

# 1,2,4-Triazolo[4,3-*a*]quinoxalin-1-one: A Versatile Tool for the Synthesis of Potent and Selective Adenosine Receptor Antagonists

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4-Amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (**1**) has been found to be an A<sub>2A</sub> versus A<sub>1</sub> selective antagonist (Colotta et al. *Arch. Pharm. Pharm. Med. Chem.* **1999**, 332, 39–41). In this paper some novel triazoloquinoxalin-1-ones **4–25** bearing different substituents on the 2-phenyl and/or 4-amino moiety of the parent 4-amino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (**3**) have been synthesized and tested in radio-ligand binding assays at bovine A<sub>1</sub> and A<sub>2A</sub> and cloned human A<sub>3</sub> adenosine receptors (AR). Moreover, the binding activities at the above-mentioned AR subtypes of the 1,4-dione parent compounds **26–31** and their 5-*N*-alkyl derivatives **33–37** were also evaluated. The substituent on the 2-phenyl ring exerted a different effect on AR subtypes, while replacement of a hydrogen atom of the 4-amino group with suitable substituents yielded selective A<sub>1</sub> or A<sub>3</sub> antagonists. Replacement of a hydrogen atom of the 4-NH<sub>2</sub> with an acyl group, or replacement of the whole 4-NH<sub>2</sub> with a 4-oxo moiety, shifted the binding activity toward the A<sub>3</sub> AR. The binding results allowed elucidation of the structural requirements for the binding of these novel tricyclic derivatives at each receptor subtype. In particular, A<sub>1</sub> and A<sub>2A</sub> binding required the presence of a proton donor group at position-4, while for A<sub>3</sub> affinity the presence of a proton acceptor in this same region was of paramount importance.

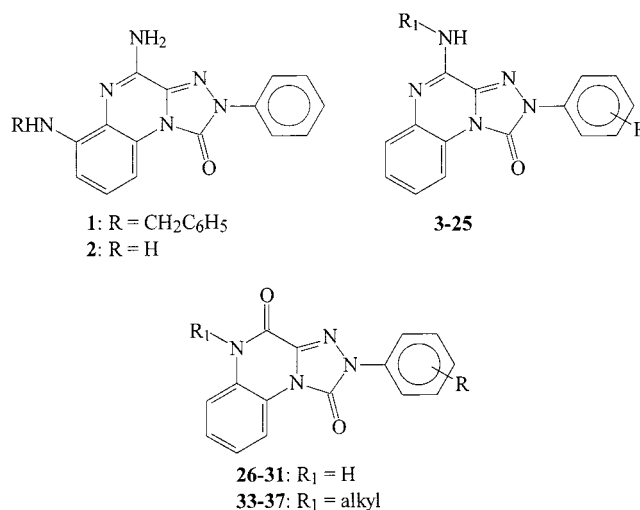
## Introduction

Adenosine is a ubiquitous neuromodulator in both the periphery and the central nervous system. The effects elicited by adenosine are mediated by its interactions with four receptor subtypes, termed A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>, belonging to the G-protein-coupled receptor family.<sup>1,2</sup> All four adenosine receptor (AR) subtypes have been identified on a pharmacological level as well as on a molecular level.<sup>3</sup> ARs from different species show amino acid sequence homology (82–93%) with the only exception being the A<sub>3</sub> subtype which only exhibits 74% primary sequence homology between rat and human or sheep.<sup>4–6</sup>

In the last two decades, many efforts have been invested in the synthesis of selective AR ligands for their potential therapeutic use. This research has resulted in the synthesis of a number of AR agonists and antagonists.<sup>7–9</sup> Particularly, selective AR subtype antagonists are sought as renal protective,<sup>10,11</sup> anti-Parkinson,<sup>12</sup> antiinflammatory, antiasthmatic, and antiischemic agents.<sup>13–16</sup>

In recent years some studies in our laboratory have been directed toward the synthesis and structure–activity relationship (SAR) studies of AR antagonists.<sup>17–21</sup> A recent paper<sup>21</sup> reported the synthesis of 4-amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (**1**) and its 6-*N*-desbenzyl derivative **2** (Chart 1). In preliminary binding screenings at the

Chart 1

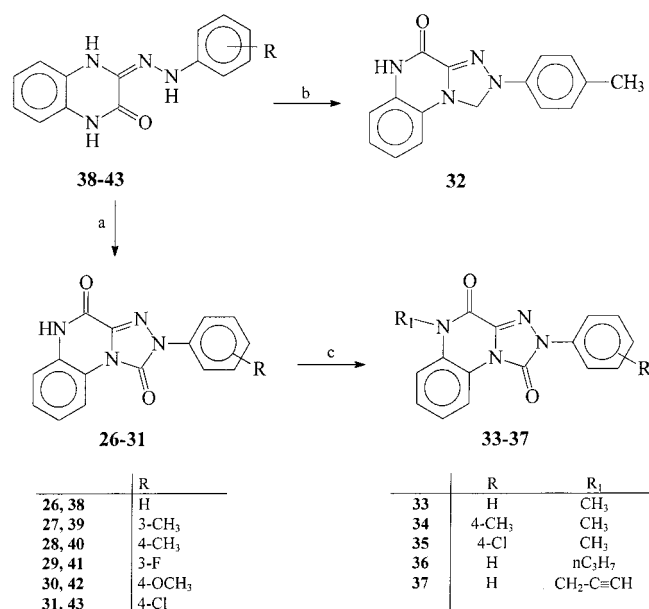


A<sub>1</sub> and A<sub>2A</sub> ARs, compound **1** was a potent and selective A<sub>2A</sub> versus A<sub>1</sub> antagonist, while **2** was 2-fold more selective for the A<sub>1</sub> versus A<sub>2A</sub> subtype. To investigate the SAR on the new triazoloquinoxalin-1-one system, we here describe the synthesis and the A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> binding activities of some novel triazoloquinoxalin-1-ones **3–25** bearing different substituents on the 2-phenyl and/or 4-amino moiety. Moreover, the binding activities at the above-mentioned AR subtypes of the 1,4-dione parent compounds **26–31**, of their 5-*N*-alkyl derivatives **33–37**, and of the 1,2,4,5-tetrahydro-2-(4-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinoxalin-4-one (**32**) are also evaluated.

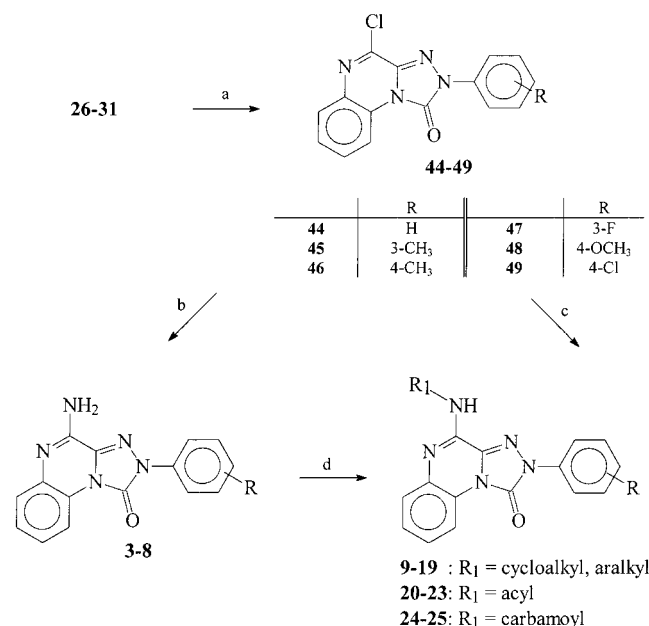
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Scheme 1<sup>a</sup>

<sup>a</sup> (a) (Cl<sub>3</sub>CO)<sub>2</sub>CO, THF; (b) 40% HCHO, ethylene glycol; (c) R<sub>1</sub>-halide, NaH, DMF.

Scheme 2<sup>a</sup>

<sup>a</sup> R and R<sub>1</sub> are defined in Tables 1 and 2. (a) PCl<sub>5</sub>/POCl<sub>3</sub>, pyridine; (b) NH<sub>3</sub>(g), EtOH; (c) R<sub>1</sub>NH<sub>2</sub>, NEt<sub>3</sub>, EtOH; (d) R<sub>2</sub>COCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, or R<sub>2</sub>NCO, THF.

## Chemistry

The synthesis of the novel triazoloquinoxalin-1-one derivatives **3–37** is illustrated in Schemes 1 and 2. Scheme 1 shows the preparation of the 1,4-dione parent compounds **26–31**, their 5-*N*-alkyl derivatives **33–37**, and the 1,2,4,5-tetrahydro-1-(4-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinoxalin-4-one (**32**), while in Scheme 2 the synthesis of the 4-amino-1-ones **3–8** and the 4-amino-substituted-1-ones **9–25** is described.

Briefly, compound **43** was prepared by reacting the commercially available *o*-phenylenediamine with *N*-(4-chlorophenyl)hydrazono-*N*<sup>2</sup>-chloroacetate<sup>22</sup> following the procedure described to prepare compounds **38–42**.<sup>23</sup> The

2-(4-chlorophenyl)-1,2,4-triazolo[4,3-*a*]quinoxalin-1,4-dione (**31**) was obtained from the corresponding 3-aryl-hydrazonoquinoxalin-2-one **43** as described for the preparation of **26–30**.<sup>23</sup> The 1,2,4,5-tetrahydro-1-(4-methylphenyl)-1,2,4-triazolo[4,3-*a*]quinoxalin-4-one (**32**) was obtained by cyclizing the corresponding hydrazonoquinoxaline **40**<sup>23</sup> with formaldehyde. The 5-*N*-alkyl-2-aryl-1,2,4,5-tetrahydro-1,2,4-triazolo[4,3-*a*]quinoxalin-1,4-diones **33–37** were prepared by reacting the key intermediates **26**, **28**, and **31** with alkyl halides (Scheme 1).

By reacting the key intermediates **26–31** with phosphorus pentachloride and phosphorus oxychloride, the unstable 2-aryl-4-chloro-1,2-dihydro-1,2,4-triazolo[4,3-*a*]quinoxalin-1-ones **44–49** were isolated (Scheme 2). Reaction of **44–49** with ammonia or amines yielded the final 4-amino-substituted derivatives **3–19**. Allowing the 4-amino-2-phenyl-1,2-dihydro-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (**3**) to react with either acyl chlorides or aryl isocyanates, the 4-amido **20–23** or 4-ureido derivatives **24–25** were obtained, respectively.

## Biochemistry

Compounds **3–37** were tested for their ability to displace [<sup>3</sup>H]*N*<sup>6</sup>-cyclohexyladenosine ([<sup>3</sup>H]CHA) from A<sub>1</sub> AR in bovine cerebral cortical membranes, [<sup>3</sup>H]-2-[[4-(2-carboxyethyl)phenethyl]amino]-5'-(*N*-ethylcarbamoyl)adenosine ([<sup>3</sup>H]CGS 21680) from A<sub>2A</sub> AR in bovine striatal membranes, and [<sup>125</sup>I]*N*<sup>6</sup>-(4-amino-3-iodobenzyl)-5'-(*N*-methylcarbamoyl)adenosine ([<sup>125</sup>I]AB-MECA) from cloned human A<sub>3</sub> AR stably expressed in HEK-293 cells. In fact, due to the species differences in A<sub>3</sub> primary amino acid sequence, new A<sub>3</sub> AR ligands had to be tested on cloned human A<sub>3</sub> ARs.<sup>4–6</sup> On the contrary, for A<sub>1</sub> and A<sub>2A</sub> AR subtypes there is a good amino acid sequence homology,<sup>9</sup> since standard antagonists, such as theophylline and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), showed an affinity at bovine A<sub>1</sub> and A<sub>2A</sub> ARs comparable to those reported at the cloned human ones.<sup>24–26</sup>

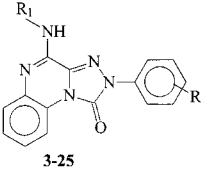
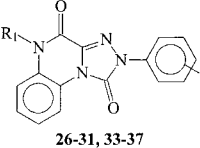
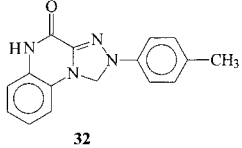







The binding results of **3–37** are shown in Table 1. In this table the binding activity at bovine A<sub>1</sub> and A<sub>2A</sub> and human cloned A<sub>3</sub> ARs of the previously synthesized<sup>21</sup> compounds **1** and **2** are also reported together with those of theophylline and DPCPX, included as antagonist reference compounds.

## Results and Conclusions

The results on the binding activities of compounds **1–37** displayed in Table 1 show that we have produced some potent and selective AR subtype antagonists. It is worth noting that a more careful screening of the A<sub>1</sub> affinity of **1** revealed a higher affinity (*K*<sub>i</sub> = 730 nM) than that reported (*K*<sub>i</sub> = 17 500 nM).<sup>21</sup> Nevertheless, due to its inactivity at the A<sub>3</sub> subtype (*I*% = 30), compound **1** is still a potent and selective A<sub>2A</sub> antagonist. Compound **2**, on the contrary, is a nonselective AR antagonist displaying nanomolar affinity at all three receptor subtypes.

With the aim of defining the SAR in the 1,2,4-triazoloquinoxalin-1-one system, we synthesized the 4-amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (**3**) which is the parent compound of the whole series. Compound **3** was equipotent to **2** at the A<sub>1</sub>, less potent

**Table 1.** Binding Activity at Bovine A<sub>1</sub> and A<sub>2A</sub> and Human A<sub>3</sub> ARs

 3-25						 26-31, 33-37						 32					
Ki (nM) or % inhibition <sup>a</sup>						Ki (nM) or % inhibition <sup>a</sup>						Ki (nM) or % inhibition <sup>a</sup>					
compd	R	R <sub>1</sub>	A <sub>1</sub> <sup>b</sup>	A <sub>2A</sub> <sup>c</sup>	A <sub>3</sub> <sup>d</sup>	compd	R	R <sub>1</sub>	A <sub>1</sub> <sup>b</sup>	A <sub>2A</sub> <sup>c</sup>	A <sub>3</sub> <sup>d</sup>	compd	R	R <sub>1</sub>	A <sub>1</sub> <sup>b</sup>	A <sub>2A</sub> <sup>c</sup>	A <sub>3</sub> <sup>d</sup>
1			730 ± 61	6.5 ± 0.38	30%	21	H	COCH <sub>2</sub> CH <sub>3</sub>	9.3 ± 0.79	2818 ± 199	15.8 ± 1.2						
2			9.2 ± 0.83	18.7 ± 1.53	54.0 ± 4.2	22	H	COC <sub>6</sub> H <sub>5</sub>	89.6 ± 7.2	53%	1.47 ± 0.11						
3	H	H	11.0 ± 0.9	49.0 ± 3.7	36%	23	H	COCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	6.3 ± 0.48	62%	3.75 ± 0.20						
4	3-CH <sub>3</sub>	H	20.0 ± 1.8	14.6 ± 1.2	28.5 ± 1.9	24	H	CONHC <sub>6</sub> H <sub>5</sub>	50.8 ± 4.2	2300 ± 219	276 ± 21						
5	4-CH <sub>3</sub>	H	19.5 ± 1.6	85.8 ± 7.4	48.3 ± 3.6	25	H	CONHC <sub>6</sub> H <sub>4</sub> -4OCH <sub>3</sub>	2600 ± 229	2800 ± 240	960 ± 86						
6	3-F	H	28.5 ± 2.2	72.0 ± 6.1	157 ± 11	26	H	H	515 ± 43	64%	80.0 ± 6.3						
7	4-OCH <sub>3</sub>	H	312 ± 27	376 ± 30	45.3 ± 3.8	27	3-CH <sub>3</sub>	H	436 ± 36	5%	91.0 ± 7.8						
8	4-Cl	H	426 ± 38	37%	329 ± 28	28	4-CH <sub>3</sub>	H	155 ± 11	0%	25.0 ± 1.6						
9	H		1.43 ± 0.1	1370 ± 118	506 ± 43	29	3-F	H	200 ± 17	20%	63.0 ± 4.5						
10	3-CH <sub>3</sub>		4.2 ± 0.23	41%	548 ± 43	30	4-OCH <sub>3</sub>	H	934 ± 85	0%	16 ± 1.2						
11	3-F		4.9 ± 0.31	66.0 ± 5.2	44.2 ± 3.5	31	4-Cl	H	1015 ± 97	5.5%	114 ± 9.7						
12	4-Cl		80.1 ± 7.1	0%	56.1 ± 4.2	32			48 %	18%	197 ± 14.2						
13	H		0.42 ± 0.03	986 ± 82	55.4 ± 4.2	33	H	CH <sub>3</sub>	309 ± 2.5	53%	36.6 ± 2.5						
14	3-CH <sub>3</sub>		1.21 ± 0.10	1400 ± 125	27.5 ± 1.9	34	4-CH <sub>3</sub>	CH <sub>3</sub>	22%	0%	504 ± 43						
15	3-F		1.1 ± 0.09	148 ± 13.1	173 ± 14	35	4-Cl	CH <sub>3</sub>	29%	8%	137 ± 11.8						
16	H	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	55.0 ± 4.3	59%	1700 ± 138	36	H	n-C <sub>3</sub> H <sub>7</sub>	406 ± 36	44%	1246 ± 110						
17	H	(CH <sub>2</sub> ) <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	4.8 ± 0.30	59%	201 ± 14	37	H	CH <sub>2</sub> C=CH	2200 ± 140	0%	479 ± 34						
18	H	(CH <sub>2</sub> ) <sub>3</sub> C <sub>6</sub> H <sub>5</sub>	17.9 ± 1.4	61%	40.9 ± 3.3	<b>Theophylline</b>			3800 ± 340	21000 ± 1800	86000 ± 7800						
19	H	CH <sub>2</sub> CH(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>	54%	50%	1020 ± 99	<b>DPCPX</b>			0.5 ± 0.03	337 ± 28	1300 ± 125						
20	H	COCH <sub>3</sub>	4.3 ± 0.38	70%	2.0 ± 0.13												

<sup>a</sup> The K<sub>i</sub> values are means ± SEM of four separate assays, each performed in triplicate. <sup>b</sup> Displacement of specific [<sup>3</sup>H]CHA binding in bovine brain membranes or percentage of inhibition (%) of specific binding at 20 μM concentration. <sup>c</sup> Displacement of specific [<sup>3</sup>H]CGS 21680 binding in bovine striatal membranes or percentage of inhibition (%) of specific binding at 20 μM concentration. <sup>d</sup> Displacement of specific [<sup>125</sup>I]AB-MECA binding at human A<sub>3</sub> ARs expressed in HEK-293 cells or percentage of inhibition (%) of specific binding at 1 μM concentration.

at the A<sub>2A</sub>, and inactive at the A<sub>3</sub> ARs. The first variation on the structure of the parent compound **3** was the introduction of simple substituents at the 3- or 4-position of the 2-phenyl ring (compounds **4–8**). In fact, nothing was known about the influence of a substituent on the 2-phenyl ring toward the AR affinity. None of these 2-phenyl-substituted derivatives **4–8** exceeded the affinity of **3** at the A<sub>1</sub> and A<sub>2A</sub> ARs, with the exception of 2-(3-methylphenyl) derivative **4** which was about 3-fold more potent than **3** at the A<sub>2A</sub> subtype. The generally negative effect of the substituent on the 2-phenyl ring toward A<sub>1</sub> and A<sub>2A</sub> affinity is stressed in the 2-(4-methoxyphenyl) (**7**) and 2-(4-chlorophenyl) (**8**) derivatives which showed dramatically reduced A<sub>1</sub> and A<sub>2A</sub> binding activities. On the contrary, the presence of the substituent on the 2-phenyl ring has a favorable effect for A<sub>3</sub> receptor–ligand interaction. In fact, compounds **4–8** displayed higher A<sub>3</sub> receptor affinity than that of the parent compound **3**. Among these 2-phenyl-substituted 4-amino derivatives **4–8** the 2-(3-methylphenyl) compound (**4**) showed the highest A<sub>3</sub> receptor affinity (K<sub>i</sub> = 28.5 nM), while the best A<sub>3</sub> receptor

selectivity was achieved with the 2-(4-methoxyphenyl) substituent (**7**). In fact compound **7** was about 6- and 8-fold more potent on A<sub>3</sub> than on A<sub>1</sub> and A<sub>2A</sub> AR subtypes, respectively. These data suggest that in A<sub>1</sub> and A<sub>2A</sub> ARs the lipophilic region that accommodates the 2-aryl moiety has different structural requirements with respect to those of the A<sub>3</sub> area.

The second modification we performed on the parent structure **3** concerned the replacement of a hydrogen atom of the 4-amino group with suitable substituents, such as cycloalkyl, aralkyl, and acyl, to obtain A<sub>1</sub> or A<sub>3</sub> subtype selective antagonists. The 4-cycloalkylamino derivatives **9–15** were prepared as potential A<sub>1</sub> selective antagonists since the cycloalkyl substituent in several tricyclic systems of similar size and shape yielded A<sub>1</sub> selective ligands.<sup>8,27,28</sup> As expected, the 4-aminocycloalkyl derivatives **9–15** displayed nanomolar A<sub>1</sub> affinity. However, compounds **9–15** were also active at the A<sub>3</sub> ARs, although the A<sub>1</sub> affinities were on the whole higher than the A<sub>3</sub> ones. The A<sub>2A</sub> affinities of **9–15** were low or null, with the exception of the 2-(3-fluorophenyl) derivatives **11** and **15** which displayed an A<sub>2A</sub> affinity



in the nanomolar range ( $K_i$  values of 66 and 148 nM, respectively). The negative effect of the substituent on the 2-phenyl ring toward the  $A_1$  binding activity is present in this series also, as shown by the decreased binding activity at this receptor of the 4-*N*-cyclohexyl (**10–12**) and 4-*N*-cyclopentyl (**14, 15**) derivatives, as compared to those of their corresponding 2-phenyl derivatives **9** and **13**, respectively. The generally favorable effect of the presence of a substituent on the 2-phenyl moiety toward  $A_3$  affinity is also confirmed in these 4-*N*-cycloalkyl derivatives **9–15**. Indeed, the 2-aryl compounds **11, 12**, and **14** are more potent than the corresponding 2-phenyl derivatives **9** and **13**, respectively.

Replacement of a hydrogen atom of the 4-amino group of the parent structure **3** with an aralkyl substituent (**16–19**) had contrasting effects depending on AR subtype. The 4-*N*-aralkylamino-2-phenyl derivatives **16–19** were all inactive at the  $A_{2A}$  AR. The *N*-benzyl **16** was less potent at the  $A_1$  ( $K_i = 55.0$  nM) and more potent at the  $A_3$  ( $K_i = 1700$  nM) than **3**. Homologation of the *N*-alkyl chain (compound **17**) produced a strong increment in  $A_1$  ( $K_i = 4.8$  nM) and  $A_3$  ( $K_i = 201$  nM) affinities. Indeed, the *N*-phenylethyl derivative **17** is a potent and selective  $A_1$  antagonist ( $A_1/A_3 = 41$ ). Further homologation of the *N*-alkyl chain (**18**) reduced, by about 4-fold, the  $A_1$  affinity ( $K_i = 17.9$  nM) while it increased, by the same order, the  $A_3$  affinity ( $K_i = 40.9$  nM). The affinity at the  $A_1$  and  $A_3$  ARs dropped significantly when a second phenyl ring (**19**) was present in the ethylene spacer chain of **17**.

Replacement of a hydrogen atom of the 4-amino group of **3** with an acyl moiety (**20–23**) yielded, in agreement with the literature data,<sup>29–31</sup> a strong increment in  $A_3$  potency. Compounds **20–23** were all inactive at the  $A_{2A}$  AR. It has to be noted that the aliphatic 4-acetylamide **20** and 4-propionylamide **21** were potent ( $K_i$  values 2.0 and 15.8 nM, respectively) but not  $A_3$  selective since they displayed  $K_i$  values of 4.3 and 9.3 nM on  $A_1$ , respectively. On the contrary, the aromatic 4-benzoylamide **22** was 60-fold more potent at human  $A_3$  ( $K_i = 1.4$  nM) than at bovine  $A_1$  subtype. Homologation of **22** afforded the 4-phenylacetylamide **23** which, like **20** and **21**, was an  $A_1$  and  $A_3$  potent nonselective antagonist. In our series of triazoloquinoxalin-1-ones the importance of the presence of the C=O amide group at position-4 in  $A_3$  receptor–ligand interaction was stressed by the comparison of the  $A_3$  affinity of the 4-*N*-benzoylamide **22** ( $K_i = 1.47$  nM) versus 4-*N*-benzylamino **16** ( $K_i = 1700$  nM) and the 4-*N*-phenylacetylamido **23** ( $K_i = 3.75$  nM) versus 4-*N*-phenethylamino **17** ( $K_i = 201$  nM). Since we presume that the exocyclic N-4 region of the triazoloquinoxalin-1-ones corresponds to that of the N-6 of the adenosine, the improvement in  $A_3$  potency of **20–23** could be due (i) to the enhanced acidity of the NH proton donor because of the presence of the electron-withdrawing C=O group and/or (ii) to the presence in the  $A_3$  subtype of a proton donor site which binds to the C=O acceptor. In contrast, the 4-C=O amide group it is not necessary for  $A_1$  receptor–ligand interaction since the 4-*N*-benzylamino **16** and 4-*N*-benzoylamido **22** showed the same order of  $A_1$  affinity ( $K_i$  values of 55.0 and 89.6 nM, respectively) as the 4-*N*-phenethylamino **17** and 4-*N*-phenylacetylamido **23** ( $K_i$  values of 4.8 and 6.3 nM,

respectively). The similar  $A_1$  affinity of **16, 22** and **17, 23** suggests that high affinity at this receptor subtype depends on the number of carbon atoms of the spacer between the phenyl moiety and the NH group.

Finally, the synthesis of the 4-*N*-carbamoyl derivatives **24** and **25** was pursued due to the  $A_3$  affinity in the low nanomolar range of some adenosine agonists.<sup>32</sup> Nevertheless, in the present series a ureido group at position-4 did not offer any advantage in receptor–ligand interaction. In fact, compounds **24** and **25** were much less active at all three receptor subtypes than the corresponding amides **20–23**.

Evaluation of the importance of the 4-amino proton donor group was the rationale for testing the intermediate 1,4-diones **26–31**. As Table 1 shows, these xanthine-like compounds are completely inactive at the  $A_{2A}$  AR and less active at the  $A_1$  AR than the corresponding 4-amino derivatives **3–8** confirming the importance of the 4-amino donor group in  $A_1$  and  $A_{2A}$  receptor recognition.<sup>19</sup> This is not the case for receptor–ligand interaction at the  $A_3$  subtype since the 1,4-diones **26–31** display at this subtype a higher affinity than the corresponding 4-amino derivatives **3–8**, with the only exception being **27** which was less active than its corresponding 4-amino derivative **4**. Moreover, comparison of the  $A_1$  and  $A_3$  affinity of the 2-phenyl-unsubstituted **26** with those of the 2-phenyl-substituted **27–31** indicated that the substituent on the 2-phenyl ring increased both  $A_1$  and  $A_3$  binding activities, with the exception of the 2-(4-methoxyphenyl) (**30**) and 2-(4-chlorophenyl) (**31**) derivatives which, in agreement with the results mentioned above, showed a decreased  $A_1$  affinity. The 4-methoxy and 4-chloro groups have however contrasting effects on the  $A_3$  affinity. In fact, while the 2-(4-methoxyphenyl) **30** was a potent and selective  $A_3$  ligand, the 2-(4-chlorophenyl) **31** showed the lowest  $A_3$  binding activity among the 1,4-diones **26–31**. The  $A_1$  and  $A_3$  affinities of **30** confirmed the different structural requirements of the  $A_1$  and  $A_3$  subtypes in the region that binds the 2-aryl moiety of the triazoloquinoxaline system.

Evaluation of the effect of the 5-*N*-alkylation on the 1,4-diones **26, 28**, and **31** was the rationale for the synthesis of the 5-*N*-alkylated-1,4-diones **33–37**. The 5-*N*-alkylation offered no advantage on  $A_{2A}$  affinity since compounds **33–37** are devoid of affinity at this receptor subtype, while it is advantageous for  $A_1$  and  $A_3$  affinities only in the case of the 5-*N*-methyl derivative **33**. In fact, compound **33** was more active at the  $A_1$  ( $K_i$  value of 309 nM) and  $A_3$  ( $K_i$  value of 36.6 nM) than its 5-*N*-desmethyl analogue **26**. Elongation of the 5-*N*-alkyl chain (**36**) or the presence of a triple bond (**37**) decreased  $A_1$  and  $A_3$  potency. It has to be noted that in these 5-*N*-alkyl derivatives the negative effect of the substituent on the 2-phenyl ring (compounds **34, 35**) appears not only for  $A_1$  affinities but also for  $A_3$  affinities.

The 1-descarbonyl derivative **32** was devoid of  $A_1$  and  $A_{2A}$  binding activity but maintained some  $A_3$  affinity ( $K_i = 197$  nM). These data showed that the presence of the C=O proton acceptor at position-1<sup>19,21</sup> is essential for  $A_1$  and  $A_{2A}$  affinity but is not necessary for  $A_3$  receptor–ligand recognition.

In conclusion, the synthesis of these novel triazoloquinoxalin-1-ones has allowed us to elucidate the struc-

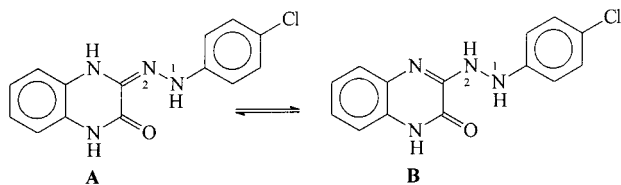
tural requirements for the binding of this new tricyclic system at each AR subtype. The AR affinities of compounds **9–18** showed that the presence of a 4-*N*-cycloalkyl or 4-*N*-aralkyl group gives rise to A<sub>1</sub> potent and selective antagonists. The introduction in the triazoloquinoxaline moiety of a 4-*N*-amido (compounds **20–23**) or 4-oxo (compounds **26–31**, **33–37**) function affords selective and/or potent A<sub>3</sub> receptor antagonists. These findings indicate that a C=O group, either extranuclear (as in the 4-amido **20–23**) or nuclear (as in the 1,4-diones **26–31**, **33–37**) is necessary for A<sub>3</sub> affinity. This suggests the importance for A<sub>3</sub> receptor–ligand interaction of (i) a strong acidic NH proton donor and/or (ii) a C=O proton acceptor able to engage a hydrogen bond with a proton donor present on the A<sub>3</sub> recognition site. Examination of the AR affinity of **26–31** and **33–37** suggests that the 4-NH<sub>2</sub> proton donor group is essential for A<sub>1</sub> and A<sub>2A</sub> receptor–ligand interaction while it is not necessary for A<sub>3</sub> receptor recognition. Finally, the binding results of the 2-aryl derivatives, in both the 4-amino (**3–15**) and 4-oxo (**26–31**) series, indicate that the presence and the nature of the substituent on the 2-phenyl moiety affect the A<sub>1</sub> and A<sub>3</sub> receptor affinities differently. Thus, the introduction of suitable groups on the 2-phenyl ring can be used to shift the selectivity toward A<sub>1</sub> or A<sub>3</sub> ARs.

In conclusion, the triazoloquinoxalin-1-one core seems to be a versatile tool to obtain potent and selective AR antagonists.

## Experimental Section

**(A) Chemistry.** Silica gel plates (Merck F<sub>254</sub>) were used for analytical chromatography. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N, and the results were within  $\pm 0.4\%$  of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm<sup>-1</sup>. The <sup>1</sup>H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in  $\delta$  (ppm) and are relative to the central peak of the solvent. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, and ar = aromatic protons. Physical data of the newly synthesized compounds are listed in Table 2.

**3-(4-Chlorophenyl)hydrazono-1,2,3,4-tetrahydroquinoxalin-2-one (43).** The title compound was obtained from ethyl *N*-(4-chlorophenyl)hydrazono-*N*<sup>2</sup>-chloroacetate<sup>22</sup> (9 mmol), *o*-phenylenediamine (9 mmol) and triethylamine (10.8 mmol) as described in ref 23 to prepare **38–42**. Compound **43** may exist, like **38–42**,<sup>23</sup> in either one of the two tautomeric forms **A** and **B**:



Tautomer **A** was easily distinguished from tautomer **B** since in the former each exchangeable proton was present as singlet, while in the latter the two hydrazine protons appeared as doublets. The <sup>1</sup>H NMR spectrum of compound **43** revealed the existence of both tautomers **A** and **B** (ratio 1:2) since there are six protons which exchange with D<sub>2</sub>O: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 6.73–7.34 (m, ar), 8.05 (d, NH of tautomer **B**, *J* = 1.4 Hz),

**Table 2.** Physical Data of the Newly Synthesized Compounds

comp	R	R <sub>1</sub>	mp, °C	cryst. solv. <sup>a</sup>	% yield
3	H	H	255–257	A	75
4	3-CH <sub>3</sub>	H	266–268	B	85
5	4-CH <sub>3</sub>	H	264–267	C	60
6	3-F	H	265–268	B	70
7	4-OCH <sub>3</sub>	H	238–240	A	75
8	4-Cl	H	>300	B	85
9	H		160–161	A	85
10	3-CH <sub>3</sub>		160–162	A	70
11	3-F		161–162	A	80
12	4-Cl		186–188	D	70
13	H		126–127	E	55
14	3-CH <sub>3</sub>		125–126	A	58
15	3-F		131–132	E	43
16	H	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	187–189	D	73
17	H	(CH <sub>2</sub> ) <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	168–170	A	70
18	H	(CH <sub>2</sub> ) <sub>3</sub> C <sub>6</sub> H <sub>5</sub>	131–132	D	92
19	H	CH <sub>2</sub> CH(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>	174–175	F	90
20	H	COCH <sub>3</sub>	248–250	G	84
21	H	COCH <sub>2</sub> CH <sub>3</sub>	224–225	A	72
22	H	COC <sub>6</sub> H <sub>5</sub>	234–235	G	75
23	H	COCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	236–237	G	60
24	H	CONHC <sub>6</sub> H <sub>5</sub>	214–215	G	80
25	H	CONHC <sub>6</sub> H <sub>4</sub> -4OCH <sub>3</sub>	237–238	G	87
31	4-Cl		>300	G	73
32			262–264 dec	H	80
33	H	CH <sub>3</sub>	232–233	G	90
34	4-CH <sub>3</sub>	CH <sub>3</sub>	295–297	G	90
35	4-Cl	CH <sub>3</sub>	>300	G	75
36	H	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	165–167	A	75
37	H	CH <sub>2</sub> C=CH	254–256	G	75
43	4-Cl		268–269 dec	G	93

<sup>a</sup> Recrystallization solvents: A = ethanol, B = dioxane, C = methanol, D = ethyl acetate, E = cyclohexane, F = acetonitrile, G = glacial acetic acid, H = ethylene glycol.

8.89 (s, NH of tautomer **A**), 9.45 (d, NH of tautomer **B**, *J* = 1.4 Hz), 9.63 (s, NH of tautomer **A**), 11.15 (s, lactam NH of tautomer **A**), 12.30 (s, lactam NH of tautomer **B**).

**2-(4-Chlorophenyl)-1,2,4,5-tetrahydro-1,2,4-triazolo-[4,3-*a*]quinoxaline-1,4-dione (31).** The title compound was obtained from **43** (4 mmol) and triphosgene (4 mmol) as described in ref 23 to prepare **26–30**: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 7.28–7.40 (m, 3H, ar), 7.65 (d, 2H, ar, *J* = 8.9 Hz), 8.06 (d, 2H, ar, *J* = 8.9 Hz), 8.60 (d, 1H, ar, *J* = 7.7 Hz), 12.01 (br s, 1H, NH).

**2-(4-Methylphenyl)-1,2,4,5-tetrahydro-1,2,4-triazolo-[4,3-*a*]quinoxalin-4-one (32).** A mixture of **40**<sup>23</sup> (0.89 mmol) in ethylene glycol (3 mL) and aqueous formaldehyde (40%, 0.4 mL) was heated at reflux for 2–3 min. Dilution with water (10 mL) yielded a yellow solid which was collected, washed with water and crystallized: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 2.25 (s, 3H, CH<sub>3</sub>), 5.72 (s, 2H, CH<sub>2</sub>), 6.84–7.18 (m, 8H, ar), 11.48 (br s, 1H, NH); IR 1670, 3160.

**General Procedure To Prepare 2-Aryl-4-chloro-1,2-dihydro-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 44–49.** A mixture of **26–31**<sup>23</sup> (2 mmol) and phosphorus pentachloride (1 mmol) in phosphorus oxychloride (30 mL) and anhydrous pyridine (0.2 mL) was heated at reflux until the disappearance (TLC monitoring) of the starting material (2–8 h). Evaporation at reduced pressure of the excess of phosphorus oxychloride yielded a residue which was treated with water (50 mL), collected and washed with cyclohexane. These 4-chloro derivatives were very unstable; however they were pure enough to be characterized and used without further purification. Compound **44** displayed the following: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 7.41 (t, 1H, ar, *J* = 7.2 Hz), 7.55–7.65 (m, 3H, ar), 7.77 (t, 1H, ar, *J* = 6.6 Hz), 7.90 (dd, 1H, ar, *J* = 8.0, 1.3 Hz), 8.06 (dd, 2H, ar, *J* = 7.4, 1.3 Hz), 8.77 (dd, 1H, ar, *J* = 8.0, 1.3 Hz).

**General Procedure To Prepare 4-Amino-2-aryl-1,2-dihydro-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 3–8.** A mixture of **44–49** (2 mmol) in absolute ethanol (30 mL) saturated with ammonia was heated overnight at 120 °C in a sealed tube. Upon cooling, a solid precipitated which was collected, washed with water and crystallized. Compound **3** displayed the following spectral data: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 7.25–7.50 (m, 3H, ar), 7.51–7.63 (m, 5H, ar + NH<sub>2</sub>), 8.09 (d, 2H, ar, *J* = 8.2 Hz), 8.64 (d, 1H, ar, *J* = 8.1 Hz); IR 1660, 1735, 3020–3220, 3320, 3460.

**General Procedure To Prepare 4-Cyclohexylamino-2-aryl-1,2-dihydro-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 9–12.** A mixture of **44, 45, 47, 49** (1 mmol), cyclohexylamine (1.2 mmol) and triethylamine (2 mmol) in absolute ethanol (5 mL) was heated overnight at 120 °C in a sealed tube. Upon cooling, a solid was obtained which was collected, washed with water and crystallized. Compound **9** displayed the following spectral data: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 1.10–2.05 (m, 10H, aliphatic protons), 4.13–4.18 (m, 1H, aliphatic proton), 7.22–7.67 (m, 7H, 6 ar + NH), 8.10 (d, 2H, ar, *J* = 8.5 Hz), 8.63 (d, 1H, ar, *J* = 7.8 Hz); IR 1730, 3420.

**General Procedure To Prepare 4-Cyclopentylamino-2-aryl-1,2-dihydro-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 13–15.** The title compounds were prepared from **44, 45, 47** (1 mmol) and cyclopentylamine (1.2 mmol) following the experimental conditions described above to obtain **9–12**. Compound **13** displayed the following spectral data: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 1.29–1.78 (m, 8H, aliphatic protons), 4.20–4.35 (m, 1H, aliphatic proton), 6.94–7.33 (m, 6H, 5 ar + NH), 7.54 (d, 1H, ar, *J* = 7.3 Hz), 7.82 (d, 2H, ar, *J* = 8.3 Hz), 8.34 (d, 1H, ar, *J* = 7.9 Hz).

**General Procedure To Prepare 4-Aralkylamino-2-phenyl-1,2-dihydro-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 16–19.** The title compounds were prepared from **44** and aralkylamine following the experimental conditions described above to obtain **9–12**. Compound **16** displayed the following spectral data: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 4.75 (d, 2H, CH<sub>2</sub>, *J* = 5.8 Hz), 7.23–7.61 (m, 11H, ar), 8.08 (d, 2H, ar, *J* = 8.5 Hz), 8.51–8.65 (m, 2H, 1H ar + NH).

**General Procedure To Prepare 4-Amido-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 20–23.** A solution of acyl chloride (2 mmol) in anhydrous dichloromethane (2 mL) was slowly added at 0 °C to a suspension of **3** (1.1 mmol) in anhydrous dichloromethane (6 mL) and anhydrous pyridine (0.4 mL). During the addition the temperature of the mixture was kept at 0 °C. The mixture was stirred at room-temperature overnight. Evaporation at reduced pressure of the solvent yielded a residue which was treated with ethanol (10 mL), collected and crystallized. Compound **20** displayed the following spectral data: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 2.37 (s, 3H, CH<sub>3</sub>), 7.39 (t, 1H, ar, *J* = 7.0 Hz), 7.52–7.64 (m, 4H, ar), 7.53 (dd, 1H, ar, *J* = 7.3, 1.1 Hz), 8.13 (d, 2H, ar, *J* = 8.6 Hz), 8.73 (d, 1H, ar, *J* = 7.9 Hz), 10.57 (br s, 1H, NH); IR 1700, 1750, 3220.

**General Procedure To Prepare 4-Arylureido-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-a]quinoxalin-1-ones 24–25.** Aryl isocyanate (1.65 mmol) was added to a suspension of **3** (1.1 mmol) in anhydrous tetrahydrofuran (50 mL). The mixture was refluxed for 30 min under nitrogen atmosphere.

The resulting solid was collected and crystallized. Compound **24** displayed the following: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 7.10–7.94 (m, 11H, ar), 8.20 (d, 2H, ar, *J* = 8.0 Hz), 8.68–8.74 (m, 1H, ar), 10.24 (s, 1H, NH), 11.68 (s, 1H, NH).

**General Procedure To Prepare 5-N-Alkyl-2-aryl-1,2,4,5-tetrahydro-1,2,4-triazolo[4,3-a]quinoxaline-1,4-diones 33–37.** The suitable alkyl halide (1.65 mmol of methyl iodide or propargyl bromide, 4 mmol of *n*-propyl bromide) and sodium hydride (80% dispersion in mineral oil, 2.42 mmol) were added to a suspension of **26, 28, 31** (1.1 mmol) in anhydrous dimethylformamide (DMF) (3 mL). The mixture was stirred at room temperature for 90 min in the case of methyl iodide and propargyl bromide or for 36 h in the case of the less reactive *n*-propyl bromide. Addition of water (40 mL) to the mixture afforded a solid which was collected and crystallized. Compound **33** displayed the following spectral data: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 3.61 (s, 3H, CH<sub>3</sub>), 7.31–7.65 (m, 6H, ar), 8.03 (d, 2H, ar, *J* = 7.5 Hz), 8.75 (d, 1H, ar, *J* = 7.8 Hz); IR 1690, 1720.

**(B) Biochemistry. A<sub>1</sub> and A<sub>2A</sub> receptor binding:** Displacement of [<sup>3</sup>H]CHA from A<sub>1</sub> AR in bovine cortical membranes and [<sup>3</sup>H]CGS 21680 from A<sub>2A</sub> AR in bovine striatal membranes was performed as described.<sup>33</sup>

**A<sub>3</sub> receptor binding:** The displacement of [<sup>125</sup>I]AB-MECA in membranes prepared from HEK-293 cells (Sigma-Aldrich, Milano) stably expressing the human A<sub>3</sub> AR was performed as described.<sup>34</sup> The assay medium consisted of a buffer containing 50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, and 1 mM EDTA at pH 8.12. The glass incubation tubes, containing 20 μL of the membrane suspension (0.2 mg of protein/mL, stored at –80 °C in the same buffer), 20 μL of [<sup>125</sup>I]AB-MECA (final concentration 0.2 nM), and 10 μL of the tested ligand, were incubated for 60 min at 25 °C in a total volume of 100 μL. After incubation the samples were filtered on Whatman GF/C filters presoaked for 1 h in 0.5% poly(ethylenimine) followed by three washes with 5 mL of ice-cold incubation buffer. Nonspecific binding was determined in the presence of 200 μM NECA. Specific binding was obtained by subtracting nonspecific binding from total binding.

Compounds were dissolved in DMSO (buffer/concentration of 2%) and added to the assay mixture. Blank experiments were carried out to determine the effect of solvent on binding. Protein estimation was based on a reported method,<sup>35</sup> after solubilization with 0.75 N sodium hydroxide, using bovine serum albumin as standard.

The concentration of the tested compound that produced 50% inhibition of specific [<sup>3</sup>H]CHA, [<sup>3</sup>H]CGS 21680, or [<sup>125</sup>I]AB-MECA binding (IC<sub>50</sub>) was calculated using a nonlinear regression method implemented in the InPlot program (Graph-Pad, San Diego, CA) with five concentrations of displacer, each performed in triplicate. Inhibition constants (*K<sub>i</sub>*) were calculated according to the Cheng–Prusoff equation.<sup>36</sup> The dissociation constants (*K<sub>d</sub>*) of [<sup>3</sup>H]CHA, [<sup>3</sup>H]CGS 21680, and [<sup>125</sup>I]AB-MECA were 1.2, 14, and 0.86 nM,<sup>37</sup> respectively.

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