2′). *Anal.* (C, H, N) for $C_{11}H_7N_5OClF$.

*5.1.10. 1-(2-Fluorobenzyl)-4-(m-toluidino)-7-hydroxy-1,2,3-triazolo[4,5-d]pyridazine (**4c.4**)*

A mixture of the 4-monochloro derivative **7c** (0.250 g, 0.954 mmol) and *m*-toluidine (0.20 ml, 1.90 mmol) was heated in a closed tube at 140°C for 12 h. The reaction mixture was treated with H_2O and extracted with $CHCl_3$; the chloroform layer was washed with 5% HCl, H_2O, dried and evaporated. The crude residue was purified by flash-chromatography through silica gel eluting with $CHCl_3$/Et_2NH/MeOH, 10:0.1:0.1 (Table 1).

*5.1.11. 1-(2-Fluorobenzyl)-4-amino-substituted-7-chloro-1,2,3-triazolo[4,5-d]pyridazine derivatives (**6c.1–6**)*

To a solution of the dichloro derivative **5c** (0.300 g, 1.00 mmol) in 5 ml of toluene and 3 ml of triethylamine, 1.00 mmol of the suitable amine (cyclopentyl, cyclohexyl, (±)-α-methylbenzyl, (±)-α-methylphenetyl amine, *m*-toluidine or *p*-toluidine) was added and the mixture was heated in a closed tube at 120°C for 12 h. The mixture was evaporated in vacuo, the residue treated with $CHCl_3$ and the organic layer washed with 10% HCl, 10% NaOH and H_2O. The solution was dried and evaporated and the residue crystallized to give the title compounds (Table 1).

*5.1.12. 1-(2-Chlorobenzyl)-4-amino-substituted-7-chloro-1,2,3-triazolo[4,5-d]pyridazine derivatives (**6e.1–7**)*

To a solution of the dichloro derivative **5e** [4] (0.300 g, 0.95 mmol) in 1.5 ml of DMF and 3 ml of triethylamine, 0.95 mmol of the suitable amine (cyclopentyl, cyclohexyl, (±)-α-methylbenzyl, (±)-α-methylphenetyl amine, *m*-toluidine, *p*-toluidine or 3-pentylamine) were added and the mixture was heated in a closed tube at 160°C for 12 h. The mixture was worked up as described above (Table 1). Compounds **6e.5–7** were isolated by flash-chromatography through silica gel eluting with an ethyl acetate/40–60° petroleum ether/diethylamine, 1.5:10:0.5 mixture (Table 1).

*5.1.13. 1-(2-Chlorobenzyl)-4-(3-pentylamino)-7-hydroxy-1,2,3-triazolo[4,5-d]pyridazine (**4e.1**)*

A solution of 0.300 g (1.08 mmol) of 4,7-dihydroxytriazolopyridazine **3e** [4] in 1.2 ml (6.02 mmol) of HMDS was heated at 140°C for 1 h. After cooling, 0.050 g of $(NH_4)_2SO_4$ and 0.70 ml (6.02 mmol) of 3-pentylamine were added and the mixture was heated again at 140°C for 20 h. The reaction mixture was treated with $CHCl_3$ and the chloroform was washed with 10% HCl, 10% NaOH and H_2O, then dried and evaporated to give a residue which was crystallized (Table 1).

Acknowledgements

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References

[1] G. Biagi, I. Giorgi, O. Livi, V. Scartoni, C. Martini, P. Tacchi, S. Merlino, M. Pasero, 1,2,3-Triazolo[4,5-d]pyridazines. II. New derivatives tested on adenosine receptors, Farmaco 49 (1994) 175–181.

[2] G. Biagi, I. Giorgi, O. Livi, V. Scartoni, A. Lucacchini, G. Senatore, P.L. Barili, 1,2,3-Triazolo[4,5-d]pyridazines. III. Synthesis of new 4-amino derivatives and their affinity toward adenosine receptors, Farmaco 49 (1994) 357–362.

[3] G. Biagi, I. Giorgi, O. Livi, V. Scartoni, S. Velo, C. Martini, G. Senatore, P.L. Barili, 1,2,3-Triazolo[4,5-d]pyridazines. IV. Preparation and adenosine receptor binding of new 4- and/or 7-amino derivatives, Farmaco 50 (1995) 99–105.
[4] G. Biagi, I. Giorgi, O. Livi, C. Manera, V. Scartoni, A. Lucacchini, G. Senatore, 1,2,3-Triazolo[4,5-d]pyridazines. V. Preparation and adenosine receptor binding of new 4-amino derivatives, Farmaco 51 (1996) 601–608.
[5] L. Betti, G. Biagi, G. Giannaccini, I. Giorgi, O. Livi, A. Lucacchini, C. Manera, V. Scartoni, Novel 3-aralkyl-7-(amino-substituted)-1,2,3-triazolo[4,5-d]pyrimidines with high affinity toward A_1 adenosine receptors, J. Med. Chem. 41 (1998) 668–673.
[6] G. Biagi, I. Giorgi, O. Livi, A. Lucacchini, C. Martini, V. Scartoni, P. Tacchi, N(6)-Substituted 2-*n*-butyl-9-benzyl-8-azaadenines. Affinity for adenosine A_1 and A_2 receptors. IV, Farmaco 49 (1994) 183–186.
[7] G. Biagi, I. Giorgi, O. Livi, A. Lucacchini, C. Martini, V. Scartoni, P. Tacchi, N(6)-Substituted 2-phenyl-9-benzyl-8-azaadenines. Affinity for adenosine A_1 and A_2 receptors. A comparison with 2-*n*-butyl analogous derivatives. V, Farmaco 49 (1994) 187–191.
[8] G. Biagi, I. Giorgi, O. Livi, V. Scartoni, A. Lucacchini, C. Martini, P. Tacchi, Synthesis of new N(6)-substituted 2-phenyl-8-azaadenosines. Their affinity for adenosine A_1 and A_2 receptors. A comparison with the corresponding 2-phenyl-9-benzyl-8-azaadenines, Farmaco 50 (1995) 13–19.
[9] G. Biagi, C. Breschi, I. Giorgi, O. Livi, C. Martini, V. Scartoni, R. Scatizzi, N(6)- or N(9)-Substituted 2-phenyl-8-azaadenines: affinity for A_1 adenosine receptors. VII, Farmaco 50 (1995) 659–667.
[10] G. Biagi, I. Giorgi, O. Livi, A. Lucacchini, V. Scartoni, N(9)-Substituted 2-phenyl-N(6)-benzyl-8-azaadenines: A_1 adenosine receptor affinity. A comparison with the corresponding N(6)-substituted 2-phenyl-N(9)-benzyl-8-azaadenines, Farmaco 51 (1996) 395–399.
[11] M.W. Dudley, N. Peet, D. Demeter, H.J.R. Weintraub, Ad.P. Ijzerman, G. Nordvall, P.J.M. van Galen, K.A. Jacobson, Adenosine A_1 receptor and ligand molecular modeling, Drug Dev. Res. 28 (1993) 237–243.
[12] W. Ried, J. Laoutidis, Synthesis neuer stickstoffreicher heterocyclen, Chem. Ztg. 114 (1990) 246–248.
[13] T. Aubert, M. Farnier, R. Guilard, New method for synthesis of isoindolinones and isoquinolines by condensation of iminophosphoranes with *o*-phthalaldehyde: reactions, mechanisms and structural study, Can. J. Chem. 68 (1990) 842–851.
[14] R. Kreher, U. Bergman, Nitrogen eliminations by electrophiles — Part 9 — Investigations on the formation of cyclic N-diazonium ions, Tetrahedron Lett. 47 (1976) 4259–4262.
[15] P.G. Baraldi, S. Manfredini, D. Simoni, L. Zappaterra, C. Zocchi, S. Dionisotti, E. Ongini, Synthesis of new pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine and 1,2,3-triazolo[4,5-e]1,2,4-triazolo[1,5-c]pyrimidine displaying potent and selective activity as A_{2A} adenosine receptor antagonists, Bioorg. Med. Chem. Lett. 4 (1994) 2539–2544.
[16] H. Vorbruggen, K. Krolikiewicz, Silylation-amination of hydroxy N-heterocycles, Chem. Ber. 117 (1984) 1523–1541.
[17] L. Andersen, F.E. Nielsen, E.B. Pedersen, Synthesis of new 1,2,3-triazolo[4,5-d]pyridazine derivatives, Chem. Scr. 29 (1989) 45–49.
[18] G. Biagi, I. Giorgi, O. Livi, C. Manera, V. Scartoni, 1,2,3-Triazolo[4,5-d]-1,2,4-triazolo[4,3-b]pyridazines, J. Heterocycl. Chem. 34 (1997) 65–69.
[19] A.M. Bianucci, M.U. Bigi, G. Biagi, I. Giorgi, O. Livi, V. Scartoni, A 3D model of the human A_1 adenosine receptor. An evaluation of the binding free-energy with ligands, Drug Des. Discovery 15 (1998) 149–156.