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_{2A} versus A_1 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 radioligand binding assays at bovine A_1 and A_{2A} and cloned human A_3 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_1 or A_3 antagonists. Replacement of a hydrogen atom of the 4-NH_2 with an acyl group, or replacement of the whole 4-NH_2 with a 4-oxo moiety, shifted the binding activity toward the A_3 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_1 and A_{2A} binding required the presence of a proton donor group at position-4, while for A_3 affinity the presence of a proton acceptor in this same region was of paramount importance.

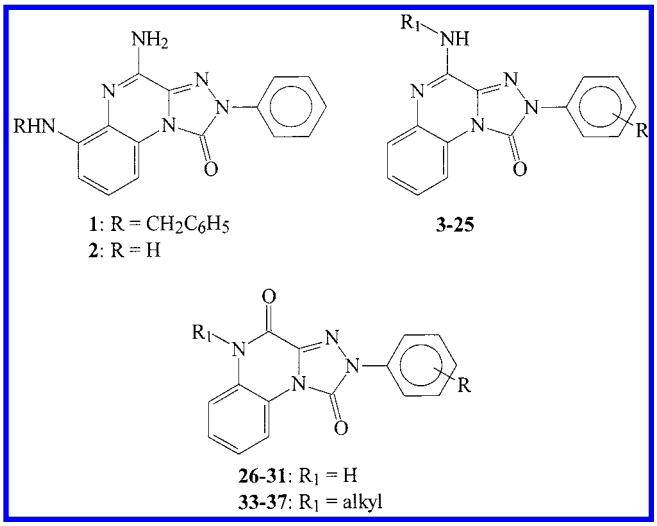
Introduction

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_1, A_{2A}, A_{2B}, and A_3, belonging to the G-protein-coupled receptor family.[1,2] All four adenosine receptor (AR) subtypes have been identified on a pharmacological level as well as on a molecular level.[3] ARs from different species show amino acid sequence homology (82−93%) with the only exception being the A_3 subtype which only exhibits 74% primary sequence homology between rat and human or sheep.[4−6]

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.[7−9] Particularly, selective AR subtype antagonists are sought as renal protective,[10,11] anti-Parkinson,[12] antiinflammatory, antiasthmatic, and antiischemic agents.[13−16]

In recent years some studies in our laboratory have been directed toward the synthesis and structure−activity relationship (SAR) studies of AR antagonists.[17−21] A recent paper[21] 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 A_1 and A_{2A} ARs, compound **1** was a potent and selective A_{2A} versus A_1 antagonist, while **2** was 2-fold more selective for the A_1 versus A_{2A} subtype. To investigate the SAR on the new triazoloquinoxalin-1-one system, we here describe the synthesis and the A_1, A_{2A}, and A_3 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.

Chart 1



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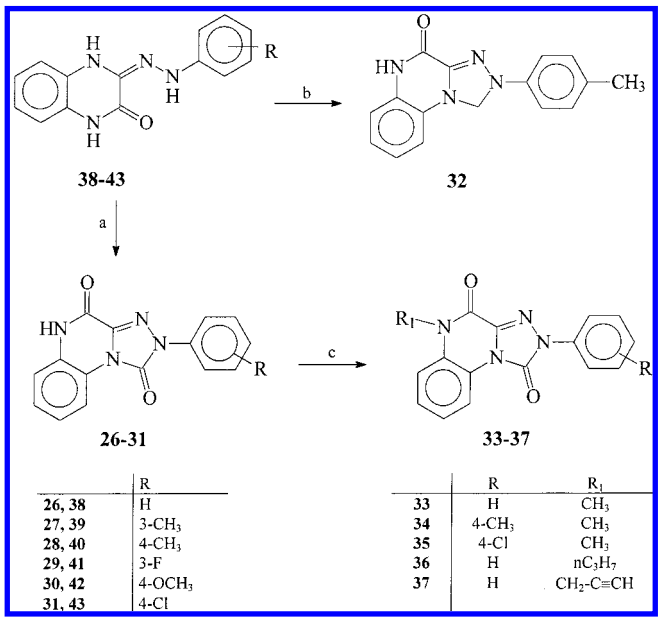
Scheme 1[a]

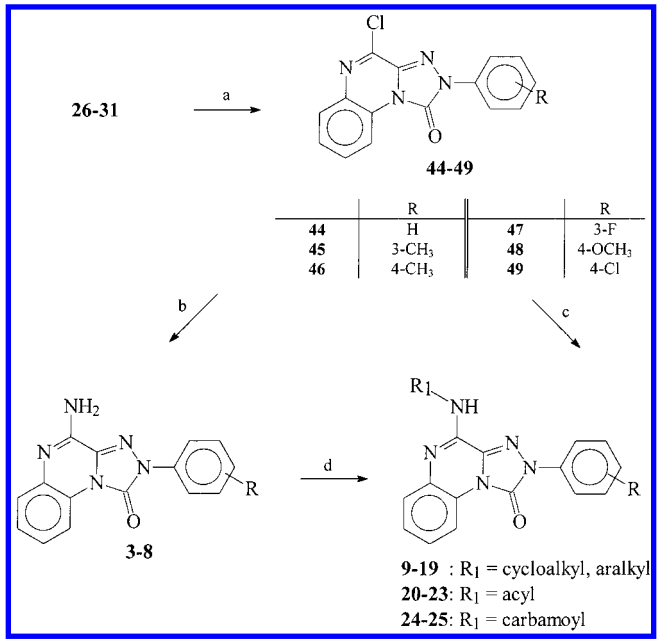


	R
26, 38	H
27, 39	3-CH_3
28, 40	4-CH_3
29, 41	3-F
30, 42	4-OCH_3
31, 43	4-Cl

	R	R_1
33	H	CH_3
34	4-CH_3	CH_3
35	4-Cl	CH_3
36	H	nC_3H_7
37	H	CH_2-C≡CH

[a] (a) $(Cl_3CO)_2CO$, THF; (b) 40% HCHO, ethylene glycol; (c) R_1-halide, NaH, DMF.

Scheme 2[a]



	R		R
44	H	47	3-F
45	3-CH_3	48	4-OCH_3
46	4-CH_3	49	4-Cl

[a] R and R_1 are defined in Tables 1 and 2. (a) $PCl_5/POCl_3$, pyridine; (b) NH_3(g), EtOH; (c) R_1NH_2, NEt_3, EtOH; (d) R_2COCl, pyridine, CH_2Cl_2, or R_2NCO, 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^1-(4-chlorophenyl)hydrazono-N^2-chloroacetate[22] following the procedure described to prepare compounds **38**−**42**.[23] The 2-(4-chlorophenyl)-1,2,4-triazolo[4,3-*a*]quinoxaline-1,4-dione **(31)** was obtained from the corresponding 3-arylhydrazonoquinoxalin-2-one **43** as described for the preparation of **26**−**30**.[23] 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**[23] with formaldehyde. The 5-*N*-alkyl-2-aryl-1,2,4,5-tetrahydro-1,2,4-triazolo[4,3-*a*]quinoxaline-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 [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) from A_1 AR in bovine cerebral cortical membranes, [^{3}H]-2-[[4-(2-carboxyethyl)phenethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680) from A_{2A} AR in bovine striatal membranes, and [^{125}I]N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine ([^{125}I]AB-MECA) from cloned human A_3 AR stably expressed in HEK-293 cells. In fact, due to the species differences in A_3 primary amino acid sequence, new A_3 AR ligands had to be tested on cloned human A_3 ARs.[4−6] On the contrary, for A_1 and A_{2A} AR subtypes there is a good amino acid sequence homology,[9] since standard antagonists, such as theophilline and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), showed an affinity at bovine A_1 and A_{2A} ARs comparable to those reported at the cloned human ones.[24−26]

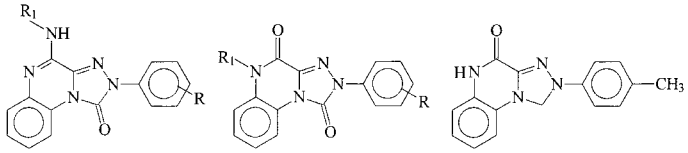
The binding results of **3**−**37** are shown in Table 1. In this table the binding activity at bovine A_1 and A_{2A} and human cloned A_3 ARs of the previously synthesized[21] compounds **1** and **2** are also reported together with those of theophilline and DPCPX, included as antagonist reference 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_1 affinity of **1** revealed a higher affinity (K_i = 730 nM) than that reported (K_i = 17 500 nM).[21] Nevertheless, due to its inactivity at the A_3 subtype (*I*% = 30), compound **1** is still a potent and selective A_{2A} 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_1, less potent

Table 1. Binding Activity at Bovine A_1 and A_{2A} and Human A_3 ARs



3-25 **26-31, 33-37** **32**

			Ki (nM) or % inhibition[a]						Ki (nM) or % inhibition[a]		
compd	**R**	**R_1**	**A_1[b]**	**A_{2A}[c]**	**A_3[d]**	**compd**	**R**	**R_1**	**A_1[b]**	**A_{2A}[c]**	**A_3[d]**
1			730 ± 61	6.5 ± 0.38	30%	**21**	H	$COCH_2CH_3$	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_6H_5	89.6 ± 7.2	53%	1.47 ± 0.11
3	H	H	11.0 ± 0.9	49.0 ± 3.7	36%	**23**	H	$COCH_2C_6H_5$	6.3 ± 0.48	62%	3.75 ± 0.20
4	3-CH_3	H	20.0 ± 1.8	14.6 ± 1.2	28.5 ± 1.9	**24**	H	$CONHC_6H_5$	50.8 ± 4.2	2300 ± 219	276 ± 21
5	4-CH_3	H	19.5 ± 1.6	85.8 ± 7.4	48.3 ± 3.6	**25**	H	$CONHC_6H_4$-4OCH_3	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_3	H	312 ± 27	376 ± 30	45.3 ± 3.8	**27**	3-CH_3	H	436 ± 36	5%	91.0 ± 7.8
8	4-Cl	H	426 ± 38	37%	329 ± 28	**28**	4-CH_3	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_3		4.2 ± 0.23	41%	548 ± 43	**30**	4-OCH_3	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_3	309 ± 2.5	53%	36.6 ± 2.5
14	3-CH_3		1.21 ± 0.10	1400 ± 125	27.5 ± 1.9	**34**	4-CH_3	CH_3	22%	0%	504 ± 43
15	3-F		1.1 ± 0.09	148 ± 13.1	173 ± 14	**35**	4-Cl	CH_3	29%	8%	137 ± 11.8
16	H	$CH_2C_6H_5$	55.0 ± 4.3	59%	1700 ± 138	**36**	H	n-C_3H_7	406 ± 36	44%	1246 ± 110
17	H	$(CH_2)_2C_6H_5$	4.8 ± 0.30	59%	201 ± 14	**37**	H	$CH_2C{\equiv}CH$	2200 ± 140	0%	479 ± 34
18	H	$(CH_2)_3C_6H_5$	17.9 ± 1.4	61%	40.9 ± 3.3	**Theophilline**			3800 ± 340	21000 ± 1800	86000 ± 7800
19	H	$CH_2CH(C_6H_5)_2$	54%	50%	1020 ± 99	**DPCPX**			0.5 ± 0.03	337 ± 28	1300 ± 125
20	H	$COCH_3$	4.3 ± 0.38	70%	2.0 ± 0.13						

[a] The K_i values are means ± SEM of four separate assays, each performed in triplicate. [b] Displacement of specific [^{3}H]CHA binding in bovine brain membranes or percentage of inhibition (*I*%) of specific binding at 20 μM concentration. [c] Displacement of specific [^{3}H]CGS 21680 binding in bovine striatal membranes or percentage of inhibition (*I*%) of specific binding at 20 μM concentration. [d] Displacement of specific [^{125}I]AB-MECA binding at human A_3 ARs expressed in HEK-293 cells or percentage of inhibition (*I*%) of specific binding at 1 μM concentration.

at the A_{2A}, and inactive at the A_3 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_1 and A_{2A} ARs, with the exception of 2-(3-methylphenyl) derivative **4** which was about 3-fold more potent than **3** at the A_{2A} subtype. The generally negative effect of the substituent on the 2-phenyl ring toward A_1 and A_{2A} affinity is stressed in the 2-(4-methoxyphenyl) **(7)** and 2-(4-chlorophenyl) **(8)** derivatives which showed dramatically reduced A_1 and A_{2A} binding activities. On the contrary, the presence of the substituent on the 2-phenyl ring has a favorable effect for A_3 receptor−ligand interaction. In fact, compounds **4**−**8** displayed higher A_3 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_3 receptor affinity (K_i = 28.5 nM), while the best A_3 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_3 than on A_1 and A_{2A} AR subtypes, respectively. These data suggest that in A_1 and A_{2A} ARs the lipophilic region that accommodates the 2-aryl moiety has different structural requirements with respect to those of the A_3 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_1 or A_3 subtype selective antagonists. The 4-cycloalkylamino derivatives **9**−**15** were prepared as potential A_1 selective antagonists since the cycloalkyl substituent in several tricyclic systems of similar size and shape yielded A_1 selective ligands.[8,27,28] As expected, the 4-aminocycloalkyl derivatives **9**−**15** displayed nanomolar A_1 affinity. However, compounds **9**−**15** were also active at the A_3 ARs, although the A_1 affinities were on the whole higher than the A_3 ones. The A_{2A} affinities of **9**−**15** were low or null, with the exception of the 2-(3-fluorophenyl) derivatives **11** and **15** which displayed an A_{2A} 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,[29−31] 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*-benzoylamido **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.[32] 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.[19] 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 mantained some A_3 affinity (K_i = 197 nM). These data showed that the presence of the C═O proton acceptor at position-1[19,21] 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-

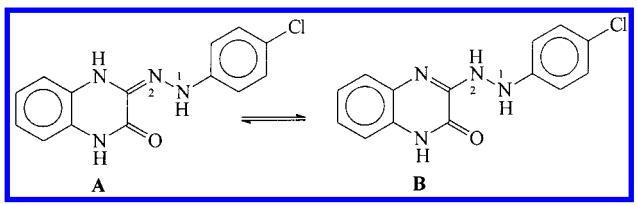
tural requirements for the binding of this new tricyclic system at each AR subtype. The AR affinities of compounds **9**−**18** showed that the presence of a 4-*N*-cycloalkyl or 4-*N*-aralkyl group gives rise to A_1 potent and selective antagonists. The introduction in the triazoloquinoxaline moiety of a 4-*N*-amido (compounds **20**−**23**) or 4-oxo (compounds **26**−**31**, **33**−**37**) function affords selective and/or potent A_3 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_3 affinity. This suggests the importance for A_3 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_3 recognition site. Examination of the AR affinity of **26**−**31** and **33**−**37** suggests that the 4-NH_2 proton donor group is essential for A_1 and A_{2A} receptor−ligand interaction while it is not necessary for A_3 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_1 and A_3 receptor affinities differently. Thus, the introduction of suitable groups on the 2-phenyl ring can be used to shift the selectivity toward A_1 or A_3 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_{254}) 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 ±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^{-1}. The ^1H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in δ (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^1-(4-chlorophenyl)hydrazono-N^2-chloroacetate[22] (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**,[23] 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 ^1H 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_2O: ^1H NMR (DMSO-d_6) 6.73−7.34 (m, ar), 8.05 (d, NH of tautomer **B**, J = 1.4 Hz), 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**).

Table 2. Physical Data of the Newly Synthesized Compounds

comp	R	R_1	mp, °C	cryst. solv.[a]	% yield
3	H	H	255-257	A	75
4	3-CH_3	H	266-268	B	85
5	4-CH_3	H	264-267	C	60
6	3-F	H	265-268	B	70
7	4-OCH_3	H	238-240	A	75
8	4-Cl	H	>300	B	85
9	H		160-161	A	85
10	3-CH_3		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_3		125-126	A	58
15	3-F		131-132	E	43
16	H	$CH_2C_6H_5$	187-189	D	73
17	H	$(CH_2)_2C_6H_5$	168-170	A	70
18	H	$(CH_2)_3C_6H_5$	131-132	D	92
19	H	$CH_2CH(C_6H_5)_2$	174-175	F	90
20	H	$COCH_3$	248-250	G	84
21	H	$COCH_2CH_3$	224-225	A	72
22	H	COC_6H_5	234-235	G	75
23	H	$COCH_2C_6H_5$	236-237	G	60
24	H	$CONHC_6H_5$	214-215	G	80
25	H	$CONHC_6H_4$-4OCH_3	237-238	G	87
31	4-Cl		>300	G	73
32			262-264 dec	H	80
33	H	CH_3	232-233	G	90
34	4-CH_3	CH_3	295-297	G	90
35	4-Cl	CH_3	>300	G	75
36	H	n-C_3H_7	165-167	A	75
37	H	$CH_2C{\equiv}CH$	254-256	G	75
43	4-Cl		268-269 dec	G	93

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

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**: ^1H NMR (DMSO-d_6) 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**[23] (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: ^1H NMR (DMSO-d_6) 2.25 (s, 3H, CH_3), 5.72 (s, 2H, CH_2), 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**[23] (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: ^{1}H NMR (DMSO-d_6) 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: ^{1}H NMR (DMSO-d_6) 7.25−7.50 (m, 3H, ar), 7.51−7.63 (m, 5H, ar + NH_2), 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: ^{1}H NMR (DMSO-d_6) 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: ^{1}H NMR (DMSO-d_6) 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 prepare from **44** and aralkylamine following the experimental conditions described above to obtain **9**−**12**. Compound **16** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 4.75 (d, 2H, CH2, 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 anydrous 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: ^{1}H NMR (DMSO-d_6) 2.37 (s, 3H, CH_3), 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 anydrous 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: ^{1}H NMR (DMSO-d_6) 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: ^{1}H NMR (DMSO-d_6) 3.61 (s, 3H, CH_3), 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_1 and A_{2A} receptor binding: Displacement of [^{3}H]CHA from A_1 AR in bovine cortical membranes and [^{3}H]CGS 21680 from A_{2A} AR in bovine striatal membranes was performed as described.[33]

A_3 receptor binding: The displacement of [^{125}I]AB-MECA in membranes prepared from HEK-293 cells (Sigma-Aldrich, Milano) stably expressing the human A_3 AR was performed as described.[34] The assay medium consisted of a buffer containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 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 [^{125}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,[35] 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 [^{3}H]CHA, [^{3}H]CGS 21680, or [^{125}I]-AB-MECA binding (IC_{50}) was calculated using a nonlinear regression method implemented in the InPlot program (GraphPad, San Diego, CA) with five concentrations of displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the Cheng−Prusoff equation.[36] The dissociation constants (K_d) of [^{3}H]CHA, [^{3}H]CGS 21680, and [^{125}I]AB-MECA were 1.2, 14, and 0.86 nM,[37] respectively.

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