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Synthesis of 4-Amino-6-(hetero)arylalkylamino-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one Derivatives as Potent A_{2A} Adenosine Receptor Antagonists

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Abstract—In previous papers (Colotta, V. et al. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 39. Colotta, V. et al. *J. Med. Chem.* **2000**, *43*, 1158) we reported the synthesis and binding affinity at bovine (b) A_1 and A_{2A} and human (h) A_3 adenosine receptors (ARs) of the 4-amino-6-benzylamino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (compound **A**) which resulted in a potent and selective A_{2A} AR antagonist. Compound **A** provided the lead compound of a series of 6- or 8-(hetero)arylalkylamino-4-amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives (compounds **1**–**20**) which are the object of this paper. Most of the newly synthesized compounds are inactive at hA_3 ARs while they possess both nanomolar bA_{2A} affinities and different degrees of bA_{2A} versus bA_1 selectivity. The binding data show that hydrophilic substituents on the benzyl moiety are the most profitable for bA_{2A} receptor affinity. Furthermore, their steric hindrance seems to play an important role for the bA_{2A} AR interaction, thus suggesting that the 6-aralkylamino moiety of these ligands interacts with a size-limited binding pocket of this AR subtype. Thus, the SAR studies provided us some new insights about the structural requirements of the bA_{2A} AR recognition site.

Introduction

Adenosine receptors (ARs) belong to the G-protein coupled receptor family and presently are classified into A_1, A_{2A}, A_{2B} and A_3 subtypes.[1] In recent years, much effort has been directed towards the study of potent and selective AR antagonists which are needed to define the specific requirements of each AR subtype. Moreover, selective AR antagonists have attracted attention for their potential therapeutic use.[2,3] In particular, over the last few years there is a growing interest toward the development of A_{2A} selective antagonists since they are sought as novel therapeutics for the treatment of Parkinson's disease, both for their capability to alleviate parkinsonian symptoms in animal models and for their chronic neuroprotective actions.[4,5] In fact, A_{2A} AR antagonists show neuroprotective effects in disease models, such as cerebral ischemia, caused by excitotoxic mechanism.[6,7] Although the mechanism responsible for such effects has yet to be elucidated, A_{2A} ARs seem to be involved in the regulation of glutamatergic transmission.[6,7] Furthermore, recent data support the hypothesis that blockade of A_{2A} ARs produces anti-nociceptive[8] and antidepressant[9] effects.

In our laboratory much effort has been directed towards the study of AR antagonists.[10–14] As a part of this program, we reported the synthesis and binding affinity at bovine (b) A_1 and A_{2A} and human (h) A_3 ARs of a series of 4-amino-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives among which the 4-amino-6-benzylamino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one **A**[15,16] was a potent and selective A_{2A} antagonist (Chart 1). SAR studies on this class of derivatives indicated that the presence of the 6-benzylamino moiety was an important requisite for obtaining high A_{2A} affinity and selectivity, in accordance with reported data on different classes of potent and selective A_{2A} AR antagonists, such as the 2-(2-furyl)-1,2,4-triazolo[2,3-*a*]-1,3,5-triazino derivative **ZM 241385**[18] and the 2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives of the **SCH series**[19,20] (Chart 1). In fact, due to the similar size and shape of

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these latter derivatives and compound **A**, we hypothesized that the appended aralkyl chain of the **SCH series** derivatives could interact with the A_{2A} receptor subsite which binds the benzyl moiety of **A**. Thus, taking **A** as lead compound, we prepared and tested at ARs a set of 6- or 8-(hetero)arylalkylamino-4-amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives (compounds **1**–**20**, Chart 2) which are the object of this paper. We performed various structural modifications on the benzyl group of **A**. The first was introduction of different substituents at different positions on the phenyl moiety. Most of the substituents (F, OMe, OH, COOR) were chosen on the basis of their capability to engage hydrogen bonds since it was reported that in the **SCH series** the presence on the appended aryl moiety of a group possessing this feature often increased A_{2A} AR affinity and selectivity.[19,20] In addition, some of these groups (OMe, OH, COOH), due to their hydrophilic properties, may be able to improve the water solubility of compounds and to make the binding assays easier. The second structural modification was replacement of the phenyl ring of the benzyl chain with an heteroaryl ring such as furyl, thienyl or pyridil. This change was made because these heterocyclic substituents, isosters of the benzene ring, are able to act as hydrogen bond acceptors. Thus, they were expected to reinforce the anchoring to the A_{2A} receptor binding site. Moreover, the pyridine ring, possessing a hydrophilic character, could increase the water solubility of compounds. The third modification performed on the benzyl moiety of **A** was homologation of the alkyl spacer. This change was made in order to evaluate the importance of the distance between the 6-amino-triazoloquinoxalin-1-one framework and the lipophilic area constituted by the benzene ring. Finally, the benzylamino substituent of **A** was moved from the 6- to the 8-position.

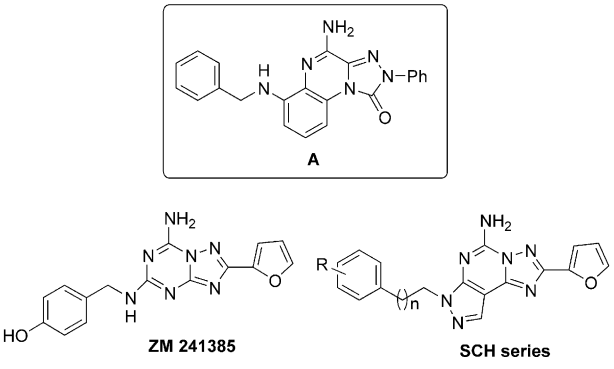


Chart 1. Previously reported A_{2A} AR selective antagonists.

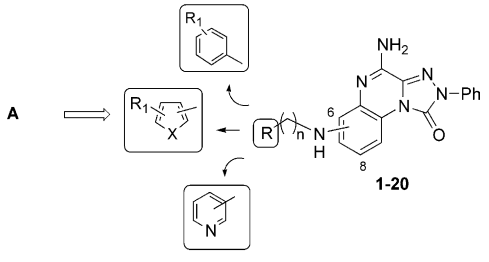
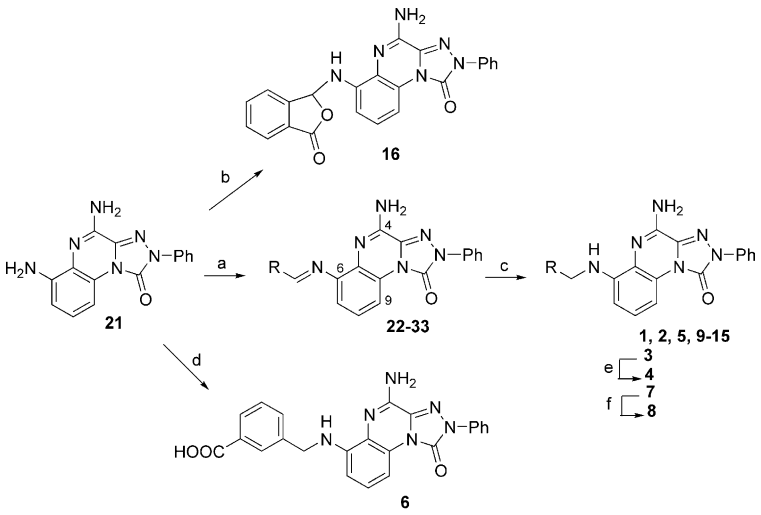


Chart 2. Presently reported 1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives.

Chemistry

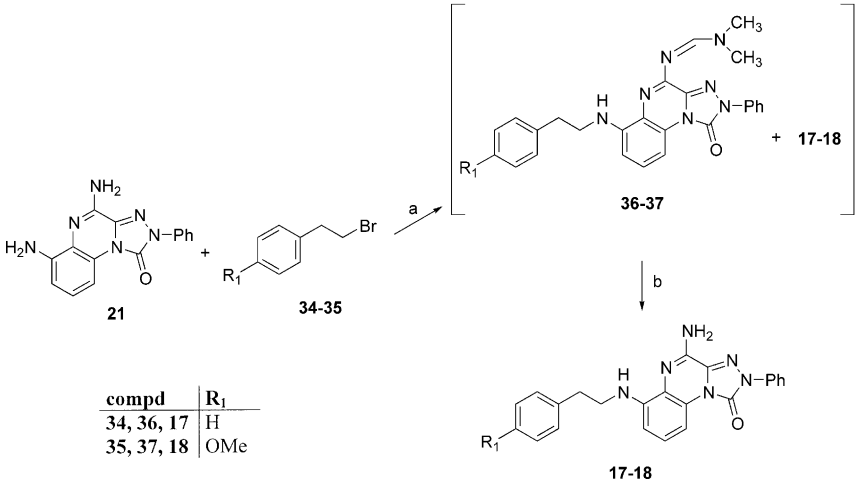
The target compounds **1**–**20** were prepared as depicted in Schemes 1–3. Scheme 1 shows the synthesis of the 6-[(hetero)arylmethyl]amino-substituted derivatives **1**–**15**. Reaction of the 4,6-diamino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one **21**[16,17] with suitable (hetero)aryl aldehydes produced the Schiff's bases **22**–**33**. The 6-[(hetero)arylmethylene]amino-structure of **22**–**33** was assigned by comparing the ^{1}H NMR spectra of **22**–**33** with that of the 4,6-diamino derivative **21**. The ^{1}H NMR spectrum of **21** shows two signals at 5.36 and 7.28 ppm, assigned to the 6-amino and the 4-amino group, respectively, this last substituent being more deshielded due to the electron-withdrawing effect of the N-5 atom. In the ^{1}H NMR spectra of compounds **22**–**33** the signal of the amino group appears at 7.2–7.6 ppm, thus this substituent is at the 4-position and, consequently, the [(hetero)arylmethylene]amino group is at the 6-position. The structure of **22**–**33** was also confirmed by the downfield shift of their H-9 signal (about 8.5 ppm), with respect to that of the 6-amino derivative **21** (7.88 ppm). This difference of chemical shift clearly indicates the transformation of the electron-donating 6-amino group of **21** into the electron-withdrawing 6-azomethine function of **22**–**33**. In fact, it is well established that the H-9 signal of the 1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives is the most deshielded aromatic proton (over 8.50 ppm) with the exceptions being those compounds containing the electron-donating 6- or 8-amino group (under 8.0 ppm).[15–17]

Reduction of **22**–**33** with sodium borohydride yielded the desired 6-[(hetero)arylmethyl]amino derivatives **1**–**3**, **5**, **7**, **9**–**15**. The ^{1}H NMR spectra of these compounds shows the H-9 signal under 8.0 ppm. The upfield shift of these signals, with respect to the H-9 signals of the corresponding Schiff's bases **22**–**33**, was due to the change of the electronic properties of the 6-substituent and it further confirmed the 6-position of the appended substituents. Moreover, the 6-substitued structure of **1**–**3**, **5**, **7**, **9**–**15** was consistent with the chemical shifts of the two amino group proton(s) which are easily identified for their different multiplicity and integral value. In fact, the NH signals appear as triplets ($J = 5.5$–6.2 Hz) at 5.8–6.2 ppm; those of NH_2 as broad singlets at about 7.3–7.4 ppm. Reaction of compound **21** with either the 2-formylbenzoic acid or the 3-formylbenzoic acid, performed in the experimental conditions followed to prepare **22**–**33**, did not afford the corresponding Schiff's bases. In fact, in the first case, the 6-(1,3-dihydro-3-oxo-isobenzofuran-1-yl)amino-substituted derivative **16** was obtained, while in the latter, the 3-formylbenzoate of the amine **21**, was isolated. The synthesis of the desired 6-(3-carboxybenzyl)amino derivative **6** was achieved by reductive amination of the 3-formylbenzoic acid with compound **21**, in the presence of sodium triacetoxyborohydride. The 6-arylmethyl-substituted structure of **6** was assigned on the basis of the chemical shifts of the two amino group proton(s). In fact, the NH signal appears as a triplet ($J = 5.9$ Hz) at 6.12 ppm while the NH_2 signal as a broad singlet at about 7.3 ppm. These significantly different chemical shifts, in accordance with



compd	R	compd	R
22, 1	C_6H_4-2F	27, 9	2-furyl
23, 2	C_6H_4-4F	28, 10	2-(5-methylfuryl)
24, 3	C_6H_4-4OMe	29, 11	3-furyl
4	C_6H_4-4OH	30, 12	2-thienyl
25, 5	C_6H_4-4Cl	31, 13	3-thienyl
26, 7	C_6H_4-4COOMe	32, 14	3-pyridil
8	C_6H_4-4COOH	33, 15	4-pyridil

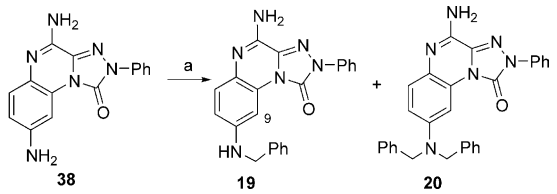
Scheme 1. (a) RCHO, $ZnCl_2$, THF; (b) 2-formylbenzoic acid, $ZnCl_2$, THF; (c) $NaBH_4$, MeOH; (d) 3-formylbenzoic acid, $ZnCl_2$, $NaB(O_2CCH_3)_3$, THF; (e) BBr_3, CH_2Cl_2; (f) NaOH, H_2O/MeOH.



compd	R_1
34, 36, 17	H
35, 37, 18	OMe

Scheme 2. (a) K_2CO_3, DMF; (b) H_2O, HCl.

the spectral data of compound **21** and of the 6-[(hetero)arylmethyl]amino- derivatives **1**–**3**, **5**, **7**, **9**–**15** (see above), indicate that the NH and NH_2 groups are, respectively, at the 6- and the 4-positions. The 6-(4-methoxybenzyl)amino-triazoloquinoxalin-1-one derivative **3** was demethylated to give the corresponding 6-(4-hydroxybenzyl)amino compound **4**. Alkaline hydrolysis of the methyl ester **7** gave the corresponding carboxylic acid **8**. The 6-(2-phenylethyl)amino derivative **17** and the 6-[2-(4-methoxyphenyl)ethyl]amino compound **18** were prepared as described in Scheme 2, that is by reacting the 4,6-diamino compound **21** with, respectively, 2-phenylethyl bromide **34** and 2-(4-methoxyphenyl)ethyl bromide **35**[21] in DMF at 70° C in the presence of potassium carbonate. These conditions afforded a mixture of **17** or **18** and the corresponding 4-(*N*,*N*-dimethylaminomethylene)amino derivatives **36** or **37**. These latter compounds were neither isolated nor characterized but were identified in the ^1H NMR spectra of the mixtures. By treatment of the crude mixture of **17** and **36** or **18** and **37** with aqueous hydrochloric acid the transformation of **36** and **37** into **17** and **18**, respectively, was achieved. The structure of both compounds **17** and **18** was assigned on the basis of the chemical shifts of the two amino group proton(s), similarly to compound **6** structure attribution (see the discussion reported above). In fact, the NH signal of **17** and **18** appears as a triplet ($J = 7.0$ Hz) at 5.6 ppm while that of



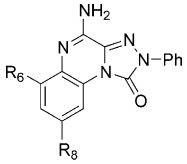
Scheme 3. (a) Benzyl bromide, K_2CO_3, DMF.

NH_2 as a broad singlet at about 7.3 ppm. These significantly different chemical shifts indicate that the NH and NH_2 groups are, respectively, at the 6- and the 4-positions. Finally, when the 4,8-diamino-triazoloquinoxalin-1-one derivative **38**[17] was reacted with benzyl bromide, both the 8-benzylamino derivative **19** and the 8-dibenzylamino compound **20** were obtained. It was not possible to assign the 8-benzylamino-substituted structure of **19** merely on the basis of the different chemical shifts of its two amino groups. In fact, the chemical shifts of the NH (about 6.6 ppm) and NH_2 (6.87 ppm) protons were too close to be unambiguously assigned to 4-amino and 8-amino groups, respectively. Thus, the structure of **19** was attributed by means of $^1H/^1H$ nuclear Overhauser enhancement (NOE) experiment. Preirradiation of the methylene protons (4.31 ppm) caused a significant enhancement of the H-9 proton signal (7.97 ppm) together with the two ortho benzyl (about 7.4 ppm) and NH (6.64 ppm) proton signals. The 8-dibenzylamino structure of compound **20** was assigned on the basis of both the structure of **19** and the chemical shift of the NH_2 signal (7.00 ppm) of **20**.

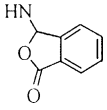
Biochemistry

Compounds **1–20** were tested for their ability to displace [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) from A_1 ARs in bovine cerebral cortical membranes, [^{3}H]2-{[4-(2-carboxyethyl)phenethyl]amino}-5′-(*N*-ethyl-carbamoyl)adenosine ([^{3}H]CGS 21680) from A_{2A} ARs in bovine striatal

Table 1. Binding activity at bovine A_1 and A_{2A} and human A_3 ARs



1-20

Compd	R_6	R_8	K_i (nM)[a] or I%		
			A_1[b]	A_{2A}[c]	A_3[d]
A[e]	$NHCH_2$–C_6H_5	H	730±75.1	6.5±0.7	30%
1	$NHCH_2$–C_6H_4–2F	H	93.9±8.8	152±16.4	31%
2	$NHCH_2$–C_6H_4–4F	H	37±3.9	74±6.9	750±76.8
3	$NHCH_2$–C_6H_4–4OMe	H	365±39.2	42.5±3.9	830±81.1
4	$NHCH_2$–C_6H_4–4OH	H	650±55.4	49.7±5.2	23.9%
5	$NHCH_2$–C_6H_4–4Cl	H	74%	229±23.5	44%
6	$NHCH_2$–C_6H_4–3COOH	H	92±7.8	15.2±1.6	817±79.6
7	$NHCH_2$–C_6H_4–4COOMe	H	627±59.4	74±6.6	52%
8	$NHCH_2$–C_6H_4–4COOH	H	94±8.3	22±1.9	41%
9	$NHCH_2$-2-furyl	H	189.4±22.4	8.66±0.9	6%
10	$NHCH_2$-2-(5-methylfuryl)	H	281±27.2	22±2.4	50%
11	$NHCH_2$-3-furyl	H	90±8.5	15±1.4	327±29.4
12	$NHCH_2$-2-thienyl	H	171±15.9	18.6±1.7	37%
13	$NHCH_2$-3-thienyl	H	259±16.2	10±1.9	31.5%
14	$NHCH_2$-3-pyridil	H	124±10.5	26±1.9	40.8%
15	$NHCH_2$-4-pyridil	H	3260±343	65.2±7.2	38.7%
16		H	16±1.5	31.5±2.9	25±2.2
17	$NH(CH_2)_2$–C_6H_5	H	116±10.5	151±13.9	36%
18	$NH(CH_2)_2$–C_6H_4–4OCH_3	H	204±19.6	42.3±5.1	0%
19	H	$NHCH_2$–C_6H_5	9.4±0.8	549±48.6	62±5.9
20	H	$N(CH_2$–$C_6H_5)_2$	56±4.8	2086±199	120±11.4
	Theophylline		3800±340	21,000±1800	86,000±7800
	DPCPX		0.5±0.03	337±28	1300±125

[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 from 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 receptors expressed in CHO cells or percentage of inhibition (I%) of specific binding at 1 μM concentration.
[e]Refs 15 and 16.

membranes and [^{125}I]N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine ([^{125}I]AB-MECA) from human cloned A_3 receptors stably expressed in CHO cells. In fact, due to the high species differences in the A_3 primary amino acid sequence,[22–24] we tested our A_3 AR ligands on cloned human A_3 receptors.

The binding results of **1–20** are shown in Table 1 together with those of the lead **A**. The binding data of theophylline and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), included as antagonist reference compounds, are also reported.

Results and Discussion

The binding results reported in Table 1 show that we have produced some potent bA_{2A} antagonists endowed with an A_{2A} affinity in the low nanomolar range. Nevertheless, none of the newly synthesized compounds **1–20** exceeded the A_{2A} affinity and A_{2A} versus A_1 selectivity of the parent compound **A** (K_i ratio $A_1/A_{2A} = 112$). Instead, most of compounds **1–20** are inactive or scarcely active at the hA_3 AR, similar to **A**. There are only three exceptions: the 6-(1,3-dihydro-3-oxo-isobenzofuran-1-yl)amino-substituted derivative **16**, the 8-benzylamino- derivative **19** and the 8-dibenzylamino- compound **20** which showed good A_3 AR affinity. All these data indicate that the presence of a 6-(hetero)aralkylamino moiety on the 4-amino-1,2,4-triazoloquinoxalin-1-one core is well tolerated by the bA_1 and bA_{2A} ARs while, in general, it is detrimental for anchoring to the hA_3 receptor. On the contrary, the presence of the 8-benzylamino- group (compound **19**) and the 8-dibenzylamino-substituent (compound **20**) significantly reduced the A_{2A} affinity while it is profitable for anchoring to the A_1 AR.

The first modification performed on **A** was introduction of various substituents on the phenyl ring of its 6-benzylamino group (compounds **1–8**). Some substituents, such as the *para*-hydroxy (compound **4**) and the *para*-carboxymethyl (compound **7**) afforded comparable A_1 affinity, with respect to **A**. All the others increased from 2- to 8-fold the A_1 affinity of **A**, while only the *para*-chloro substituent (compound **5**) significantly reduced the A_1 binding activity of the lead **A**. The A_{2A} affinities of compounds **1–8** were comparable or lower than that of **A**. Consequently, these derivatives were scarcely A_{2A} versus A_1 selective, suggesting that the A_1 and A_{2A} lipophilic areas which accommodate the 6-(hetero)-aralkylamino group possess similar requirements. The best substituents for A_{2A} receptor–ligand interaction were the hydrophilic ones, such as methoxy, hydroxy or carboxy group (compounds **3**, **4**, **6**, **8**). In particular, a free carboxylic group, at either the *meta*- or *para*- position (compounds **6** and **8**, respectively), produced high A_{2A} affinities, only slightly decreased with respect to that of the lead compound **A**. Nevertheless, although these groups are able to engage hydrogen bonds, their presence did not increase the A_{2A} affinity of **A**. A similar consideration applies to compound **16** which shows a 5-fold reduced A_{2A} affinity, with respect to **A**, although possessing a hydrogen bond acceptor, that is the carbonyl oxygen of the lattone moiety. These results could suggest that a hydrogen bonding interaction does not play an important role for anchoring of the 6-aralkylamino moiety of our triazoloquinoxaline derivatives to the A_{2A} receptor, differently from what reported for the **SCH series**.[19,20] Alternatively, we can suppose that the 6-appended moiety of these ligands interacts with a size-limited binding pocket and hence only the 6-benzylamino group would possess the right steric bulk to best fit the A_{2A} receptor subsite. The importance of the steric hindrance of the 6-substituent seems to be confirmed by the 3-fold reduced A_{2A} affinity of compound **7**, ensuing by replacement of the *para*-carboxylic function of **8** with the bulkier *para*-carboxymethyl group. Nevertheless, the difference in A_{2A} affinity of **8** and **7** could also be ascribed to the reduced hydrophilicity of the ester group with respect to the carboxylic group. The negative influence of steric hindrance and/or lipophilic properties of the substituent seems to be confirmed by the binding datum of compound **5**, bearing a *para*-chloro substituent, which possesses a 35-fold reduced A_{2A} binding activity, compared to **A**. The same applies to the *para*-fluoro substituted compound **2**, even though to a minor extent and probably due to either lower lipophilicity or steric hindrance of fluorine with respect to chlorine atom. The *ortho*-fluoro-substituted derivative **1** also exibits a significantly reduced A_{2A} affinity (23-fold), compared to **A**. This result could be explained by assuming that the *ortho*-fluorine atom stabilizes a bioinactive conformation of the molecule as it may engage an intramolecular hydrogen bond with the 6-amino group.

Better results in terms of A_{2A} affinity were obtained among the 6-(heteroarylmethyl)amino derivatives **9–15**. In fact, the 6-[(2-furylmethyl)]amino derivative **9**, the 6-[(3-furylmethyl)]amino derivative **11** and their corresponding thienyl derivatives **12** and **13** show comparable or only slightly reduced ($K_i = 9–19$ nM) A_{2A} affinity with respect to that of **A**. Compounds **9** and **13** also display some A_{2A} versus A_1 selectivity (21- and 25-fold, respectively). The above cited influence of the steric hindrance of the 6-appended moiety on the A_{2A} affinity was also observed among these heterocycle-substituted derivatives. In fact, the 6-[2-(5-methylfuryl)methyl]-amino-substituted derivative **10** exibits a 2.5-fold reduction of A_{2A} affinity with respect to the less bulky 6-[(2-furylmethyl)]amino- derivative **9**. The 6-(pyridylmethyl)amino-substituted derivatives **14** and **15** show, among the heterocycle-substituted derivatives **9–15**, the lowest A_{2A} AR affinities. Nevertheless, the 6-(4-pyridylmethyl)amino- derivative **15**, due to its very low A_1 affinity, possesses the highest A_{2A} versus A_1 selectivity (selectivity ratio = 50) among the herein reported antagonists.

Homologation of the alkyl chain of the lead compound **A** and derivative **3** (compounds **17** and **18**, respectively) elicited contrasting effects on A_{2A} affinity and selectivity. In fact, while the 6-phenethylamino- derivative **17** shows a reduced (23-fold) A_{2A} affinity and a loss of A_{2A} versus A_1 selectivity, compared to **A**, compound **18**

Table 2. Analytical data of the newly synthesized compounds

Compd	Formula	C Calcd–found	H Calcd–found	N Calcd–found
1	$C_{22}H_{17}FN_6O$	65.98–65.67	4.29–4.48	20.99–21.15
2	$C_{22}H_{17}FN_6O$	65.98–65.75	4.29–4.37	20.99–21.23
3	$C_{23}H_{20}N_6O_2$	66.97–66.78	4.90–4.85	20.38–20.25
4	$C_{22}H_{18}N_6O_2$	66.31–66.52	4.56–4.35	21.10–20.99
5	$C_{22}H_{17}ClN_6O$	63.38–63.59	4.12–4.34	20.16–20.01
6	$C_{23}H_{18}N_6O_3$	64.77–64.61	4.26–4.12	19.71–19.89
7	$C_{24}H_{20}N_6O_3$	65.43–65.32	4.59–4.41	19.08–19.22
8	$C_{23}H_{18}N_6O_3$	64.77–64.59	4.26–4.03	19.71–19.99
9	$C_{20}H_{16}N_6O_2$	64.50–64.69	4.34–4.52	22.57–22.34
10	$C_{21}H_{18}N_6O_2$	65.26–65.01	4.70–4.96	21.75–21.58
11	$C_{20}H_{16}N_6O_2$	64.50–64.41	4.34–4.20	22.57–22.89
12	$C_{20}H_{16}N_6OS$	61.83–61.69	4.16–4.34	21.64–21.81
13	$C_{20}H_{16}N_6OS$	61.83–61.63	4.16–4.01	21.64–21.49
14	$C_{21}H_{17}N_7O$	65.77–65.60	4.48–4.67	25.58–25.35
15	$C_{21}H_{17}N_7O$	65.77–65.53	4.48–4.30	25.58–25.75
16	$C_{23}H_{16}N_6O_3$	65.08–65.25	3.81–3.97	19.80–19.64
17	$C_{23}H_{20}N_6O$	69.67–69.48	5.09–5.27	21.20–21.46
18	$C_{24}H_{22}N_6O_2$	67.58–67.85	5.21–5.01	19.71–19.60
19	$C_{22}H_{18}N_6O$	69.08–69.27	4.75–4.96	21.98–21.74
20	$C_{29}H_{24}N_6O$	73.70–73.65	5.13–5.30	17.79–17.58

exibits similar A_{2A} affinity and selectivity with respect to **3**. These opposing data regarding A_{2A} affinity do not permit us to draw any conclusion about the influence of the distance between the 6-amino-triazoloquinoxaline framework and the lipophilic aryl ring for the anchoring to this receptor subtype.

Movement of the benzylamino chain of **A** from the 6- to the 8-position yielded compound **19** which showed a dropped A_{2A} and a significantly increased A_1 affinity. Consequently, **19** showed a reversed selectivity with respect to **A**. These data indicate that the presence of a bulky substituent at the 8-position of the 1,2,4-triazoloquinoxaline framework, while being well tolerated by the A_1 subtype, it is deleterious for anchoring to the A_{2A} AR. The detrimental effect of the steric hindrance of the 8-substituent is confirmed by the very low A_{2A} affinity ($K_i = 2086$ nM) of compound **20** bearing the bulky 8-dibenzylamino moiety.

To summarize, the binding data show that hydrophilic substituents on the benzyl moiety of the lead compound **A** are the most profitable for the A_{2A} AR affinity, and the best is the carboxy group. Nevertheless, although these substituents could engage hydrogen bonds, their presence, as stated above, do not reinforce the binding to the A_{2A} receptor. These results suggest that: (i) a hydrogen bonding interaction does not play a crucial role for anchoring of the 6-aralkylamino moiety of our triazoloquinoxaline derivatives to the bA_{2A} receptor, and (ii) the 6-appended moiety of these ligands may interact with a size-limited binding pocket. In fact, among the 6-aralkylamino-substituents (compounds **A**, **1**–**8**), the 6-benzylamino group of **A** seems to possess the best balance of hydrophobic and steric properties to permit a good fit with the receptor pocket. The existence of strict steric and lipophilic requirements can be confirmed by the high binding affinity of compounds **9**, **11**–**13** in which the phenyl ring of **A** was replaced by the less hindered bioisoster furyl or thienyl rings.

In conclusion, some of our modifications on the 6-benzylamino moiety of the lead compound **A** have maintained high nanomolar A_{2A} affinity, although reducing the A_{2A} versus A_1 selectivity. Moreover, the SAR study of compounds **1**–**20** gave us some useful insights about the structural requirements of the bA_2 AR subtype which may be taken into consideration in the design of new A_{2A} AR antagonists.

Experimental

Chemistry

Silica gel plates (Merck F_{254}) and silica gel 60 (Merck, 70–230 mesh) were used for analytical and column chromatography, respectively. 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 (Table 2). 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 that is always DMSO-d_6. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, ar = aromatic protons.

General procedure for the synthesis of 4-amino-6-[(hetero)arylmethylene]amino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-ones 22–33

A mixture of compound **21** (2.05 mmol),[16,17] the suitable aldehyde (2.46 mmol) and anhydrous zinc chloride (4.10 mmol) was refluxed in anhydrous tetrahydrofuran (30 mL), under nitrogen atmosphere, until the disappearance (TLC monitoring) of the starting material (4–8 h). The solid was filtered, washed with water and dried. The Schiff's bases **22**–**33**, obtained in high overall yields (70–95%), were instable upon recrystallization, nevertheless they were pure enough to be used without purification. Compounds **22**–**33** displayed the following ^1H NMR data.

4-Amino-1,2-dihydro-6-[(2-fluorophenyl)methylene]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (22). 7.08 (d, 1H, ar, $J = 6.4$ Hz), 7.28–7.47 (m, 4H, 2 ar + NH_2), 7.50–7.63 (m, 5H, ar), 8.16–8.20 (m, 3H, ar), 8.58 (d, 1H, H-9, $J = 6.7$ Hz), 8.76 (s, 1H, =CH).

4-Amino-1,2-dihydro-6-[(4-fluorophenyl)methylene]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (23). 7.00 (d, 1H, ar, $J = 7.1$ Hz), 7.20–7.59 (m, 8H, 6 ar + NH_2), 7.98–8.08 (m, 4H, ar), 8.51–8.57 (m, 2H, H-9 + =CH).

4-Amino-1,2-dihydro-6-[(4-methoxyphenyl)methylene]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (24). 3.85 (s, 3H, OMe), 6.98 (d, 1H, ar, $J = 7.7$ Hz), 7.09 (d, 2H, ar, $J = 8.1$ Hz), 7.22–7.39 (m, 2H, ar),

7.52–7.60 (m, 4H, 2 ar + NH_2), 7.91 (d, 2H, ar, J = 8.4 Hz), 8.07 (d, 2H, ar, J = 8.4 Hz), 8.48 (s, 1H, =CH), 8.52 (d, 1H, H-9, J = 8.4 Hz).

4-Amino - 1,2 - dihydro - 6 - [(4 - chlorophenyl)methylene] amino-2-phenyl-1,2,4-triazolo[4,3-*a*] quinoxalin-1-one (25). 7.04 (d, 1H, ar, J = 7.7 Hz), 7.24–7.39 (m, 2H, ar), 7.53–7.65 (m, 6H, 4 ar + NH_2), 7.99 (d, 2H, ar, J = 8.4 Hz), 8.08 (d, 2H, ar, J = 8.4 Hz), 8.55 (d, 1H, H-9, J = 8.1 Hz), 8.61 (s, 1H, =CH).

Methyl 4-[(4-amino-1,2-dihydro-1-oxo-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-6-yl)amino] methylene benzoate (26). 3.89 (s, 3H, CH_3), 7.05 (d, 1H, ar, J = 7.9 Hz), 7.24–7.38 (m, 2H, ar), 7.51–7.59 (m, 4H, 2 ar + NH_2), 8.04–8.10 (m, 6H, ar), 8.55 (d, 1H, H-9, J = 8.1 Hz), 8.59 (s, 1H, =CH).

4-Amino-1,2-dihydro-6-(2-furylmethylene)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (27). 6.79–6.80 (m, 1H, ar), 7.01–7.12 (m, 1H, ar), 7.20–7.49 (m, 4H, 2 ar + NH_2), 7.52–7.63 (m, 3H, ar), 8.02–8.12 (m, 3H, ar), 8.40–8.55 (m, 1H, =CH), 8.55 (d, 1H, H-9, J = 8.2 Hz).

4-Amino-1,2-dihydro-6-[2-(5-methylfuryl)methylene]amino - 2 - phenyl - 1,2,4 - triazolo[4,3-*a*]quinoxalin-1-one (28). 2.42 (s, 3H, CH_3), 6.39 (br s, 1H, furane proton), 6.92–7.18 (m, 2H, ar), 7.20–7.40 (m, 2H, ar), 7.42–7.60 (m, 4H, 2 ar + NH_2), 8.05 (d, 2H, ar, J = 8.4 Hz), 8.25 (br s, 1H, =CH), 8.51 (d, 1H, H-9, J = 6.3 Hz).

4-Amino-1,2-dihydro-6-(3-furylmethylene)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (29). 6.96–7.01 (m, 2H, ar), 7.20–7.40 (m, 2H, ar), 7.48–7.60 (m, 4H, 2 ar + NH_2), 7.83 (br s, 1H, furane proton), 8.06 (d, 2H, ar, J = 8.4 Hz), 8.31 (br s, 1H, furane proton), 8.48–8.56 (m, 2H, H-9 + =CH).

4-Amino - 1,2 - dihydro - 6 - (2 - thienylmethylene)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (30). 7.06 (d, 1H, ar, J = 7.0 Hz), 7.22–7.43 (m, 4H, 2 ar + NH_2), 7.50–7.91 (m, 5H, ar), 8.09 (d, 2H, ar, J = 8.2 Hz), 8.55 (d, 1H, H-9, J = 8.1 Hz), 8.78 (s, 1H, =CH).

4-Amino - 1,2 - dihydro - 6 - (3 - thienylmethylene)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (31). 6.98 (d, 1H, ar, J = 7.2 Hz), 7.20–7.40 (m, 2H, ar), 7.42–7.68 (m, 6H, 4 ar + NH_2), 8.05 (d, 2H, ar, J = 8.1 Hz), 8.18–8.20 (m, 1H, ar), 8.48–8.58 (m, 2H, H-9 + =CH).

4-Amino - 1,2 - dihydro - 6 - (3 - pyridylmethylene)amino - 2 - phenyl - 1,2,4 - triazolo[4,3-*a*]quinoxalin-1-one (32). 7.07 (d, 1H, ar, J = 6.6 Hz), 7.24–7.38 (m, 2H, ar), 7.45–7.62 (m, 5H, 3 ar + NH_2), 8.05 (d, 2H, ar, J = 8.4 Hz), 8.35 (d, 1H, ar, J = 6.6 Hz), 8.55 (d, 1H, H-9, J = 8.4 Hz), 8.67–8.76 (m, 2H, ar + =CH), 9.07 (s, 1H, pyridine proton).

4-Amino - 1,2 - dihydro - 6 - (4 - pyridylmethylene)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (33). 7.08 (d, 1H, ar, J = 7.1 Hz), 7.22–7.28 (m, 2H, ar), 7.51–7.55 (m, 4H, 2 ar + NH_2), 7.89 (d, 2H, pyridine proton, J = 5.9 Hz), 8.05 (d, 2H, ar, J = 8.1 Hz), 8.55 (d, 1H, H-9, J = 8.4 Hz), 8.67 (s, 1H, =CH), 8.76 (d, 2H, pyridine proton, J = 5.9 Hz).

General procedure for the synthesis of 4-amino-6-[(hetero) arylmethyl]amino - 1,2 - dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-ones 1–3, 5, 7, 9–16

Sodium borohydride (4.6 mmol) was added portionwise, over 10 min, to a boiling suspension of compounds **22–33** (2.3 mmol) in anhydrous methanol (20 mL), under nitrogen atmosphere. The mixture was refluxed until the disappearance (TLC monitoring) of the starting material (2–3 h), cooled at room temperature and then quenched with ice water (15 mL). The solid was collected, washed with water and recrystallized.

4-Amino-1,2-dihydro-6-[(2-fluorophenyl)methyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (1). Yield 70%; mp 228–229 °C (CH_3CN); ^{1}H NMR 4.52 (d, 2H, CH_2, J = 6.2 Hz), 6.02 (t, 1H, NH, J = 6.2 Hz), 6.58 (d, 1H, J = 8.0 Hz), 7.02–7.48 (m, 8H, 6 ar + NH_2), 7.57 (t, 2H, ar, J = 7.4 Hz), 7.93 (d, 1H, ar, J = 8.2 Hz), 8.08 (d, 2H, ar, J = 8.5 Hz); IR 1710, 3315, 3405, 3495. Anal. ($C_{22}H_{17}FN_6O$) C, H, N.

4-Amino - 1,2 - dihydro-6-[(4-fluorophenyl)methyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (2). Yield 74%; mp 204–205 °C (DMF); ^{1}H NMR 4.42 (d, 2H, CH_2, J = 5.9 Hz), 6.12 (t, 1H, NH, J = 5.8 Hz), 6.56 (d, 1H, ar, J = 7.3 Hz), 6.98–7.19 (m, 3H, ar), 7.23–7.59 (m, 7H, 5 ar + NH_2), 7.89 (d, 1H, ar, J = 8.8 Hz), 8.1 (d, 2H, ar, J = 8.1 Hz); IR 1709, 3338, 3360, 3417, 3463. Anal. ($C_{22}H_{17}FN_6O$) C, H, N.

4-Amino-1,2-dihydro-6-[(4-methoxyphenyl)methyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (3). Yield 85%; mp 210–212 °C (EtOH); ^{1}H NMR 3.74 (s, 3H, OCH_3), 4.35 (d, 2H, CH_2, J = 5.9 Hz), 5.89 (t, 1H, NH, J = 5.9 Hz), 6.57 (d, 1H, ar, J = 8.1 Hz), 6.92 (d, 2H, ar, J = 8.7 Hz), 7.05 (t, 1H, ar, J = 8.4 Hz), 7.29–7.36 (m, 5H, 3 ar + NH_2), 7.56 (t, 2H, ar, J = 7.3 Hz), 7.90 (d, 1H, H-9, J = 7.3 Hz), 8.06 (d, 2H, ar, J = 8.1 Hz); IR 1670, 3290, 3400. Anal. ($C_{23}H_{20}N_6O_2$) C, H, N.

4-Amino-1,2-dihydro-6-[(4-chlorophenyl)methyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (5). Yield 80%; mp 235–237 °C (EtOAc); ^{1}H NMR 4.46 (d, 2H, CH_2, J = 6.2 Hz), 6.09 (t, 1H, NH, J = 6.2 Hz), 6.48 (d, 1H, ar, J = 8.1 Hz), 7.02 (d, 1H, ar, J = 8.4 Hz), 7.31–7.40 (m, 7H, 5 ar + NH_2), 7.56 (t, 2H, ar, J = 7.7 Hz), 7.90 (d, 1H, H-9, J = 8.1 Hz), 8.07 (d, 2H, ar, J = 8.4 Hz); IR 1715, 3330, 3410, 3460. Anal. ($C_{22}H_{17}ClN_6O$) C, H, N.

Methyl 4-[(4-amino-1,2-dihydro-1-oxo-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-6-yl)amino]methyl benzoate (7). Yield 65%; mp 208–210 °C (CH_3CN); ^{1}H NMR 3.81 (s, 3H, CH_3), 4.53 (d, 2H, CH_2, J = 6.2 Hz), 6.18 (t, 1H, NH, J = 6.2 Hz), 6.41 (d, 1H, ar, J = 8.1 Hz), 6.98 (t, 1H, ar, 8.7 Hz), 7.36–7.57 (m, 7H, 5 ar + NH_2), 7.82–7.88 (m, 3H, ar), 8.05 (d, 2H, ar, J = 7.7 Hz); IR 1627, 1714, 3300, 3348, 3443. Anal. ($C_{24}H_{20}N_6O_3$) C, H, N.

4-Amino - 1,2 - dihydro - 6 - (2 - furylmethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (9). Yield 80%; mp 209–211 °C (CH_3CN); ^{1}H NMR 4.46 (d, 2H, CH_2,

$J = 5.9$ Hz), 5.90 (t, 1H, NH, $J = 5.9$ Hz), 6.31–6.42 (m, 2H, ar), 6.73 (d, 1H, ar, $J = 8.1$ Hz), 7.11 (t, 1H, ar, $J = 8.2$ Hz), 7.30–7.50 (m, 3H, 1 ar + NH_2), 7.51–7.78 (m, 3H, ar,), 7.95 (d, 1H, ar, $J = 8.2$ Hz), 8.09 (d, 2H, ar, $J = 8.1$ Hz); IR 1705, 3320, 3400, 3500. Anal. ($C_{20}H_{16}N_6O_2$) C, H, N.

4-Amino-1,2-dihydro-6-[2-(5-methylfuryl)methyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (10). Yield 76%; mp 190–192 °C (Isopropyl alcohol); ^{1}H NMR 2.22 (s, 3H, CH_3), 4.35 (d, 2H, CH_2, $J = 5.9$ Hz), 5.80 (t, 1H, NH, $J = 5.9$ Hz), 5.90–6.01 (m, 1H, furane proton), 6.19–6.20 (m, 1H, furane proton), 6.67 (d, 1H, ar, $J = 7.3$ Hz), 7.07 (t, 1H, ar, $J = 8.4$ Hz), 7.33–7.58 (m, 5H, 3 ar + NH_2), 7.90 (d, 1H, ar, $J = 7.3$ Hz), 8.04 (d, 2H, ar, $J = 7.7$ Hz); IR 1718, 3300, 3436, 3475. Anal. ($C_{21}H_{18}N_6O_2$) C, H, N.

4-Amino-1,2-dihydro-6-(3-furylmethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (11). Yield 78%; mp 208–210 °C (DMF); ^{1}H NMR 4.22 (d, 2H, CH_2, $J = 5.5$ Hz), 5.49 (t, 1H, NH, $J = 5.5$ Hz), 6.51 (s, 1H, furane proton), 6.65 (d, 1H, ar, $J = 7.7$ Hz), 7.06 (t, 1H, ar, $J = 7.7$ Hz), 7.25–7.36 (m, 3H, 2 ar + NH_2), 7.53 (t, 2H, ar, $J = 8.1$ Hz), 7.64 (d, 2H, ar, $J = 8.8$ Hz), 7.89 (d, 1H, ar, $J = 7.7$ Hz), 8.05 (d, 2H, ar, $J = 7.7$ Hz); IR 1705, 3186, 3321, 3393, 3446. Anal. ($C_{20}H_{16}N_6O_2$) C, H, N.

4-Amino-1,2-dihydro-6-(2-thienylmethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (12). Yield 72%; mp 208–209 °C (CH_3CN); ^{1}H NMR 4.61 (d, 2H, CH_2, $J = 6.2$ Hz), 6.01 (t, 1H, NH, $J = 6.2$ Hz), 6.66 (d, 1H, ar, $J = 7.7$ Hz), 6.90–7.10 (m, 3H, ar), 7.30–7.40 (m, 4H, 2 ar + NH_2), 7.55 (t, 2H, ar, $J = 7.7$ Hz), 7.92 (d, 1H, ar, $J = 7.3$ Hz), 8.06 (d, 2H, ar, $J = 7.6$ Hz); IR 1720, 3375, 3470. Anal. ($C_{20}H_{16}N_6SO$) C, H, N.

4-Amino-1,2-dihydro-6-(3-thienylmethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (13). Yield 70%; mp 211–212 °C (DMF); ^{1}H NMR 4.40 (d, 2H, CH_2, $J = 5.8$ Hz), 5.85 (t, 1H, NH, $J = 5.8$ Hz), 6.62 (d, 1H, ar, $J = 8.4$ Hz), 7.04–7.18 (m, 2H, ar), 7.29–7.41 (m, 4H, 2 ar + NH_2), 7.49–7.58 (m, 3H, ar), 7.90 (d, 1H, ar, $J = 8.4$ Hz), 8.05 (d, 2H, ar, $J = 8.4$ Hz); IR 1710, 3357, 3460. Anal. ($C_{20}H_{16}N_6SO$) C, H, N.

4-Amino-1,2-dihydro-6-(3-pyridylmethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (14). Yield 75%; mp 259–260 °C (2-Methoxyethanol); ^{1}H NMR 4.49 (d, 2H, CH_2, $J = 6.2$ Hz), 6.12 (t, 1H, NH, $J = 6.2$ Hz), 6.54 (d, 1H, ar, $J = 8.1$ Hz), 7.02 (t, 1H, ar, $J = 8.1$ Hz), 7.37–7.66 (m, 4H, 2 ar + NH_2), 7.53 (t, 2H, ar, $J = 7.3$ Hz), 7.75 (d, 1H, ar, $J = 7.7$ Hz), 7.89 (d, 1H, ar, $J = 8.1$ Hz), 8.05 (d, 2H, ar, $J = 8.4$ Hz), 8.45 (d, 1H, pyridine proton, $J = 4.7$ Hz), 8.59 (s, 1H, pyridine proton); IR 1702, 3381. Anal. ($C_{21}H_{17}N_7O$) C, H, N.

4-Amino-1,2-dihydro-6-(4-pyridylmethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (15). Yield 72%; mp 279–280 °C (DMF); ^{1}H NMR 4.51 (d, 2H, CH_2, $J = 6.2$ Hz), 6.24 (t, 1H, NH, $J = 6.2$ Hz), 6.38 (d, 1H, ar, $J = 8.1$ Hz), 6.99 (t, 1H, ar, $J = 8.1$ Hz), 7.31–7.37 (m, 5H, 3 ar + NH_2), 7.54 (t, 2H, ar, $J = 7.3$ Hz), 7.88 (d, 1H, ar, $J = 8.4$ Hz), 8.05 (d, 2H, ar, $J = 8.4$ Hz), 8.42 (d, 2H, pyridine protons); IR 1711, 3320, 3390, 3457. Anal. ($C_{21}H_{17}N_7O$) C, H, N.

Synthesis of 3-[(4-amino-1,2-dihydro-1-oxo-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-6-yl)amino]methylbenzoic acid (6). A mixture of compound **21** (0.68 mmol), 3-formylbenzoic acid (0.82 mmol), anhydrous zinc chloride (1.36 mmol) and sodiumtriacetoxyborohydride (3.06 mmol) in anhydrous tetrahydrofuran (20 mL) and glacial acetic acid (0.05 mL) was refluxed under nitrogen atmosphere for 6 h. After cooling at room temperature, the suspension was diluted with water and acidified with glacial acetic acid. The solid was collected by filtration, washed with water and then ethanol. Yield 95%; mp 253–255 °C (EtOH/AcOH); ^{1}H NMR 4.49 (d, 2H, CH_2, $J = 5.9$ Hz), 6.12 (t, 1H, NH, $J = 5.9$ Hz), 6.50 (d, 1H, ar, $J = 9.1$ Hz), 7.00 (t, 1H, ar, $J = 7.7$ Hz), 7.23–7.57 (m, 7H, 5 ar + NH_2), 7.74–8.06 (m, 5H, ar), 12.50 (br s, 1H, OH); IR 1690, 1727, 3396, 3506. Anal. ($C_{23}H_{18}N_6O_3$) C, H, N.

Synthesis of 4-amino-1,2-dihydro-6-(1,3-dihydro-3-oxo-isobenzofuran-1-yl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (16). The title compound was obtained by reacting compound **21**[16,17] (2.05 mmol) with 2-formyl benzoic acid (2.46 mmol), following the procedure above described to prepare Schiff's bases **22**–**33**. Yield 87%; mp 282–284 °C (2-Methoxyethanol); ^{1}H NMR 6.59 (d, 1H, NH, $J = 11.3$ Hz), 7.19–7.58 (m, 8H, 5 ar + CH + NH_2), 7.73–7.93 (m, 4H, ar), 8.04 (d, 2H, ar, $J = 7.7$ Hz), 8.16 (d, 1H, ar, $J = 7.5$ Hz); IR 1736, 1754, 3367, 3489. Anal. ($C_{23}H_{16}N_6O_3$) C, H, N.

Synthesis of 4-amino-1,2-dihydro-6-[(4-hydroxyphenyl)-methyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (4). 1 M solution of BBr_3 in dichloromethane (2.06 mL) was slowly added at 0 °C, under nitrogen atmosphere, to a suspension of compound **3** (1.02 mmol) in anhydrous dichloromethane (20 mL). The mixture was stirred at 0 °C for 2 h and then at room temperature for 24 h. The mixture was diluted with water (10 mL) and neutralized with a saturated solution of sodium bicarbonate. The solid, which was filtered, washed with water and dried, was a mixture of compound **4** and **21** (ratio about 2:1 from ^{1}H NMR spectrum). The crude solid was chromatographed on silica gel column, eluting system cyclohexane/ethyl acetate 1:1. Evaporation of the first and the second eluates afforded compound **4** and **21**, respectively. **4**: Yield 50%; mp 141–143 °C (EtOAc); ^{1}H NMR 4.28 (d, 2H, CH_2, $J = 6.0$ Hz), 5.78 (t, 1H, NH, $J = 6.0$ Hz), 6.58 (d, 1H, ar, $J = 8.1$ Hz), 6.74 (d, 2H, ar, $J = 8.4$ Hz), 7.06 (t, 1H, ar, $J = 8.1$ Hz), 7.20 (d, 2H, ar, $J = 8.4$ Hz), 7.36–7.40 (m, 3H, 2 ar + NH_2), 7.56 (t, 2H, ar, $J = 8.1$ Hz), 7.90 (d, 1H, ar, $J = 8.1$ Hz), 8.06 (d, 2H, ar, $J = 7.7$ Hz), 9.35 (s, 1H, OH); IR 1690, 3340, 3400, 3480. Anal. ($C_{22}H_{18}N_6O_2$) C, H, N.

Synthesis of 4-[(4-amino-1,2-dihydro-1-oxo-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-6-yl)amino]methylbenzoic acid (8). A solution of NaOH (1.8 M, 5 mL) was added to a suspension of the methyl ester **7** (0.76 mmol) in

methanol (10 mL). The mixture was refluxed for 3 h. After cooling at room temperature, the suspension was acidified to pH = 6 with HCl 6 N. The solid was filtered and recrystallized. Yield 80%; mp 284–286 °C (EtOH/AcOH); ^{1}H NMR 4.54 (d, 2H, CH_2, J = 6.1 Hz), 6.15 (t, 1H, NH, J = 6.1 Hz), 6.44 (d, 1H, ar, J = 8.4 Hz), 6.99 (t, 1H, ar, J = 8.4 Hz), 7.35–7.57 (m, 7H, ar, 5 ar + NH_2), 7.89–8.04 (m, 5H, ar), 12.8 (br s, 1H, OH); IR 1698, 1723, 3295, 3426. Anal. ($C_{23}H_{18}N_6O_3$) C, H, N.

Synthesis of 4-amino-1,2-dihydro-6-(2-phenylethyl)amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (17) and 4-amino-1,2-dihydro-6-[2-(4-methoxyphenyl)ethyl]amino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (18). 2-Phenylethyl bromide or 2-(4-methoxyphenyl)ethyl bromide[21] (2.65 mmol) was added to a suspension of compound **21**[16,17] (1.03 mmol) and potassium carbonate (2.06 mmol) in anhydrous DMF (6 mL). The mixture was stirred at 70° C for 3 h, then cooled at room temperature, diluted with water (20 mL) and extracted with ethyl acetate (15 mL × 3). The organic layers were evaporated and the residue, made up of a mixture of **17** and **36** or **18** and **37**, was diluted with water (20 mL) and HCl 6 N (3–4 mL) and refluxed for 3 h. After cooling at room temperature, the solution was neutralized with NaOH 10% and extracted with ethyl acetate (10 mL × 3). The organic layers were dried (Na_2SO_4), evaporated and the residue recrystallized.

17. Yield 41%; mp 208–209 °C (EtOAc); ^{1}H NMR 2.88 (t, 2H, CH_2, J = 7.1 Hz), 3.25–3.41 (m, 2H, CH_2), 5.63 (t, 1H, NH, J = 7.1 Hz), 6.61 (d, 1H, ar, J = 7.7 Hz), 7.09–7.37 (m, 9H, 7 ar + NH_2), 7.53 (t, 2H, ar, J = 7.7 Hz), 7.85 (d, 1H, ar, J = 7.6 Hz), 8.01 (d, 2H, ar, J = 7.7 Hz); IR 1720, 3400, 3500. Anal. ($C_{23}H_{20}N_6O$) C, H, N.

18. Yield 46%; mp 207–208 °C (EtOAc/cyclohexane); ^{1}H NMR 2.87 (t, 2H, CH_2, J = 7.3 Hz), 3.25–3.38 (m, 2H, CH_2), 3.72 (s, 3H, OCH_3), 5.60 (t, 1H, NH, J = 7.3 Hz), 6.61 (d, 1H, ar, J = 8.1 Hz), 6.88 (d, 2H, ar, J = 8.4 Hz), 7.10–7.24 (m, 3H, ar), 7.26–7.38 (m, 3H, 1 ar + NH_2), 7.55 (t, 2H, ar, J = 7.7 Hz), 7.90 (d, 1H ar, J = 7.7 Hz), 8.06 (d, 2H, ar, J = 7.7 Hz); IR 1730, 3360, 3430, 3480. Anal. ($C_{24}H_{22}N_6O_2$) C, H, N.

Synthesis of 4-amino-1,2-dihydro-8-benzylamino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (19) and 4-amino-1,2-dihydro-8-dibenzylamino-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one (20). Benzyl bromide (1.6 mmol) was added to a suspension of compound **38**[17] (1.6 mmol) and potassium carbonate (2.4 mmol) in anhydrous DMF (0.6 mL). The mixture was stirred at room temperature for 6 days, then it was diluted with water (10 mL). The solid, made up of the 8-dibenzylamino derivative **20**, was filtered. Further dilution with water (10 mL) of the clear solution yielded a solid which was filtered and washed with water and then ethanol. This crude product was a mixture of compound **19** and the starting product **38**, which were separated on silica gel column, eluting system cyclohexane/ethyl acetate, 3:7. Evaporation of the first and the second eluates afforded compounds **19** and **38**, respectively.

19. Yield 30%; mp 237–239 °C (DMF/MeOH); ^{1}H NMR 4.31 (d, 2H, CH_2, J = 5.5 Hz), 6.60–6.65 (m, 2H, 1 ar + NH), 6.87 (br s, 2H, NH_2), 7.14–7.41 (m, 7H, ar), 7.54 (t, 2H, ar, J = 7.7 Hz), 7.97 (d, 1H, H-9, J = 2.6 Hz), 8.04 (d, 2H, ar, J = 7.7 Hz); IR 1730, 3300, 3490. Anal. ($C_{22}H_{18}N_6O$) C, H, N.

20. Yield 51%; mp 232–234 °C (CH_3NO_2); ^{1}H NMR 4.76 (s, 4H, 2 CH_2), 6.70 (dd, 1H, ar, J = 7.9, 2.9 Hz), 7.00 (s, 2H, NH_2), 7.18–7.37 (m, 12H, ar), 7.54 (t, 2H, ar, J = 7.7 Hz), 8.01 (d, 2H, ar, J = 7.7 Hz), 8.17 (d, 1H, H-9, J = 2.6 Hz). IR 1680, 3200, 3385. Anal. ($C_{29}H_{24}N_6O$) C, H, N.

Biochemistry

Bovine A_1 and A_{2A} receptor binding. Displacement of [^{3}H]CHA from A_1 ARs in bovine cerebral cortical membranes and [^{3}H]CGS 21680 from A_{2A} ARs in bovine striatal membranes was performed as described in ref 25.

Human A_3 receptor binding. Displacement of [^{125}I]AB-MECA from hA_3 ARs stably expressed in CHO cells was performed as previously described.[14]

The concentration of the tested compounds 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 non-linear regression method implemented in the InPlot program (Graph-Pad, San Diego, CA, USA) with five concentrations of displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the Cheng–Prusoff equation.[26] The dissociation constant (K_d) of [^{3}H]CHA and [^{3}H]CGS 21680 in cortical and striatal bovine brain membranes were 1.2 and 14 nM, respectively. The K_d value of [^{125}I]AB-MECA in hA_3 ARs in CHO cell membranes was 1.4 nM.

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References and Notes

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