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Original article

N^6-Cycloalkyl-2-phenyl-3-deaza-8-azaadenines: a new class of A_1 adenosine receptor ligands. A comparison with the corresponding adenines and 8-azaadenines

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Abstract

Several 9-benzyl-N^6-cycloalkyl-2-phenyladenines, 9-benzyl-N^6-cycloalkyl-2-phenyl-8-azaadenines and 4-cycloalkylamino-1-benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridines were prepared and assayed as A_1 adenosine receptor ligands. The 1H-1,2,3-triazolo[4,5-c]pyridines were obtained starting from *N*,*N*-diethyl-1-benzyl-4-carboxyamido-5-methyl-1H-1,2,3-triazole by lithiation in anhydrous tetrahydrofurane in the presence of benzonitrile. The usual work up afforded the isolation of 1-benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridin-4-one which was treated with phosphorous oxychloride and cycloalkylamines. Some compounds showed high affinity and selectivity and the trend of K_i values corresponds to the series of 9-benzyl-N^6-cycloalkyl-2-phenyladenines and 9-benzyl-N^6-cycloalkyl-2-phenyl-8-azaadenines, therefore they can be considered bioisosteres. The affinity data permitted us to ascertain the role and the importance of the N(3) in the adenine or 8-azaadenine moiety in the receptor binding and to study the dimension of the receptor lipophilic pocket which is filled by the N^6 substituent of adenosine derivatives.

Keywords: A_1 Adenosine receptor ligands; 4-Cycloalkylamino-1-benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridines; 9-Benzyl-N^6-cycloalkyl-2-phenyladenines; 9-Benzyl-N^6-cycloalkyl-2-phenyl-8-azaadenines; Bioisosterism

1. Introduction

Adenosine is an endogenous nucleoside which mediates a variety of important physiological effects by its specific receptors: A_1 receptors were the first subtype to be identified. They are widely distributed in the central nervous system and in peripheral tissues and mediate diverse biological effects. The role of adenosine as a neuroprotective agent during hypoxia and ischemic conditions seems to be mediated by the A_1 receptors; these receptors have been involved in sedative, anti-seizure and anxiolytic effects. A_1 receptors mediate cardiac depression through negative chronotropic, dromotropic and inotropic effects. In the kidney, activation of A_1 receptors causes vasocostriction, inhibition of renin secretion, diuresis, natriuresis and other effects [1]. A great number of physiological effects have been demonstrated for A_1 receptors. For example, hundreds of agonists and antagonists have been assayed to obtain some compounds useful in therapy. A number of A_1 antagonists have been studied as novel potassium sparing diuretics with kidney-protection properties, in dementia, Alzheimer's disease, cardiac therapy and kidney disease [2].

In the course of our studies directed toward synthesis of A_1 adenosine receptor antagonists, we had occasion to prepare and test a number of 8-azaadenines and adenines having three lipophilic substituents in the positions 2, 9 and N^6 [3]. Initially, we synthesised 2-, N^6- and 9-substituted-8-azaadenines by a facile two-stage method [4]. This procedure afforded a large number of compounds with good affinity towards A_1 adenosine receptors. These had high selectivity A_1/A_2

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and some were very effective having a K_i in the nanomolar range. The most active compounds were 9-benzyl-2-phenyl-8-azaadenines N^6-cyclopentyl and N^6-cyclohexyl substituted (general formula **I**, Fig. 1), having K_i 11 [3] and 1.6 nM [5], respectively. Thus, we have completed the series, by synthesising all the N^6-cycloalkyl derivatives from three to eight carbons. We also prepared the corresponding adenine derivatives (general formula **II**, Fig. 1) in order to compare their affinity with the analogous 8-azaadenines (**I**), and to confirm the bioisosterism of the two nuclei, which we demonstrated recently [6].

In an effort to further explore other structural modifications of the purine nucleus, we sought to verify the importance of N(3) in the binding of ligands with A_1 adenosine receptors. In the past, some studies regarding the relative importance of nuclear nitrogen atoms in several deaza analogs of adenosine were synthesised [7]. More recently, *N*-cycloalkyl derivatives of adenosine and 1-deazaadenosine, were compared regarding the importance both of N-1 atom and of the dimension of the cycloalkyl inserted on N^6 [8]. Similar analogous studies on adenine derivatives have not been reported in the literature.

First we planned to prepare 9-benzyl-2-phenyl-3-deaza-8-azaadenines (general formula **III**, Fig. 1) and 9-benzyl-2-phenyl-3-deazaadenines (general formula **IV**, Fig. 1) by cyclisation of 3,4-diamino-2-chloro-6-phenylpyridine by classical methods, but this first idea was rejected because these compounds are not easy to obtain by this synthetic pathway. In fact in the literature the preparation of N^4-substituted-2-chloro-3,4-diaminopyridine [9] or 2-hydroxy-3,4-diamino-pyridine [10] is reported, but their 6-phenylsubstituted analogs are not. So we studied a new synthetic route which permitted us to prepare, starting from 1-benzyl-5-methyl-1H-1,2,3-triazole-4-carboxylic acid, 4-cycloalkyl derivatives of 7-phenyl-1-benzyl-1H-1,2,3-triazolo[4,5-c]pyridine (2-phenyl-9-benzyl-3-deaza-8-azaadenines) which, up to now, have not been reported. Comparing the capability of these compounds to bind A_1 adenosine receptors with the corresponding adenine and 8-azaadenine derivatives, we were able to study the importance of the purine N(3) atom in the binding with A_1 receptors. Synthesis of the three series of compounds (2-phenyl-9-benzyl-N^6-cycloalkyl substituted adenines, 8-azadenines and 3-deaza-8-azaadenines) was also useful to explore the steric tolerance of the A_1 receptor lipophilic pocket in front of N^6 of adenosine which is able to hold a cyclopentyl or a cyclohexyl moiety in potent agonists (as cyclohexyladenosine or cyclopentyladenosine) or antagonists as N^6-cyclohexyl- or cyclopentyl-2-phenyl-9-benzyl-8-azaadenines.

2. Chemistry

The 9-benzyl-2-phenyladenine (**4**) and its N^6-cycloalkyl derivatives (**5**–**11**) were obtained from 9-benzyl-6-chloro-2-phenylpurine (**3**) according to described procedures [6] (Fig. 2); also the 9-benzyl-N^6-cycloalkyl-2-phenyl-8-azaadenines (**14**–**18**) were prepared as previously reported [11] (Fig. 3).

Compounds **27**–**34** were obtained by a new method (Fig. 4). In fact, up to now, 1,2,3-triazolo[4,5-c]pyridines have been prepared from the corresponding 3,4-diaminopyridines by diazotation reaction [9,12]. In this paper we present a synthetic route starting from 1-benzyl-5-methyl-1H-1,2,3-triazole-4-carboxylic acid ethyl ester (**19**), obtained as described previously in the literature [14] by regiospecific and base-catalysed cycloaddition of benzylazide and ethyl acetoacetate under mild conditions; **19** was hydrolysed with sodium hydroxide to obtain the corresponding acid (compound **20**) [15] which, in turn, was converted into chloride **21** by treatment with thionyl chloride at reflux: this was converted in four stages into 1-benzyl-4-aminosubstituted-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridines. This synthetic method is based on a lithiation reaction: a first attempt was carried out on 1-benzyl-5-methyl-1H-1,2,3-triazole-4-(*N*-methyl)-carboxamide (**22**) in the presence of benzonitrile and lithium diisopropyl amide (LDA). This reaction was used by Poindexter [15] who transformed 1-methyl-2-(*N*-methyl)-carboxamido benzene into an isochinoline derivative. The chloride **21** was reacted with methyl amine to obtain the corresponding amide **22**. A treatment of **22** with benzonitrile and a 2 M solution of LDA at −74 °C, did not give the expected 1-

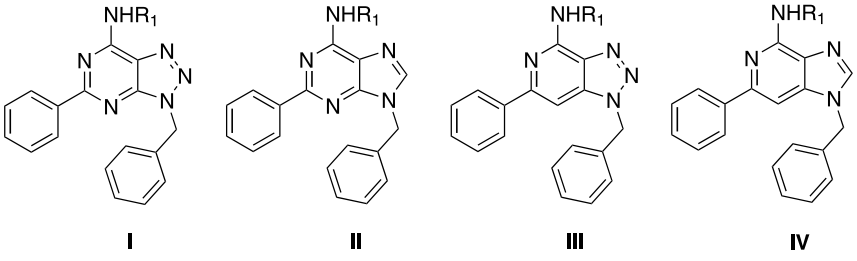
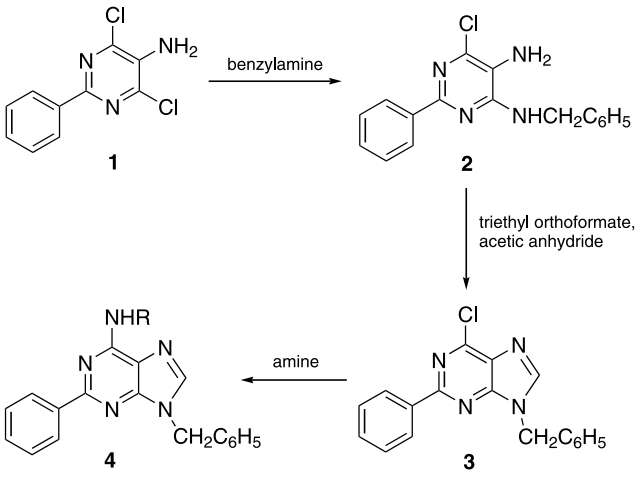


Fig. 1. General formulas of N^6-substituted 9-benzyl-2-phenyl-8-azaadenines (**I**), N^6-substituted 9-benzyl-2-phenyladenines (**II**), N^6-substituted 9-benzyl-2-phenyl-3-deaza-8-azaadenines (**III**) N^6-substituted 9-benzyl-2-phenyl-3-deazaadenines (**IV**).



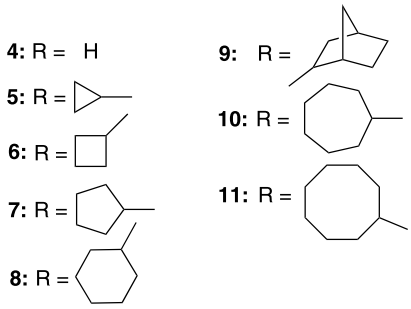


Fig. 2. Synthesis of N^6-substituted 2-phenyl-9-benzyladenines (**4**–**11**).

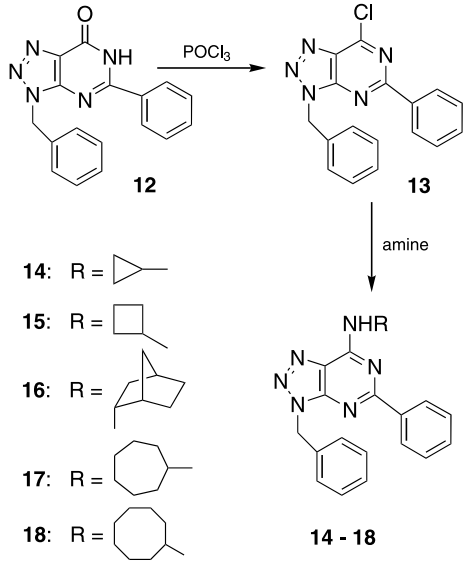


Fig. 3. Synthesis of 9-benzyl-2-phenyl-6-cycloalkylamino-8-azaadenines (**14**–**18**).

benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridine-4-one (**25**). From the reaction mixture, only the open imino intermediate adduct **23**, formed between lithiated triazole and benzonitrile, was isolated. All attempts to obtain compound **25** by heating **23** in basic or acid conditions failed. Only the starting material, sometimes contaminated with decomposition products, was recovered. A good yield (60%) of **25** was obtained employing the lithiation reaction on 1-benzyl-5-methyl-1H-1,2,3-triazole-4-(*N*,*N*-diethyl)-carboxamide (**24**), prepared from **21** by reaction with diethylamine. Examples of cyclisation by lithiation reaction using diethylamides were described by Mitsuaki et al. in 1984 [16]. 1-Benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridine-4-one (**25**) was transformed into the corresponding chloride **26** by reaction with phosphorous oxychloride. Finally, the 4-aminosubstituted compounds **27**–**34** were obtained by treatment of the chloride with the appropriated amine at 140–150 °C in a sealed tube.

3. Biological evaluation

Compounds **4**–**11**, **14**–**18** and **27**–**34** were tested in radioligand binding assays for affinity at A_1 and A_{2A} adenosine receptors in bovine brain cortical membranes and bovine striatum respectively. In Table 1 the results of A_1 adenosine receptor binding assays are reported; in A_{2A} adenosine receptor binding assays, all the compounds showed inhibition values $<60\%$ at 1 μM, and are considered inactive.

4. Results and discussion

All synthesised compounds, assayed as A_1 and A_{2A} adenosine receptor ligands, showed good affinity and selectivity for A_1 subtype. As can be seen in the histogram of Table 1, trends of K_i values in series **I** and **II** are very similar; by increasing the size of the N^6-cycloalkyl substituent, affinity increased up to five or six carbon atoms, then affinity decreased for the compounds with N^6-cycloheptyl or -cyclooctyl substituents. The biological results (Table 1) also indicate that the two series would bind to the receptor in the same site with the same orientation; the receptor pocket in front of N^6 of adenosine would be very large and capable of binding either little or large cycloalkyl substituents. The different size of the cycloalkyl substituent modulated the affinity, and cyclopentyl, cyclohexyl or norbornyl groups conferred the highest affinity to the molecules.

The comparison of the values of K_i of adenines (Table 1) with those of the corresponding 8-azaadenines (Table 1, newly developed compounds **14**–**18** and compounds **A**–**C** already reported in the literature [3,5]) showed that they are very close, either for N^6-unsubstituted as for N^6-cycloalkylsubstituted compounds. This strong similarity of affinity permits us to consider the two nuclei as bioisosteric: the N(8) did not contribute to the binding

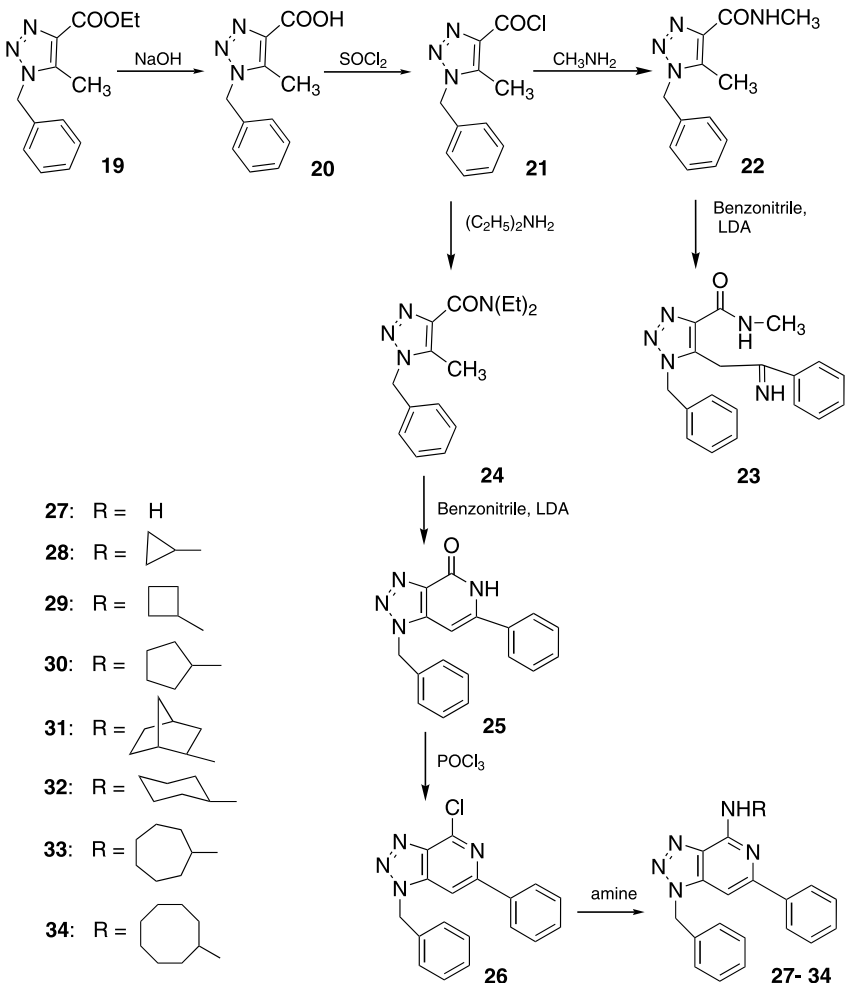


Fig. 4. Synthesis of 1-benzyl-4-amino-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridine (**27**) and 1-benzyl-4-cycloalkylamino-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridines (**28**–**34**).

of these molecules towards the receptor protein in a different manner from the C(8).

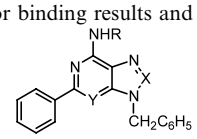
Regarding triazolopyridines (series **III**), biological results (Table 1) indicated a general lowering of A_1 affinity with respect to the analogous compounds having nitrogen in the position 3 (series **I** and **II**): most of the triazolopyridine derivatives (compounds **27**, **28**, **32**, **33**, **34**) have a $K_i > 100$ nM, and only compounds **29**, **30** and **31** are very potent A_1 ligands, having K_i values of 44, 14 and 11 nM, respectively. The trend of K_i values in series **III** is similar to that of series **I** and **II** for the first five compounds (compounds **27**–**31** vs. **4**–**7** and **A**, **14**, **15**, **B**, **16**) but it is different for the other ones, in which we can observe an abrupt lowering of activity. So, in the triazolopyridines, the size of the cycloalkyl substituent was more critical, because affinity decreased quickly when the cycloalkyl moiety was different from cyclobutyl, cyclopentyl or norbornyl group. In conclusion, from the biological data we may presume that nitrogen in the position 3 of 2-phenyl-8-azaadenines (**I**) and their bioisoster 2-phenyladenines (**II**) was important, but not crucial, to stabilise the complex between these ligands and the A_1 receptors.

5. Experimental

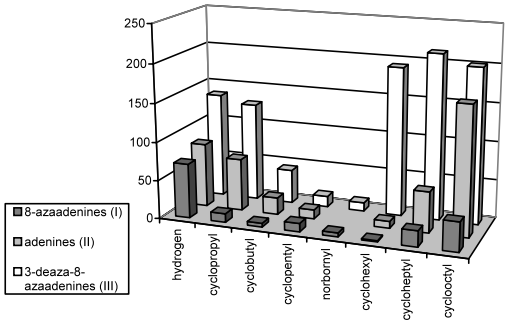
5.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 spectrometer in δ units using TMS as an internal standard; the compounds were dissolved in $CDCl_3$. Mass spectra were performed on a Hewlett–Packard GC/MS System 5988A. TLC was performed on precoated silica gel F_{254} plates (Merck). Flash-column chromatography was performed using Merck Kieselgel 60 (230–400 mesh). Microanalyses (C H N) were carried out on a Carlo Erba elemental analyser (model 1106) and were within $\pm$0.4% of the theoretical values. Petroleum–ether corresponds to fraction boiling at 40–60 °C.

Table 1
A_1 adenosine receptor binding results and related histogram



R	8-aza adenines (I§) X=N; Y=N	Ki (nM)	adenines (II§) X=C;Y=N	Ki (nM)	3-deaza-8-azaadenines (III§) X=N; Y=C	Ki (nM)
H	**A**[a]	71[a]	**4**	83±7	**27**	137±10
	14	11.3±1	**5**	68±7	**28**	128±11
	15	5±0.5	**6**	22±2	**29**	44±4
	B[a]	11[a]	**7**	12±2	**30**	14±1
	16	5±0.4	**8**	*	**31**	11.1±1
	C[b]	1.6[b]	**9**	9±1	**32**	193±20
	17	20.7±2	**10**	53±4	**33**	214±22
	18	38±4	**11**	167±10	**34**	201±19



§ See Fig. 1.

* Compound **8** is not enough soluble at the assay conditions.

[a] Ref. [3]; [b] Ref. [5].

5.1.1. General synthesis of N^6-substituted 2-phenyl-9-benzyladenines (**4**–**11**)

9-Benzyl-6-chloro-2-phenylpurine 3 [6] (0.15 g, 0.47 mmol), the suitable amine (Table 2) (6.60 mmol) and some drops of *N*,*N*-diethylaniline were dissolved in absolute ethanol (3 mL), and refluxed for 12 h. After evaporation, the mixture was diluted with chloroform, washed with 10% HCl and 5% $NaHCO_3$ and H_2O, evaporated and crystallised from diethyl ether–hexane to give the title compounds (Tables 2 and 3).

5.1.2. General synthesis of 9-benzyl-2-phenyl-6-cycloalkylamino-8-azaadenines (**14**–**18**)

To a mixture of 2-phenyl-9-benzyl-8-azahypoxanthine (**12**) [17] (1.0 g, 3.31 mmol) and *N*,*N*-diethylaniline (0.8 g, 5.36 mmol), phosphorous oxychloride (6 mL, 65.1 mmol) was added and heated at 90 °C for 4 h. Then the mixture was evaporated at reduced pressure and the resulting 6-chloro-2-phenyl-9-benzyl-8-azaadenine (**13**) [18] was used without purification for the substitution reactions. In a well-stoppered flask a mixture of **13** (0.2 g, 0.62 mmol), ethanol (5 mL), the suitable amine (Table 2) (6 mmol) and *N*,*N*-diethylaniline (0.1 g, 0.67 mmol) was refluxed for 12 h. Then the solution was evaporated at reduced pressure, diluted with chloroform and washed with 10% HCl and water. After evaporation of the organic layer, the residue was crystallised from isopropanol (Tables 2 and 3).

Table 2
Reaction and physico-chemical data

Compound	Amine	Yield (%)	M.p. (°C)	Analysis (C, H, N)
4	NH_3	85	210	$C_{18}H_{15}N_5$
5	cyclopropylamine	62	173	$C_{21}H_{19}N_5$
6	cyclobutylamine	58	225	$C_{22}H_{21}N_5$
7	cyclopentylamine	80	165–167	$C_{23}H_{23}N_5$
8	norbornylamine	47	150	$C_{25}H_{25}N_5$
9	cyclohexylamine	80	170	$C_{24}H_{25}N_5$
10	cycloheptylamine	39	140	$C_{25}H_{27}N_5$
11	cyclooctylamine	79	137–139	$C_{26}H_{29}N_5$
14	cyclopropylamine	63	193–195	$C_{20}H_{18}N_6$
15	cyclobutylamine	70	170	$C_{21}H_{20}N_6$
16	norbornylamine	58	185	$C_{24}H_{24}N_6$
17	cycloheptylamine	58	144	$C_{24}H_{26}N_6$
18	cyclooctylamine	62	176–178	$C_{25}H_{28}N_6$
27	NH_3	68	145–146	$C_{18}H_{15}N_5$
28	cyclopropylamine	93	156–157	$C_{21}H_{19}N_5$
29	cyclobutylamine	95	136	$C_{22}H_{21}N_5$
30	cyclopentylamine	92	147	$C_{23}H_{23}N_5$
31	norbornylamine	44	140–143	$C_{25}H_{25}N_5$
32	cyclohexylamine	90	138–139	$C_{24}H_{25}N_5$
33	cycloheptylamine	83	121	$C_{25}H_{27}N_5$
34	cyclooctylamine	87	106–107	$C_{26}H_{29}N_5$

5.1.3. 1-Benzyl-5-methyl-1H-1,2,3-triazol-4-carboxylic acid ethyl ester (**19**)

Title compound was obtained as described in the literature [13]. M.p. 78–79 °C (lit. 76–78 °C).

5.1.4. 1-Benzyl-5-methyl-1H-1,2,3-triazol-4-carboxylic acid (**20**)

A mixture of **19** and 30 mL of 10% NaOH was refluxed for 2 h; after cooling, the title compound, precipitated by acidification to pH of 2, was filtered, washed with water and crystallised from chloroform–hexane. M.p. 168–169 °C (lit. 168–169 °C [15]). MS (*m*/*e*): 217 [M^+], 188, 91, 65. ^{1}H-NMR ($CDCl_3$): 7.38–7.16 (m, 5H); 5.51 (s, 2H); 2.48 (s, 3H).

5.1.5. 1-Benzyl-5-methyl-1H-1,2,3-triazol-4-carbonyl chloride (**21**)

1-Benzyl-5-methyl-1H-1,2,3-triazol-4-carboxylic acid (**20**) (3.0 g, 13.8 mmol) was refluxed in $SOCl_2$ for 3 h. By evaporation under reduced pressure a solid was obtained (3.1 g, 13.3 mmol, 96% yield) which was crystallised from chloroform–hexane. M.p. 94–95 °C. MS (*m*/*e*): 235 [M^+], 206, 200, 178, 91, 65. ^{1}H-NMR

Table 3
^{1}H-NMR and MS spectral data of some synthesised compounds

Compound	^{1}H-NMR				MS (*m/z*, %)
	Aromatic H	Benzylic H	Aliphatic H	Exchang. H	
4	7.52–7.32 (m 8H), 7.77 (s 1), 8.46–8.43 (m 2H)	5.45 (m 2H)	–	5.62 (2H)	301 ([M$^+$] 28), 91 (100)
5	7.56–7.36 (m 8H), 7.84 (s 1), 8.58–8.53 (m 2H)	5.45 (m 2H)	1.02–0.86 (m 4H), 3.45 (m 1H)	–	341 ([M$^+$] 3.5), 91 (100)
6	7.58–7.38 (m 8H), 7.89 (s 1), 8.56 (m 2H)	5.46 (m 2H)	2.51–1.21 (m 7H), 5.30 (m 1H)	5.30 (1H)	355 ([M$^+$] 2), 300 (2) 236 (11), 91 (100)
7	7.63–8.41 (s 1H), 7.60–7.43 (m 8H), 8.53–8.41 (m 2H)	5.39 (m 2H)	2.16–1.73 (m 8H), 4.95 (m 1H)	5.7 (1H)	369 ([M$^+$] 5), 300(1) 333 (13), 91(100)
8	7.53–7.32 (m 8H), 7.72(s 1H), 8.54–8.49 (m 2H)	5.44 (m 2H)	1.67–0.89 (m 10H), 5.30 (m 1H)	5.86 (1H)	395 ([M$^+$] 11), 301 (16), 91 (100)
9	7.83 (m 1H), 7.58–7.50(s 8H), 8.54–8.49 (m 2H)	5.44 (m 2H)	1.67–0.89 (m 10H), 5.86 (m 1H)	6.15 (1H)	395 ([M$^+$] 11), 301 (16), 91 (100)
10	7.51–7.37 (m 8H), 7.75 (s 1H), 8.56–8.52 (m 2H)	5.44 (m 2H)	1.65 (m 10H), 2.19–2.13 (m 2H), 4.60 (m 1H)	4.91 (1H)	397 ([M$^+$] 5), 301 (32), 91 (100)
11	8.55–8.50 (m 2H), 7.71 (s 1H), 7.46–7.36 (m 8H)	5.43 (m 2H)	1.66 (m 12H), 2.13–2.03 (m 2 H), 4.64 (m 1H)	5.75 (1H)	411([M$^+$] 3), 301(25), 91 (100)
14	8.59–8.54 (m 2H), 7.58–7.53 (m 5H), 7.31 (m 3H)	5.84 (s 2H)	1.16–0.80 (m 4H) 3.68 (m 1H)	6.30 (1H)	342 ([M$^+$] 17), 315 (2), 91 (100)
15	8.55–8.52 (m 2H), 7.53–7.50 (m 5H), 7.37–7.34 (m 3H)	5.81 (s 2H)	1.94 (m 2H), 2.20 (m 2H), 2.60 (m 2H), 4.97 (m 1H)	6.32 (1H)	328 ([M$^+$] – 28 49), 300 (5.6), 91 (100)
16	8.54–8–51 (m 2H), 7.51–7.30 (m 8H)	5.80 (s 2H)	2.80 (m 1H), 2.34 (m 2H), 1.65–1.00 (m 7H), 4.66 (m 1H)	6.25 (1H)	396 ([M$^+$] 10), 339 (4), 273 (15), 91 (100)
17	8.56–8.53 (m 2H), 7.52–7.49 (m 5H), 7.33–7.31 (m 3H)	5.81 (s 2H)	1.75–1.58 (m 10H), 2.20 (m 2H), 4.560 (m 1H)	6.21 (1H)	398 ([M$^+$] 2.8), 302 (18) 273 (36), 91 (100)
18	8.60–8.57 (m 2H), 7.64–7.37 (m 8H)	5.83 (s 2H)	1.70–1.55 (m 12H), 2.10–2.04 (m 2H), 5.10 (m 1H)	6.22 (1H)	412 ([M$^+$] 10), 302 (19) 273 (42), 91 (100)
27	7.91–7.85 (m 2H), 7.51–7.31 (m 8H), 6.93 (s 1H)	5.80 (s 2H)		5.69 (1H)	301 ([M$^+$] 10), 272 (5), 91(100)
28	8.03–7.99 (m 2H), 7.48–7.30 (m 8H) 6.91 (s 1H)	5.78 (s 2H)	3.22 (m 1H), 0.95 (m 2H), 0.75 (m 2H)	6.38 (1H)	341([M$^+$] 7), 91(100), 77(13)
29	7.99–7.95 (m 2H), 7.48–7.30 (m 8H) 6.87 (s 1H)	5.77 (s 2H)	4.97 (m 1H), 2.54 (m 2H), 2.06 (m 2H), 1.84 (m 2H)	6.01 (1H)	355 ([M$^+$] 2), 326 (23), 91(100)
30	8.01–7.96 (m 2H), 7.44–7.27 (m 8H), 6.87 (s 1H)	5.77 (s 2H)	4.78 (m 1H), 2.21 (m 2H), 1.79–1.67 (m 6H)	5.94 (1H)	369 ([M$^+$] 2), 301(10) 272(5), 91(100)
31	7.99–7.94 (m 2H), 7.49–7.30 (m 8H) 6.86 (s 1H)	5.77 (s 2H)	4.64 (m 1H), 2.32–0.85 (m 10H)	5.96 (1H)	395 ([M$^+$] 15.4), 366(12) 301(30), 91(100)
32	7.99–7.94 (m 2H), 7.50–7.30 (m 8H) 6.86 (s 1H)	5.77 (s 2H)	4.35 (m 1H), 2.21 (m 2H), 1.87–1.26 (m 8H)	5.81 (1H)	383 ([M$^+$] 6), 301(72) 272(28.8) 91(100)
33	8.00–7.97 (m 2H), 7.48–7.30 (m 8H) 6.86 (s 1H)	5.77 (s 2H)	4.57 (m 1H), 2.19 (m 2H), 1.67–1.60 (m 10H)	5.82 (1H)	397([M$^+$] 7), 301(81) 272(22), 91(100)
34	8.00–7.97 (m 2H), 7.47–7.30 (m 8H) 6.86 (s 1H)	5.77 (s 2H)	4.61 (m 1H), 2.18–1.57 (m 14H)	5.82 (1H)	411([M$^+$] 2), 301(14) 272(4), 91(100) 77(6)

($CDCl_3$): 7.38–7.16 (m, 5H); 5.52 (s, 2H); 2.45 (s, 3H). Anal.: C,H,N.

5.1.6. 1-Benzyl-5-methyl-4-(N-methyl)-carboxamido-1H-1,2,3-triazole (**22**)

To an iced solution of **21** (2.9 g, 12.7 mmol) in THF (50 mL), 40% aqueous methylamine (25 mL) was added slowly. After stirring for a day at room temperature (r.t.), the mixture was concentrated at reduced pressure, then filtered and the solid washed with water and crystallised from chloroform–hexane. The pure title compound was obtained with high yield (2.7 g, 11.8 mmol, 92% yield). M.p. 134–135 °C. MS (*m/e*): 230 [M$^+$], 201, 91, 41. ^{1}H-NMR ($CDCl_3$): 7.30–7.12 (m, 5H+1H exchangeable); 5.47 (s, 2H); 2.97 (d, 3H); 2.49 (s, 3H). Anal.: C,H,N.

5.1.7. 1-Benzyl-4-(N-methyl)-carboxamido-5(1-phenyl-1-iminoethyl)-1H-1,2,3-triazole (**23**)

To a stirring solution of **22** (2.0 g, 8.68 mmol) and benzonitrile (1.1 g, 0.01 mol) in anhydrous THF (50 mL), under nitrogen and cooled to −74 °C, 10 mL of 2M LDA was added. Stirring was maintained for 30 min at −74 °C, then the mixture was allowed to reach r.t.

After evaporation at reduced pressure, the residue was dissolved in CH_2Cl_2 and the solution was washed with saturated NH_4Cl, H_2O and evaporated. The orange oil, treated at reflux with petroleum ether 40–60 to dissolve the unreacted benzonitrile, was repeatedly crystallised from toluene and finally from ethanol to give pure 23 (0.43 g, 1.35 mmol, 15% yield). M.p. 183–185 °C. MS (*m/e*): 375 [M^+], 234. ^{1}H-NMR ($CDCl_3$): 8.00 (s, 1H, exc.); 7.97 (s, 1H, exc.); 7.61–7.11 (m, 10H); 5.62 (s, 2H); 4.72 (s, 2H); 2.98 (d, 3H). Anal.: C,H,N.

5.1.8. 1-Benzyl-5-methyl-1H-4-(*N*,*N*-diethyl)-carboxamido-1,2,3-triazole (**24**)

To an iced solution of **21** (3.0 g, 12.7 mmol) in CH_2Cl_2, *N*,*N*-diethylamine (5.0 g, 33.7 mmol) was slowly added. The mixture was refluxed for 2 h, then cooled and repeatedly washed with 5% HCl. The organic layer was evaporated to give an oil which was flash-chromatographed using as eluent $CHCl_3$–MeOH 99:1. The pure title compound was obtained as an oil (2.96 g, 10.9 mmol, 85% yield). MS (*m/e*): 272 [M^+], 201, 91. ^{1}H-NMR ($CDCl_3$): 7.41–7.17 (m, 5H); 5.50 (s, 2H); 3.84 (q, 2H); 3.53 (q, 2H); 2.42 (s, 3H); 1.32 (t, 3H); 1.24 (t, 3H). Anal.: C,H,N.

5.1.9. 1-Benzyl-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridin-4-oxo (**25**)

To a stirring solution of **24** (3.0 g, 11.0 mmol) and benzonitrile (1.25 g, 12.1 mmol) in anhydrous THF (20 mL), under nitrogen and cooled to −70 °C, 8 mL of 2 M LDA was added. Then the mixture was allowed to reach r.t. and stirring was maintained overnight. After evaporation at reduced pressure, the oily residue was dissolved in CH_2Cl_2 (200 mL) and the solution was washed with saturated NH_4Cl and concentrated to 15 mL, obtaining a solid which was filtered (2.05 g, 6.78 mol, 62% yield) and crystallised from ethanol. M.p. 240–242 °C. MS (*m/e*): 302 [M^+], 273, 91, 77, 65. ^{1}H-NMR ($CDCl_3$): 9.43 (s, 1H, exc.); 7.47–7.22 (m, 10H); 6.30 (s, 1H); 5.71 (s, 2H). Anal.: C,H,N.

5.1.10. 1-Benzyl-4-chloro-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridine (**26**)

A solution of **25** (0.2 g, 0.66 mmol) and $POCl_3$ (1 mL) was refluxed for 30 min. To the mixture, iced and stirred, ethanol (15 mL) and then H_2O (30 mL) were slowly added. The solid obtained was filtered, washed with H_2O and crystallised from 1,2-dimethoxyethane–H_2O to give 0.19 g (0.61 mmol, 92% yield) of the title compound. M.p. 173–174 °C. MS (*m/e*): 320 [M^+], 291, 257, 91, 77, 65. ^{1}H-NMR($CDCl_3$): 7.97 (d, 1H); 7.93 (d, 1H); 7.49–7.31 (m, 9H); 5.91 (s, 2H). Anal.: C,H,N.

5.1.11. 1-Benzyl-4-amino-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridine (**27**)

An iced solution of **26** (0.1 g, 0.31 mmol) in a mixture of 1,2-dimethoxyethane–H_2O (1:1) was saturated with gaseous NH_3, then heated in a steel bomb at 140 °C for 36 h. After cooling, 5% HCl was added dropwise to the mixture. The solid obtained was filtered, washed with H_2O and crystallised from 1,2-dimethoxyethane–H_2O to give 0.63 g (0.21 mmol, 67% yield) of the title compound (Tables 2 and 3).

5.1.12. General synthesis of 1-benzyl-4-cycloalkylamino-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridine (**28**–**34**)

To a solution of **26** (0.31 mmol) in 1,2-dimethoxyethane, the suitable amine (Table 2) (2.0 mmol) was added, then heated in a steel bomb at150 °C for 24 h. After cooling, 20 mL of 5% HCl was added dropwise to the mixture. The solid obtained was filtered, washed with H_2O and crystallised from 1,2-dimethoxyethane–H_2O (Tables 2 and 3).

5.2. Biochemistry

5.2.1. A_1 receptor binding

Bovine cerebral cortex was homogenised in ice-cold 0.32 M sucrose containing protease inhibitors, as previously described [19]. The homogenate was centrifuged at 1000 × *g* for 10 min at 4 °C and the supernatant again centrifuged at 48 000 × *g* for 15 min at 4 °C. The final pellet was dispersed in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 U mL^{-1}) to remove endogenous adenosine at 37 °C for 60 min and then recentrifuged at 48 000 × *g* for 15 min at 4 °C. The pellet was suspended in buffer and used in the binding assay.

The [^{3}H]CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of Tris–HCl, pH 7.7, containing 2 mM $MgCl_2$, with approximately 1.2 nM [^{3}H]CHA. Nonspecific binding was defined in the presence of 50 μM *R*-PIA. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer.

5.2.2. A_{2A} receptor binding

Bovine striatum was homogenised in 20 volumes of ice-cold 50 mM Tris–HCl, pH 7.5, containing 10mM $MgCl_2$ and protease inhibitors. The membrane homogenate was centrifuged at 48 000 × *g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 U mL^{-1} of adenosine deaminase and incubated at 37 °C for 30 min. The membrane homogenate was centrifuged, and the final pellet frozen at −80 °C. Routine assays were performed in triplicate by incubation an aliquot of striatal membranes (0.2–0.3 mg of

protein) in cold 50 mM Tris–HCl, pH 7.5, containing 10 mM $MgCl_2$ with approximately 5 nM [^{3}H]CGS 21680 in a final volume of 0.5 mL. Incubation was carried out for 90 min at 25 °C. Non-specific binding was defined in the presence of 50 μM CGS 21680. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer and placed in scintillation vials. The radioactivity was counted in a 4 mL Beckman Ready-Protein scintillation cocktail in a scintillation counter. The compounds were dissolved in DMSO and added to the assay mixture to make a final volume of 0.5 mL. Blank experiments were carried out to determine the effect of the solvent (2%) on binding. The concentrations of the tested compounds to produce 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21 680 binding (IC_{50}) were determined from semilog plots of data from experiments of binding inhibition. The K_i values were calculated from the IC_{50} values using the equation $IC_{50}/(L/K_d)$ [20] ([^{3}H]CHA $K_d = 10.5$ nM and $L = 1.2$ nM; [^{3}H]CGS 21 680 $K_d = 1$ nM and $L = 5$ nM); protein estimation was based on the method reported [21] using bovine serum albumin as standard.

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