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Synthesis of some tricyclic heteroaromatic systems and their A_1 and A_{2a} adenosine binding activity

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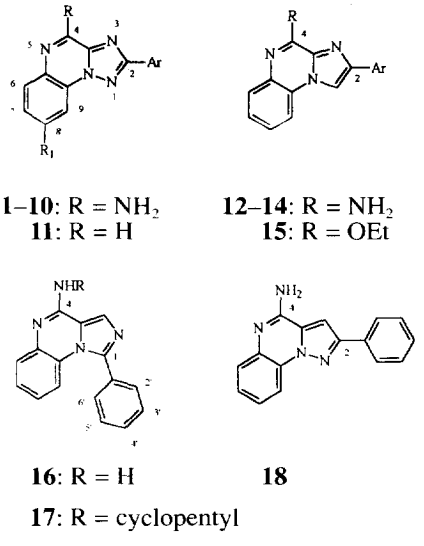
Summary — The syntheses, A_1 and A_{2a} adenosine receptor affinities and structure–activity relationships of some 2-aryl-1,2,4-triazolo[1,5-*a*]quinoxalines, 2-arylimidazo[1,2-*a*]quinoxalines, 1-arylimidazo[1,5-*a*]quinoxalines are reported and compared with that of a previously reported 2-phenylpyrazolo[1,5-*a*]quinoxaline. The results show that some triazoloquinoxalines are potent and specific A_1 adenosine receptor ligands and that the replacement of either nitrogen at position 1 or 3 of the triazoloquinoxaline moiety with a CH brought about a decrease in affinity at both adenosine receptors.

adenosine receptor ligand / 1,2,4-triazolo[1,5-*a*]quinoxaline / imidazo[1,2-*a*]quinoxaline / imidazo[1,5-*a*]quinoxaline / tricyclic heteroaromatic system

Introduction

Adenosine produces a variety of physiological responses by interacting with two major extracellular receptor subtypes, A_1AR and A_2AR, which were originally defined on the basis of whether their activation inhibited or stimulated adenyl cyclase, respectively [1]. The A_2AR has been further divided into high and low affinity subtypes, $A_{2a}AR$ and $A_{2b}AR$, respectively, after extensive structure–activity relationship studies [2, 3], biochemistry [4], binding studies [5–8] and the cloning of separate entities [9–12]. Another adenosine receptor, A_3AR, has recently been identified by cloning [13–15], while binding studies with [^{3}H]CV 1808 [16] have led to the pharmacological identification of a new adenosine receptor, tentatively named A_4AR. A_3AR displays an unusual structural diversity for species homologues, and has a broad tissue distribution in human and sheep where it binds with high affinity some xanthines with acidic side chains or NH_2 groups[14].

Receptor subtypes are generally defined by their different affinities for specific synthetic agonists and antagonists. The synthesis of new adenosine receptor ligands would thus provide a valuable tool for defining the structural requirements of each receptor subtype. With this in mind, and as a further development of our previous work on the preparation of non-xanthine antagonists at A_1AR and A_2AR [17–19], we extended our investigations to the study of the tricyclic compounds **1–18** shown in figure 1. These



1–10: R = NH_2
11: R = H

12–14: R = NH_2
15: R = OEt

16: R = H
17: R = cyclopentyl

18

Fig 1. Chemical structures of the tricyclic systems **1–18**.

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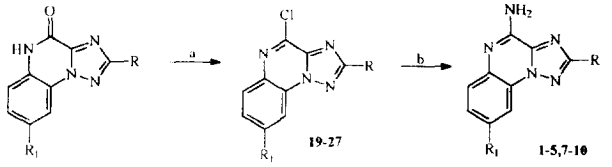
investigations resulted in the synthesis of some 2-aryl-1,2,4-triazolo[1,5-*a*]quinoxalin-4-amines **1–10**, 2-arylimidazo[1,2-*a*]quinoxalines **12–15** and 1-arylimidazo[1,5-*a*]quinoxalines **16–17**. All these newly reported compounds, together with the previously reported 2-phenyl-1,2,4-triazolo[1,5-*a*]quinoxaline [20] **11** and 2-phenylpyrazolo[1,5-*a*]quinoxalin-4-amine [21] **18**, were studied for their interactions with both A_1AR and $A_{2a}AR$.

Chemistry

The final newly reported tricyclic heteroaromatic derivatives **1–5**, **7–10**, and **12–17** were prepared from the corresponding 4-chloro key intermediates **19–30** and **34**.

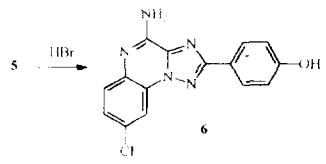
The syntheses of the 2-aryl-1,2,4-triazolo[1,5-*a*]-quinoxalines **1–10** are illustrated in scheme 1. By reacting 2-aryl-1,2,4-triazolo[1,5-*a*]quinoxalin-4-ones [20, 22] with a mixture of $POCl_3/PCl_5$, the corresponding 4-chloro derivatives **19–27** were obtained. Nucleophilic replacement of the 4-chloro group with ammonia yielded compounds **1–5** and **7–10**.



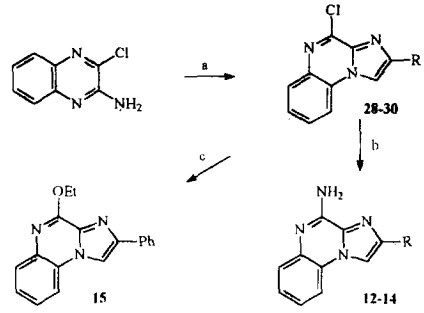
4-Cl	R	R_1	4-NH_2
19	C_6H_5	H	**1**
20	C_6H_5	Cl	**2**
21	C_6H_5	Me	**3**
22	2-FC_6H_4	Cl	**4**
23	4-$MeOC_6H_4$	Cl	**5**
24	2-Furyl	H	**7**
25	2-Furyl	Cl	**8**
26	3-Furyl	Cl	**9**
27	2-Thienyl	Cl	**10**

Scheme 1. a: $POCl_3/PCl_5$/pyridine; b: ammonia.

The 2-(4-hydroxyphenyl)-1,2,4-triazolo[1,5-*a*]quinoxalin-4-amine **6** ensued from dealkylation of the corresponding 2-(4-methoxyphenyl) derivative **5**.



The syntheses of the 2-arylimidazo[1,2-*a*]quinoxalines **12–15** are illustrated in scheme 2. By allowing 2-chloroquinoxalinamine [23] to react with bromomethyl-4-chlorophenylketone, following the procedure reported in the literature [22] for the preparation of compounds **28** and **30**, compound **29** was obtained. Treatment with ammonia of **28–30** afforded compounds **12–14**, while by reacting **28** with an ethanolic solution of sodium hydroxide the 4-ethoxy derivative **15** was prepared.



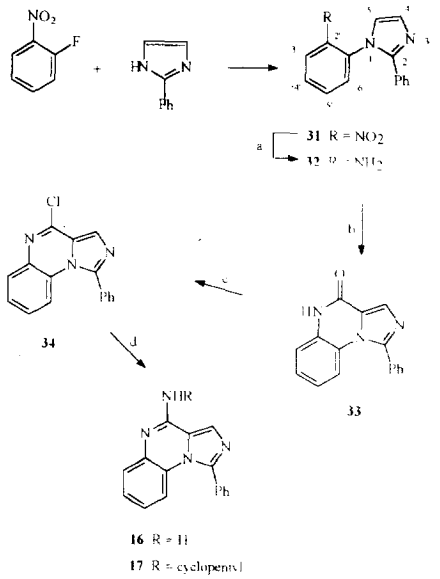
4-Cl	R	4-NH_2
28	C_6H_5	**12**
29	4-ClC_6H_4	**13**
30	2-Thienyl	**14**

Scheme 2. a: $RCOCH_2Br$; b: ammonia; c: EtOH/NaOH.

Finally, the syntheses of the 1-phenylimidazo[1,5-*a*]-quinoxalines **16–17** are shown in scheme 3. Reaction of 1-fluoro-2-nitrobenzene with 2-phenylimidazole yielded the 1-(2-nitrophenyl)-2-phenylimidazole **31**, which was catalytically reduced to the amino derivative **32**. Treatment of compound **32** with 1,1'-carbonyldiimidazole afforded the tricyclic 1-phenylimidazo[1,5-*a*]quinoxalin-4-one **33** which, with $POCl_3$, gave the 4-chloro derivative **34**. The latter with ammonia or cyclopentylamine gave the final compounds **16** and **17**, respectively.

Biochemistry

Compounds **1–18** were tested for their ability to displace [^{3}H]-N^6-cyclohexyladenosine (CHA) on A_1AR in rat cerebral cortical membranes and [^{3}H]-2-[4-((2-carboxyethyl)phenethyl)amino]-5'-*N*-ethylcarboxamidoadenosine (CGS 21680) on $A_{2a}AR$ in rat striatal membranes. The A_1AR and $A_{2a}AR$ affinities of the tested compounds, expressed as their K_i values, are listed in table I.



Scheme 3. a: H_2/Pd/C; b: Im_2CO; c: $POCl_3$; d: ammonia or cyclopentylamine.

Results and conclusions

The tricyclic heteroaromatic systems are arranged in table I according to the modifications of the pentatomic ring, which range from 1,2,4-triazole, to differently fused imidazole and pyrazole.

Table I shows that the highest A_1AR affinities are displayed by the 2-aryl-1,2,4-triazolo[1,5-*a*]quinoxalin-4-amines **1–10**. The adenosine receptor affinity within this subgroup of compounds is clearly dependent on the presence of the NH_2 group at position 4, since the 4-unsubstituted triazoloquinoxaline **11** shows a substantial reduction in A_1AR affinity. For compounds **1–10**, variation of the R_1 substituent at position 8 from hydrogen to chlorine or methyl produces little or no increase in potency. Variation in compounds **1–10** of the 2-substituent yielded different A_{2a}AR affinities. In fact, compounds **7–8** bearing a 2-(2-furyl) ring are the only ones that showed high A_{2a}AR affinity. The substituent at position 2 was also important for A_1/A_{2a} selectivity. The 2-unsubstituted phenyl ring gave the non-selective adenosine ligands **1–3**, while *ortho* or *para* substitution of the phenyl ring afforded the A_1-selective ligands **4–6**. The highly potent A_{2a} ligands **7–8** were however non-selective, displaying a 30 nM A_1 affinity. The 2-(3-furyl) and 2-(2-thienyl) substituents gave rise to the A_1-selective ligands **9** and **10**, respectively.

All the imidazo[1,2-*a*]quinoxalines **12–15** are less active at both A_1AR and A_{2a}AR than their 1-aza analogues triazolo-quinoxalines. In this subgroup of compounds the presence of the NH_2 group at position 4 is not necessary. In fact, 4-ethoxyimidazo[1,2-*a*]quinoxaline **15** displayed A_1 and A_{2a} potency comparable to that of the other 4-amino derivatives **12–14**.

Variation of the kind of condensation between the imidazole and quinoxaline moiety occurring in compounds **16–17** does not enhance the inhibitory potency at either A_1AR or A_{2a}AR.

Finally, the 2-phenylpyrazolo[1,5-*a*]quinoxalin-4-amine [21] **18**, displayed a 53-fold and 11-fold lower A_1 and A_{2a} binding activity, respectively, as compared to the 3-aza analogue 2-phenyl-1,2,4-triazolo[1,5-*a*]quinoxalin-4-amine **1**, thus discouraging further syntheses of this kind of compounds.

In conclusion, the syntheses of these tricyclic heteroaromatic systems have provided three potent and selective A_1 adenosine receptor ligands, compounds **4**, **9** and **10**, while the structure–activity relationships have shown that replacement of either nitrogen at position-1 or position-3 of the triazoloquinoxalines **1–10** with a CH resulted in a decrease in binding potency at A_1AR and/or A_{2a}AR. These findings are consistent with the chemical features of the natural ligand, the adenosine. The nitrogen atoms at position 1 and 3 of the triazole ring mimic those of position 7 and 9 of the adenosine and are thus necessary for the anchoring of a ligand to both A_1 and A_{2a} receptors.

Experimental protocols

Chemistry

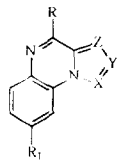
Silica-gel plates (Merck F_{254}) and silica gel 60 (Merck, 70-230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp capillary melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for 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 mull and are reported in cm^{-1}. The ^{1}H-NMR spectra were run on a Varian Gemini 200 instrument in the Fourier transform mode at 200 MHz using an acquisition time of 1.5 s, a flip angle of 37° and a spectral width of 3000 Hz. The deuterium signal of the solvent provided the field frequency lock. Chemical shifts are reported in δ (ppm) and refer to the central peak of the solvent ($CDCl_3$ = 1385 Hz, DMSO-d_6 = 792 Hz). The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, br = broad, ar = aromatic proton.

The physical data of the newly synthesized compounds are listed in table II.

2-Aryl-4-chloro-8-R_1-1,2,4-triazolo[1,5-a]quinoxalines ***19–27***

A mixture of the suitable 2-aryl-8-R_1-1,2,4-triazolo[1,5-*a*]quinoxalin-4-one [20, 22] (1 mmol), $POCl_3$ (5 mL), PCl_5 (0.3 mmol) and a catalytic amount of pyridine was heated under stirring and nitrogen flow at 120°C for 3 h. Evaporation of the excess $POCl_3$ at reduced pressure afforded a crude product which was used without further purification. The yields are calculated on the crude product. Only a small amount of crude product was recrystallized to determine the melting point and the ^{1}H-NMR.

Table I. A_1 and A_{2a} binding constants of the reported tricyclic heteroaromatic systems[a].



Compound	*R*	*X*	*Y*	*Z*	R_1	$K_i \pm SEM$ *(nM)*[b]	
						A_1[c]	A_{2a}[d]
1	NH_2	N	C-C_6H_5	N	H	57 ± 6	300 ± 28
2	NH_2	N	C-C_6H_5	N	Cl	50 ± 4	161 ± 15
3	NH_2	N	C-C_6H_5	N	Me	39 ± 3.7	107 ± 9.2
4	NH_2	N	C-$C_6H_4F(2)$	N	Cl	13 ± 1.5	>10 000
5	NH_2	N	C-$C_6H_4OMe(4)$	N	Cl	150 ± 12	>10 000
6	NH_2	N	C-$C_6H_4OH(4)$	N	Cl	36 ± 2	3170 ± 298
7	NH_2	N	C-furyl(2)	N	H	30 ± 2.3	11 ± 0.9
8	NH_2	N	C-furyl(2)	N	Cl	30 ± 2	12 ± 1.1
9	NH_2	N	C-furyl(3)	N	Cl	24 ± 2.1	>10 000
10	NH_2	N	C-thienyl(2)	N	Cl	12 ± 1	>10 000
11	H	N	C-C_6H_5	N	H	1300 ± 107	>10 000[e]
12	NH_2	CH	C-C_6H_5	N	H	230 ± 20	720 ± 81[e]
13	NH_2	CH	C-$C_6H_4Cl(4)$	N	H	530 ± 51	4430 ± 519[e]
14	NH_2	CH	C-thienyl(2)	N	H	730 ± 61	>10 000[e]
15	OEt	CH	C_6H_5	N	H	680 ± 59	450 ± 39[e]
16	NH_2	C-C_6H_5	N	CH	H	130 ± 11	310 ± 30[e]
17	NH-cyclopentyl	C-C_6H_5	N	CH	H	810 ± 73	>10 000
18	NH_2	N	C-C_6H_5	CH	H	3050 ± 298	3380 ± 328[e]

[a]The tests were carried out dissolving the tested compound in DMSO (DMSO/buffer 2%) unless otherwise stated. [b]The K_i values are means ± SEM of four separate assays, each performed in triplicate. [c]A_1 binding was measured as inhibition of [^{3}H]CHA binding as described in the *Experimental protocols*. [d]A_{2a} binding was measured as inhibition of [^{3}H]CGS 21680 binding as described in the *Experimental protocols*. [e]The tests were carried out dissolving the tested compound in ethanol (ethanol/buffer 2%).

The title compounds displayed the following ^{1}H-NMR data: **19**: ($CDCl_3$): 7.4–7.6 (m, 3H, H-3'-5'), 7.7–7.9 (m, 2H, H-7, 8), 8.14 (d, 1H, H-6, J = 8.2 Hz), 8.3–8.4 (m, 2H, H-2',6'), 8.52 (d, 1H, H-9, J = 8.2 Hz). **20**: ($CDCl_3$): 7.5–7.6 (m, 3H, H-3'-5'), 7.68 (dd, 1H, H-7, J = 8.7, 2.2 Hz), 8.08 (d, 1H, H-6, J = 8.7 Hz), 8.3–8.5 (m, 2H, H-2',6'), 8.53 (d, 1H, H-9, J = 2.2 Hz). **21**: (DMSO-d_6): 2.66 (s, 3H, CH_3), 7.6–7.7 (m, 4H, H-3'-5', 7), 8.07 (d, 1H, H-6, J = 8.7 Hz), 8.3–8.4 (m, 3H, H-2',6',9). **22**: ($CDCl_3$): 7.2–7.4 (m, 2H, H-3',5'), 7.5–7.6 (m, 1H, H-4'), 7.69 (dd, 1H, H-7, J = 8.7, 2.2 Hz), 8.09 (d, 1H, H-6, J = 8.7 Hz), 8.3–8.4 (m, 1H, H-6'), 8.55 (d, 1H, H-9, J = 2.2 Hz). **23**: ($CDCl_3$): 3.91 (s, 3H, OCH_3), 7.05 (d, 2H, H-3',5', J = 8.8 Hz), 7.66 (dd, 1H, H-7, J = 8.7, 2.2. Hz), 8.06 (d, 1H, H-6, J = 8.7 Hz), 8.33 (d, 2H, H-2',6', J = 8.8 Hz), 8.50 (d, 1H, H-9, J = 2.2 Hz). **24**: ($CDCl_3$): 6.60 (dd, 1H, H-4', J = 3.4, 1.7 Hz), 7.3–7.7 (m, 4H, H-7,8,3',5'), 7.85 (d, 1H, H-6, J = 8.2 Hz), 8.30 (d, 1H, H-9, J = 8.2 Hz). **25**: (DMSO-d_6): 6.79 (dd, 1H, H-4', J = 3.4, 1.7 Hz), 7.2–7.6 (m, 3H, H-6,7,3'), 7.98 (d, 1H, H-5', J = 1.7 Hz), 8.07 (d, 1H, H-9, J = 2.2 Hz). **26**: ($CDCl_3$): 7.18 (d, 1H, H-4', J = 2.1 Hz), 7.27 (dd, 1H, H-5', J = 2.1, 1.5 Hz), 7.46 (d, 1H, H-2', J = 1.5 Hz), 7.69 (dd, 1H, H-7, J = 8.7, 2.2 Hz), 8.08 (d, 1H, H-6, J = 8.7 Hz), 8.49 (d, 1H, H-9, J = 2.2 Hz). **27**: ($CDCl_3$): 7.21 (dd, 1H, H-4', J = 4.8, 3.8 Hz), 7.4–7.5 (m, 2H, H-7,3'), 7.72 (d, 1H, H-6, J = 8.7 Hz), 8.02 (d, 1H, H-5', J = 4.8 Hz), 8.49 (d, 1H, H-9, J = 2.2 Hz).

2-Aryl-8-R_1-1,2,4-triazolo[1,5-a]quinoxalin-4-amines ***1–5, 7–10***

A mixture of 4-chloro-derivative **19–27** (1 mmol) in absolute ethanol (30 ml) saturated with ammonia was heated at 130°C for 10 h in a sealed tube. Evaporation of the solvent at reduced pressure yielded a residue which was purified by column chromatography (eluting system conc NH_4OH/absolute ethanol/$CHCl_3$/40–60° petroleum ether 4:32.5:170:30), and then recrystallized.

The title compounds displayed the following spectral data: **1**: ^{1}H-NMR ($CDCl_3$): 5.7 (br s, 2H, NH_2), 7.4–7.6 (m, 5H, H-3'-5',7,8), 7.77 (d, 1H, H-6, J = 8.2 Hz), 8.3–8.4 (m, 3H, H-2',6',9). IR: 3320, 3460. **2**: ^{1}H-NMR ($CDCl_3$): 5.7 (br s, 2H,

NH_2), 7.5–7.6 (m, 4H, H-3'-5',7), 7.68 (d, 1H, H-6, J = 8.7 Hz), 8.3–8.4 (m, 3H, H-2',6',9). IR: 3310, 3480. **3**: ^{1}H-NMR (DMSO-d_6): 2.51 (s, 3H, CH_3), 7.38 (dd, 1H, H-7, J = 8.6, 1.5 Hz), 7.5–7.6 (m, 6H, H-6,3',5'+ NH_2), 8.04 (d, 1H, H-9, J = 1.5 Hz), 8.2–8.3 (m, 2H, H-2',6'). IR: 3310, 3470. **4**: ^{1}H-NMR ($CDCl_3$): 5.8 (br s, 2H, NH_2), 7.2–7.4 (m, 2H, H-3',5'), 7.5–7.6 (m, 2H, H-7,4'), 7.69 (d, 1H, H-6, J = 8.7 Hz), 8.2–8.3 (m, 1H, H-6'), 8.37 (d, 1H, H-9, J = 2.1 Hz). IR: 3140, 3310. **5**: ^{1}H-NMR ($CDCl_3$): 3.90 (s, 3H, OCH_3), 5.7 (br s, 2H, NH_2), 7.04 (d, 2H, H-3',5', J = 8.9 Hz), 7.48 (dd, 1H, H-7, J = 8.7, 2.2 Hz), 7.67 (d, 1H, H-6, J = 8.7 Hz), 8.2–8.3 (m, 3H, H-2',6',9). IR 3310, 3470. **7**: ^{1}H-NMR ($CDCl_3$): 5.7 (br s, 2H, NH_2), 6.62 (dd, 1H, H-4', J = 3.4, 1.8 Hz), 7.24 (d, 1H, H-3', J = 3.4 Hz), 7.4–7.6 (m, 2H, H-7,8), 7.66 (d, 1H, H-5', J = 1.8 Hz), 7.76 (d, 1H, H-6, J = 8.2 Hz), 8.35 (d, 1H, H-9, J = 8.2 Hz). IR: 3320, 3480. **8**: ^{1}H-NMR ($CDCl_3$): 5.9 (br s, 2H, NH_2), 6.41 (dd, 1H, H-4', J = 3.4, 1.8 Hz), 7.23 (d, 1H, H-3', J = 3.4 Hz), 7.50 (dd, 1H, H-7, J = 8.7, 2.1 Hz), 7.6–7.7 (m, 2H, H-6,5'); 8.34 (d, 1H, H-9, J = 2.1 Hz). IR: 3150, 3320, 3480. **9**: ^{1}H NMR ($CDCl_3$): 5.8 (br s, 2H, NH_2), 7.10 (d, 1H, H-4', J = 2.1 Hz), 7.27 (dd, 1H, H-5', J = 2.1, 1.5 Hz), 7.4–7.5 (m, 2H, H-7,2'), 7.68 (d, 1H, H-6, J = 8.7 Hz), 8.30 (d, 1H, H-9, J = 2.1 Hz). IR: 3320, 3480. **10**: ^{1}H-NMR ($CDCl_3$): 5.7 (br s, 2H, NH_2), 7.20 (dd, 1H, H-4', J = 4.8, 3.8 Hz), 7.5–7.6 (m, 2H, H-7,3'), 7.68 (d, 1H, H-6, J = 8.7 Hz), 7.93 (d, 1H, H-5', J = 4.8 Hz), 8.33 (d, 1H, H-9, J = 2.1 Hz). IR: 3160, 3310.

2-(4-Hydroxyphenyl)-1,2,4-triazolo[1,5-a]quinoxalin-4-amine ***6***
A solution of **5** (1 mmol) in glacial acetic acid (8 mL) and HBr (48%, 9 mL) was heated at reflux for 3 h. The cooled solution was neutralized with a saturated solution of Na_2CO_3. The solvents were evaporated at reduced pressure and the residue was extracted with acetone in a Soxhelet apparatus. Evaporation of the acetone at reduced pressure afforded the crude title compound which after recrystallization displayed the following spectral data: ^{1}H-NMR (DMSO-d_6): 6.96 (d, 2H, H-3',5', J = 8.7 Hz), 7.5–7.8 (m, 4H, H-6,7 + NH_2), 8.0–8.2 (m, 3H, H-2',6',9), 10.01 (s, 1H, OH). IR: 2600–3300, 3380, 3480.

2-(4'-Chlorophenyl)-4-chloroimidazo[1,2-a]quinoxaline ***29***
The title compound was obtained following the procedure described in reference [22] to prepare its 2-phenyl- (**28**) and 2-(2-thienyl)- (**30**) analogues. Compound **29** displayed the following ^{1}H-NMR (DMSO-d_6): 7.6–7.9 (m, 4H, H-7,8,3',5'), 8.0–8.1 (m, 3H, H-6,2',6'), 8.38 (d, 1H, H-9, J = 7.9 Hz), 9.54 (s, 1H, H-1).

2-Arylimidazo[1,2-a]quinoxalin-4-amines ***12–14***
A mixture of 4-chloro derivative **28–30** (0.7 mmol) in absolute ethanol (10 mL) saturated with ammonia was heated at 110°C for 20 h in a sealed tube. Upon cooling a solid precipitated; this was collected, washed with water and recrystallized.

The title compounds displayed the following spectral data: **12**: ^{1}H-NMR (DMSO-d_6): 7.2 (br s, 2H, NH_2), 7.3–7.6 (m, 6H, H-6-8,3'-5'), 8.0–8.2 (m, 3H, H-9,2',6'), 9.15 (s, 1H, H-1). IR: 3320, 3480. **13**: ^{1}H-NMR (DMSO-d_6): 7.2–7.6 (m, 7H, H-6-8,3',5' + NH_2), 8.0–8.1 (m, 3H, H-9,2',6'), 9.19 (s, 1H, H-1). IR: 3310, 3490. **14**: ^{1}H-NMR (DMSO-d_6): 7.2–7.6 (m, 8H, H-6-8,3'-5' + NH_2), 8.11 (d, 1H, H-9, J = 7.9 Hz), 9.02 (s, 1H, H-1). IR: 3160, 3320.

2-Phenyl-4-ethoxyimidazo[1,2-a]quinoxaline ***15***
A mixture of **28** (0.43 mmol) in an ethanolic solution of NaOH (4.3 mmol in 10 mL absolute ethanol) was heated at reflux for 30 min. Upon cooling a solid was obtained which was collected, washed with water and recrystallized. ^{1}H-NMR (DMSO-d_6): 1.51 (t, 3H, CH_3, J = 7.1 Hz), 4.67 (q, 2H, CH_2, J = 7.1 Hz), 7.3–7.7 (m, 5H, H-7,8,3',5'), 7.81 (d, 1H, H-6, J = 7.8 Hz), 8.0–8.1 (m, 2H, H-2',6'), 8.27 (d, 1H, H-9, J = 7.9 Hz), 9.29 (s, 1H, H-1).

1-(2'-Nitrophenyl)-2-phenylimidazole ***31***
Anhydrous K_2CO_3 (4.9 g) was added to a solution of 2-phenylimidazole (13.9 mmol) and 1-fluoro-2-nitrobenzene (34.6 mmol) in acetonitrile (20 mL). The mixture was heated at reflux for 80 h. Evaporation of the solvent at reduced pressure afforded a residue which was dissolved in $CHCl_3$ (250 mL). The organic solution was washed twice with water (200 mL each time), dried (anhydrous Na_2SO_4) and filtered through Norite. Evaporation of the $CHCl_3$ at reduced pressure afforded a resi-

Table II. Physical data of the newly synthesized compounds.

Compound	*Mp (°C) (solvent)*[a]	*Yield (%)*
1	> 300 (A)	20
2	> 300 (A)	47
3	255–257 (B)	73
4	> 300 (A)	65
5	> 300 (A)	80
6	> 300 (B)	60
7	> 300 (A)	36
8	> 300 (A)	30
9	> 300 (A)	40
10	> 300 (A)	36
12	216–218 (A)	67
13	> 300 (A)	85
14	250–251 (A)	54
15	139–141 (A)	75
16	292–293 (A)	50
17	204–205 (C)	40
19	275–276 (A)	50
20	> 300 (A)	30
21	247–248 (A)	55
22	> 300 (A)	54
23	> 300 (A)	64
24	> 300 (A)	65
25	> 300 (A)	54
26	> 300 (A)	50
27	> 300 (A)	40
29	257–258 (D)	40
31	168–169 (E)	75
32	127–128 (F)	75
33	289–290 (A)	80
34	171–173 (G)	40

[a]Recrystallization solvents: A = absolute ethanol; B = dimethylformamide; C = cyclohexane/ethyl acetate; D = column chromatography, eluting system: chloroform/ethyl acetate, 9:1; E = ethyl acetate; F = ethanol/water; G = ethanol/diethyl ether.

due which was triturated with diethyl ether containing a few drops of diethyl acetate, collected and then recrystallized. ^{1}H-NMR (DMSO-d_6): 7.2–7.3 (m, 6H, 5 ar + H-4), 7.53 (s, 1H, H-5), 7.7–7.9 (m, 3H, ar), 8.08 (d, 1H, H-3', J = 9.4 Hz).

1-(2'-Aminophenyl)-2-phenylimidazole **32**
Pd/C (0.34 mg) was added to a solution of **31** (4.3 mmol) in ethanol (200 mL). The mixture was hydrogenated in a Parr apparatus at 25 psi for 16 h. Elimination of the catalyst and evaporation of the solvent at reduced pressure afforded a waxy oil. When treated with diethyl ether, this gave rise to a white solid which was recrystallized. ^{1}H-NMR (DMSO-d_6): 4.9 (br s, 2H, NH_2), 6.5–6.6 (m, 1H, ar), 6.8–6.9 (m, 2H, ar), 7.1–7.3 (m, 6H, 4 ar + H-4,5), 7.4–7.5 (m, 2H, ar).

4,5-Dihydro-1-phenylimidazo[1,5-a]quinoxalin-4-one **33**
1,1'-Carbonyldiimidazole (Im_2CO) (4.5 mmol) was added to a solution of **32** (3 mmol) in 1,2-dichlorobenzene (20 mL). The mixture was heated at reflux under nitrogen flow for 3 h. Upon cooling a solid was obtained which was collected, washed with diethyl ether and recrystallized. ^{1}H-NMR (DMSO-d_6): 6.9–7.0 (m, 1H, ar), 7.0–7.1 (m, 1H, ar), 7.2–7.4 (m, 2H, ar), 7.5–7.7 (m, 5H, ar), 7.98 (s, 1H, H-3), 11.5 (br s, 1H, NH). IR: 1690.

2-Phenyl-4-chloroimidazo[1,5-a]quinoxaline **34**
N,N-Diethylaniline (0.3 mL) was added to a suspension of **33** (1.26 mmol) in $POCl_3$ (4 mL). The mixture was heated at reflux under nitrogen flow for 6 h. The excess of $POCl_3$ was evaporated at reduced pressure. The residue was treated twice with cyclohexane (20 mL) which was in its turn distilled off. The resulting oily residue became a crystalline white solid upon treatment with diethyl ether. ^{1}H-NMR (DMSO-d_6): 7.3–7.8 (m, 8H, ar), 7.91 (d, 1H, ar, J = 8.1 Hz), 8.18 (s, 1H, H-3).

2-Phenylimidazol[1,5-a]quinoxalin-4-amine **16**
A suspension of **34** (0.47 mmol) in isopropanol saturated with ammonia (10 mL) was heated at 110°C for 40 h in a sealed tube. By quenching the mixture with water (20 mL) a solid was obtained which was collected, washed with water and recrystallized. ^{1}H-NMR (DMSO-d_6): 6.8–6.9 (m, 1H, ar), 7.1–7.3 (m, 4H, 2 ar + NH_2), 7.4–7.5 (m, 1H, ar), 7.5–7.7 (m, 5H, ar), 8.03 (s, 1H, H-3). IR: 3160, 3340.

2-Phenylimidazo[1,5-a]quinoxalin-4-cyclopentylamine **17**
A mixture of **34** (0.6 mmol) and cyclopentylamine (0.9 mL) was heated at 120°C for 14 h in a sealed tube. Evaporation of the excess of cyclopentylamine at reduced pressure afforded a residue which was dissolved in $CHCl_3$ (20 mL). The solution was washed twice with water (20 mL each time) and dried (Na_2SO_4). Evaporation of the organic solvent at reduced pressure yielded a residue which was washed with a little diethyl ether and recrystallized. ^{1}H-NMR (DMSO-d_6): 1.5–1.8 (m, 6H, cyclopentyl protons), 2.0–2.2 (m, 2H, cyclopentyl protons), 4.5–4.6 (m, 1H, cyclopentyl proton), 6.8–6.9 (m, 1H, ar), 7.1–7.3 (m, 2H, ar), 7.5–7.7 (m, 7H, 6 ar + NH), 8.10 (s, 1H, H-3). IR: 3280.

Biochemistry

A_1 Receptor binding
Rat cerebral cortex was homogenized in ice-cold 0.32 M sucrose containing protease inhibitors (20 µg/mL soybean trypsin inhibitor, 200 µg/mL bacitracine, and 160 µg/mL benzamidine) in an ultra-turrax homogenizer. 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 resulting pellet was suspended in 10 volumes of ice-cold 40 mM Tris-HCl buffer at pH 7.7 containing 2 mM $MgCl_2$ and protease inhibitors (buffer T_1). It was then homogenized and centrifuged at 48 000 *g* for 15 min at 4°C.

The pellet was dispersed in 40 volumes of fresh T_1 buffer and incubated with adenosine deaminase (1 UI/mL) at 37°C for 60 min, then recentrifuged at 48 000 *g* for 15 min at 4°C.

The resulting pellet was frozen at –80°C until the time of assay.

The pellet was suspended in ice-cold T_1 buffer and A_1 binding assay was performed in triplicate by incubating at 25°C for 45 min in 0.5 mL T_1 buffer containing 1.3 nM [^{3}H]CHA in the absence or presence of unlabelled 10 µM *R*-phenylisopropyladenosine. The binding reaction was terminated by filtering through Whatman GF/B glass fiber filters under suction and washing twice with 5 mL ice-cold Tris-buffer. The filters were placed in scintillation vials and 4 mL Beckman Ready-Protein solvent scintillation fluid was added. The radioactivity was counted with an LS 1800 scintillation counter. Specific binding was obtained by subtracting non-specific binding from total binding and was approximated to 85–90% of the total binding.

A_{2a} receptor binding
Corpora striata were dissected from rat brain and the tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.5 containing protease inhibitors as reported above and 10 mM $MgCl_2$ (buffer T_2). The homogenate was centrifuged at 48 000 *g* for 10 min at 4°C. The pellet was then suspended in 20 volumes of Tris-buffer (T_2) containing adenosine deaminase (1 UI/mL) and incubated for 30 min at 37°C. The resulting pellet was diluted in 20 volumes of 50 mM Tris-HCl buffer at pH 7.5 containing 10 mM $MgCl_2$ and used in the binding assay.

A_{2a} binding assay was performed in triplicate, by incubating aliquots of the membrane fraction (0.2–0.3 mg protein) in Tris-HCl buffer at pH 7.5, with approximately 4 nM [^{3}H]CGS 21680 in a final volume of 0.5 mL. Incubation was carried out at 25°C for 90 min. Non-specific binding was defined in the presence of 10 µM CGS 21680. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filters were washed four times with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting non-specific binding from total binding and approximated to 85–90% of the total binding. The receptor-bound radioactivity was measured as described above.

Compounds were dissolved in ethanol or DMSO (buffer/concentration of 2%) and added to the assay mixture. Blank experiments were carried out to determine the effect of the solvent on binding.

Protein estimation was based on a reported method [24], after solubilization with 0.75 N sodium hydroxide, using bovine serum albumin as standard.

The concentration of tested compound that produce 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21680 binding (IC_{50}) was determined by log-probit analysis with seven concentrations of the displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according the equation [25]: $K_i = IC_{50}/(1 + [L]/K_d)$ where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]CHA binding to cortex membranes was 1.6 nM and K_d of [^{3}H]CGS 21680 binding to striatal membranes was 15 nM [18].

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