Tricyclic Heteroaromatic Systems. Synthesis and A_1 and A_{2a} Adenosine Binding Activities of Some 1-Aryl-1,4-dihydro-3-methyl[1]benzopyrano[2,3-c]pyrazol-4-ones, 1-Aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones, and 1-Aryl-1*H*-imidazo[4,5-*b*]quinoxalines

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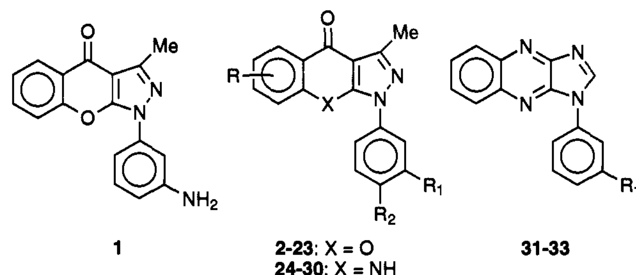
The syntheses and A_1 and A_{2a} adenosine binding activities of some new 1-aryl-1,4-dihydro-3-methyl[1]benzopyrano[2,3-c]pyrazol-4-ones, 1-aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones, and 1-aryl-1*H*-imidazo[4,5-*b*]quinoxalines are reported. Some compounds show A_1 adenosine receptor affinity and selectivity. Structure−activity relationships on these new classes of adenosine receptor ligands are defined.

Introduction

Extracellular adenosine receptors (AR) are divided into subtypes, A_1AR and A_2AR, which are distinguished by their different effect on adenyl cyclase.[1] Adenosine interaction with A_1AR inhibits adenyl cyclase, whereas interaction with A_2AR stimulates it. A_2AR can be further subdivided into high-affinity A_{2a}AR and low-affinity A_{2b}AR subtypes. Recently a fourth AR subtype, A_3AR, was cloned.[2−4] Interaction of adenosine with A_3-AR, as with A_1AR, inhibits adenyl cyclase,[2] and one of its physiological functions is to facilitate mast cell degranulation.[5]

The development of subtype-selective agonists and antagonists of AR, an active area of research, has aimed at providing tools with which to define the structural requirements of each receptor subtype. In recent years some research in our laboratory has been directed toward the synthesis of non-xanthine antagonists of the AR containing a six−six−five tricyclic ring system.[6−9] This program has led to the discovery of a new specific A_2AR antagonist,[7] 1-(3-aminophenyl)-3-methyl[1]benzopyrano[2,3-c]pyrazol-4-one (**1**) (see Chart 1). Compound 1 provided the lead of a series of analogues which are the object of this paper. In fact, to better understand the structural requirements for the anchoring of this new kind of ligand to the AR recognition sites, we hereby report the syntheses and the A_1 and A_{2a} binding activities of some new 1-aryl-1,4-dihydro-3-methyl[1]-benzopyrano[2,3-c]pyrazol-4-ones (**2−23**), their isosters 1-aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones (**24−30**), and some 1-aryl-1*H*-imidazo[4,5-*b*]quinoxalines (**31−33**).

Chart 1



Chemistry

The syntheses of the benzopyranopyrazoles **2−23** are illustrated in Schemes 1−3. Allowing the 3-acetyl-4-hydroxycoumarins **34−37**[10−11] to react with arylhydrazines, the arylhydrazones **38−44** were isolated. By heating **38−44** at reflux in glacial acetic acid, nucleophilic attack of the α-arylhydrazone nitrogen on the C-2 lactone carbonyl occurs, with consequent ring opening; thus the corresponding 1-aryl-4-(2-hydroxyaroyl)-3-methylpyrazol-5-ols **45−51** were obtained. Heating of the latter with an excess of $POCl_3$ yielded the 1-aryl-1,4-dihydro-3-methyl[1]benzopyrano[2,3-c]pyrazol-4-ones **2−8** (see Scheme 1).

The 7-methoxy- and 6-methoxybenzopyranopyrazoles **3−6** were demethylated with hydrobromic acid to yield the 7-hydroxy- and 6-hydroxy derivatives **9−12**. The 7-hydroxy derivative **10** was alkylated to give compounds **13−15** (see Scheme 2).

The 1-(3-nitroaryl) derivatives **2**, **4**, **6**, **8**, **10**, **13**, and **14** were catalytically reduced to the corresponding 1-(3-aminoaryl)-1,4-dihydro-3-methyl[1]benzopyrano[2,3-c]-pyrazol-4-ones **16−22**, while the previously reported A_2-selective antagonist 1-(3-aminophenyl)-3-methyl[1]-benzopyrano[2,3-c]pyrazol-4-one (**1**)[7] was transformed into the 1-[3-(benzylamino)phenyl] derivative **23** (see Scheme 3).

The syntheses of the pyrazoloquinolines **24−30** are illustrated in Schemes 4−5. Treatment of 2-acetonyl-4*H*-3,1-benzoxazin-4-one[12] with arylhydrazines gave the *N*-(1-aryl-3-methylpyrazol-5-yl)anthranilic acids **52−55**. By heating the latter with a mixture of P_2O_5 and poly(phosphoric acid), the 1-aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones **24−27** were obtained (see Scheme 4).

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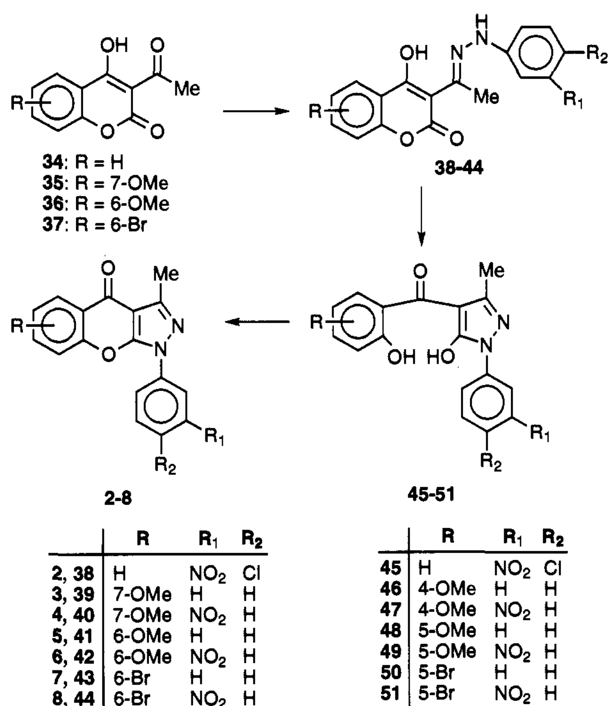
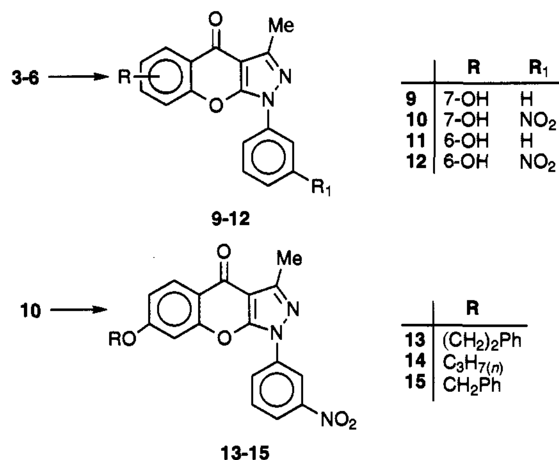
Scheme 1



	R	R_1	R_2
2, 38	H	NO_2	Cl
3, 39	7-OMe	H	H
4, 40	7-OMe	NO_2	H
5, 41	6-OMe	H	H
6, 42	6-OMe	NO_2	H
7, 43	6-Br	H	H
8, 44	6-Br	NO_2	H

	R	R_1	R_2
45	H	NO_2	Cl
46	4-OMe	H	H
47	4-OMe	NO_2	H
48	5-OMe	H	H
49	5-OMe	NO_2	H
50	5-Br	H	H
51	5-Br	NO_2	H

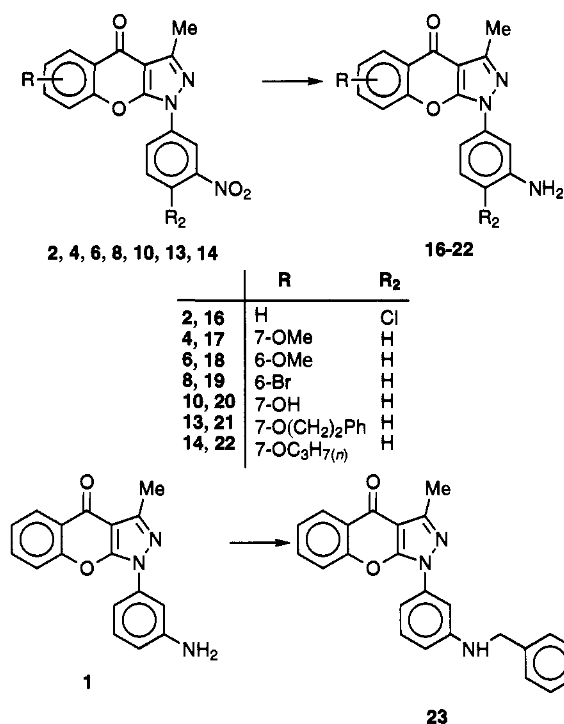
Scheme 2



	R	R_1
9	7-OH	H
10	7-OH	NO_2
11	6-OH	H
12	6-OH	NO_2

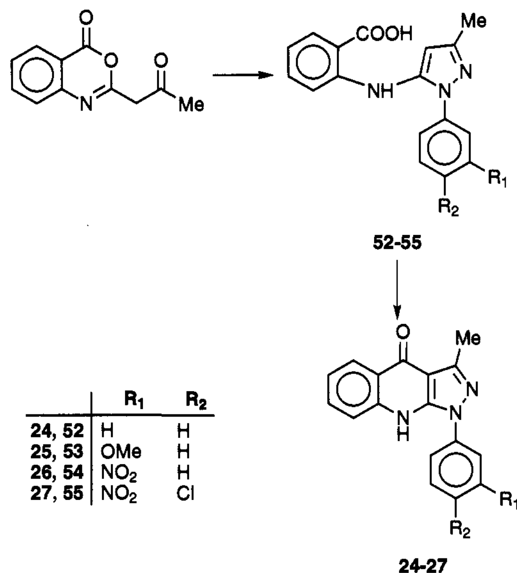
	R
13	$(CH_2)_2Ph$
14	$C_3H_{7(n)}$
15	CH_2Ph

Scheme 3



	R	R_2
2, 16	H	Cl
4, 17	7-OMe	H
6, 18	6-OMe	H
8, 19	6-Br	H
10, 20	7-OH	H
13, 21	7-O$(CH_2)_2$Ph	H
14, 22	7-O$C_3H_{7(n)}$	H

Scheme 4



	R_1	R_2
24, 52	H	H
25, 53	OMe	H
26, 54	NO_2	H
27, 55	NO_2	Cl

The 1-(3-methoxyphenyl) derivative **25** was demethylated to the corresponding 1-(3-hydroxyphenyl) compound **28**, while catalytic hydrogenation of the 1-(3-nitroaryl)pyrazoloquinolines **26** and **27** afforded the corresponding 1-(3-aminoaryl)-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones **29** and **30** (see Scheme 5).

Finally, the synthesis of the 1-arylimidazo[4,5-*b*]quinoxalines **31**–**33** is illustrated in Scheme 6. By reacting 3-chloroquinoxalin-2-amine[13] with suitable anilines, followed by treatment of the resulting hydrochloride with $NaHCO_3$, compounds **56**–**58** were isolated. Cyclization of **56**–**58** with triethyl orthoformate afforded the tricyclic derivatives **31**–**33**.

The chemical structures of all the newly synthesized compounds were determined by IR and ^{1}H NMR spectroscopy. The 4-oxo structure of compounds **24**–**30** is in agreement with the literature data.[14,15]

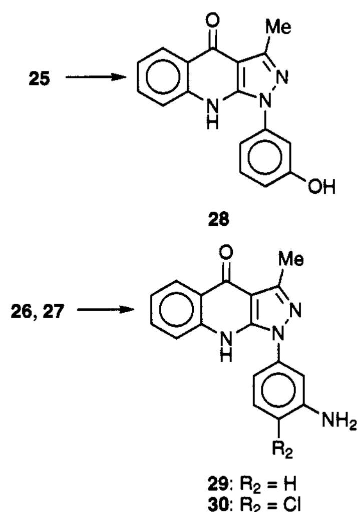
Biochemistry

Compounds **2**–**33** 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*-ethylcarbamoyl)adenosine (CGS 21680) on A_{2a}AR in rat striatal membranes. The A_1 and A_{2a} receptor affinities of the tested compounds, expressed as their K_i values, are listed in Table 1 together with that of the previously reported compound **1** which is included as a reference.

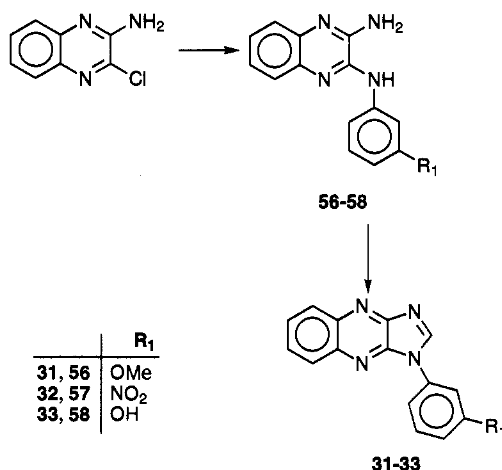
Results and Conclusions

Table 1 shows that the A_2AR affinity and selectivity of the lead structure **1** is completely lost in the compounds under study while A_1AR affinity and selectivity is widely distributed among them. The disappearance of the A_2 binding activity indicates that (i) the presence of the primary amino group not bearing a bulky ortho substituent is of paramount importance—when in **1** a hydrogen of the NH_2 is replaced with a benzyl, as in

Scheme 5



Scheme 6



23, or the NH_2 is ortho-substituted with a chlorine atom, as in **16**, there is either a 130-fold decrease in activity or complete inactivity at the A_2 receptor subtype, respectively; (ii) the presence of a substituent at the 6- or 7-position is detrimental for the A_2 binding activity of 1—compounds **17–22** show at least a 37-fold decreased A_2 affinity; clearly the 1-phenyl- and 1-(3-nitrophenyl) derivatives **2–15** are devoid of A_2 affinity; and (iii) the benzopyranopyrazolo moiety is also important for the A_2 binding activity—comparison of **1** with its isoster **29** shows that the latter has very low A_2 affinity like all the other pyrazoloquinolines (**24–28**, **30**). The 1-(3-methoxyphenyl) derivative **25** alone displays some A_2 binding activity, although compound **25** is 2.5-fold more active at the A_1AR. Similar considerations apply to the imidazoquinoxalines **31–33**.

The appearance of A_1 affinity and selectivity in most of the benzopyranopyrazoles **2–23** is clearly to be attributed to the presence of the substituent on the fused benzo moiety. This is demonstrated by comparison of the A_1 binding activity of the lead **1** with those of the 1-amino 6- or 7-substituted compounds **16–20** and **22**.

It may be hypothesized that the 6- or 7-substituent occupies a subregion of the A_1AR domain, thus shifting the affinity of the lead structure **1** from A_2 to A_1 subtype. It should be noted that in the 6- or 7-substituted benzopyranopyrazoles **3–5**, **7**, **9–12**, **17–20**, and **22** the 3-amino group on the 1-phenyl ring is not essential for the A_1 binding activity, since all the 1-phenyl (**3**, **5**, **7**, **9**, **11**) and some 1-(3-nitrophenyl) (**4**, **10**, **12**) derivatives display similar A_1 affinity to the corresponding 1-(3-aminophenyl) derivatives **17–20.**

Comparison of the A_1 affinity of the lead structure **1** with that of the 1-(3-amino-4-chlorophenyl) analogue **16** reveals that the ortho chloro on the 1-(3-aminophenyl) ring in this case is advantageous. There is a limited bulk tolerance in the accommodation of the substituents of the reported benzopyranopyrazoles on the A_1AR recognition site; the 7-methoxy compounds **3**, **4**, and **17** are well accommodated when they are bearing either a H, NO_2, or NH_2 group on the 1-phenyl ring. However the *n*-propoxy chain at position 7 is well tolerated only when there is a NH_2 on the 1-phenyl ring, as in **22**; otherwise, when the 1-phenyl ring bears the bulkier *m*-NO_2, as in **14**, the 7-*n*-propoxy chain is no longer tolerated.

These data suggest that the benzopyranopyrazole moiety occupies almost all the A_1AR recognition site and that little room is left for bulky substituents. The low A_1 binding activity of the 1-(3-aminophenyl) 7-(phenethyloxy) derivative **21** confirms this.

In conclusion, previous[8] and present data indicate that every modification made to the structure of the A_2-selective compound **1** led to loss of A_2 affinity and/or selectivity. In any event, the hereby reported analogues of **1** produced some selective and structurally novel A_1 adenosine receptor ligands which may serve as tools to further define structure–activity relationships in the anchoring of these new kinds of adenosine receptor ligands to the adenosine receptor subtypes.

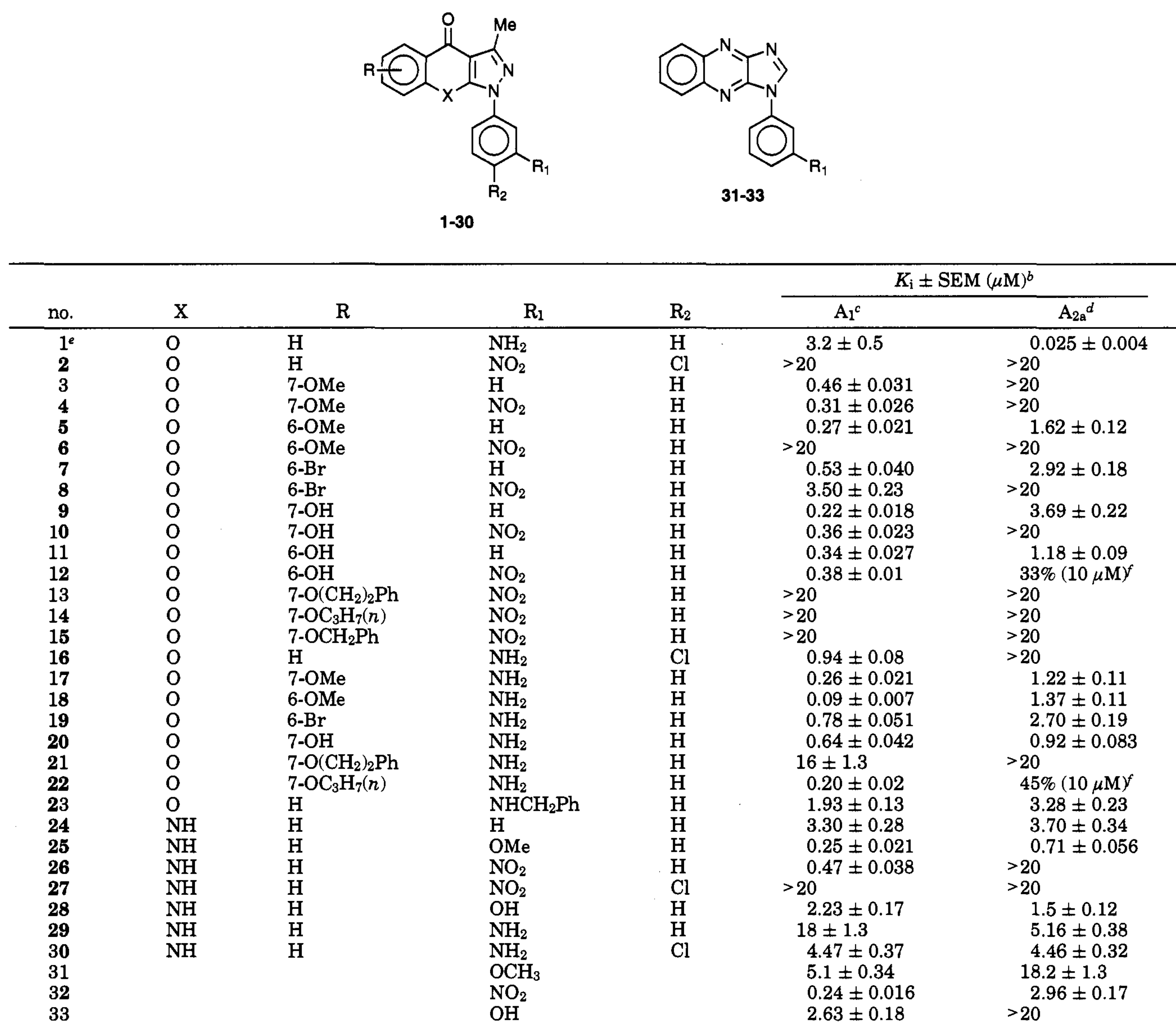
Experimental Section

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 Gallemkamp capillary melting point apparatus. The IR spectra were recorded on a Perkin-Elmer 1420 spectrometer in Nujol mull and are reported 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 using the following abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, and ar = aromatic proton(s). Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, and N, and the results are within ±0.4% of the theoretical values. The physical data of the newly synthesized compounds are listed in Table 2. Phenylhydrazine and other arylhydrazine hydrochlorides were commercially available except for (3-chloro-4-nitrophenyl)hydrazine, which was obtained as the hydrochloride following the procedure described in ref 16.

Arylhydrazones of 3-Acetyl-4-hydroxycoumarins 38–44. Phenylhydrazine or arylhydrazine hydrochloride (8.5 mmol), liberated in situ as free base with an equimolar amount of triethylamine, was added to a mixture of **34–37**[10–11] (8.5 mmol) in hot ethanol. The mixture was heated at reflux for 15 min. The resulting solid was isolated and recrystallized. Compound **38** displayed the following spectral data. IR: 3240, 1685, 1620, 1540, 1350. ^1H NMR (DMSO-d_6): 2.65 (s, 3H, CH_3), 7.15–7.25 (m, 1H, ar), 7.30–7.40 (m, 2H, ar), 7.50–7.58 (m, 1H, ar), 7.62–7.75 (m, 2H, ar), 7.95–8.05 (m, 1H, ar), 9.72 (s, 1H, NH).

1-Aryl-4-(2-hydroxyaroyl)-3-methylpyrazol-5-ols 45–51. A mixture of **38–44** (3.1 mmol) in glacial acetic acid (100 mL) was heated at reflux for 15 min. Elimination of the solvent at reduced pressure yielded a residue which was worked up with ethanol (10 mL). The resulting solid was collected and recrystallized. Compound **45** displayed the

Table 1. A_1 and A_{2a} Adenosine Binding Activity[a]



no.	X	R	R_1	R_2	K_i ± SEM (μM)[b] A_1[c]	A_{2a}[d]
1[e]	O	H	NH_2	H	3.2 ± 0.5	0.025 ± 0.004
2	O	H	NO_2	Cl	>20	>20
3	O	7-OMe	H	H	0.46 ± 0.031	>20
4	O	7-OMe	NO_2	H	0.31 ± 0.026	>20
5	O	6-OMe	H	H	0.27 ± 0.021	1.62 ± 0.12
6	O	6-OMe	NO_2	H	>20	>20
7	O	6-Br	H	H	0.53 ± 0.040	2.92 ± 0.18
8	O	6-Br	NO_2	H	3.50 ± 0.23	>20
9	O	7-OH	H	H	0.22 ± 0.018	3.69 ± 0.22
10	O	7-OH	NO_2	H	0.36 ± 0.023	>20
11	O	6-OH	H	H	0.34 ± 0.027	1.18 ± 0.09
12	O	6-OH	NO_2	H	0.38 ± 0.01	33% (10 μM)[f]
13	O	7-O(CH_2)$_2$Ph	NO_2	H	>20	>20
14	O	7-OC_3H_7(*n*)	NO_2	H	>20	>20
15	O	7-OCH_2Ph	NO_2	H	>20	>20
16	O	H	NH_2	Cl	0.94 ± 0.08	>20
17	O	7-OMe	NH_2	H	0.26 ± 0.021	1.22 ± 0.11
18	O	6-OMe	NH_2	H	0.09 ± 0.007	1.37 ± 0.11
19	O	6-Br	NH_2	H	0.78 ± 0.051	2.70 ± 0.19
20	O	7-OH	NH_2	H	0.64 ± 0.042	0.92 ± 0.083
21	O	7-O(CH_2)$_2$Ph	NH_2	H	16 ± 1.3	>20
22	O	7-OC_3H_7(*n*)	NH_2	H	0.20 ± 0.02	45% (10 μM)[f]
23	O	H	NHCH_2Ph	H	1.93 ± 0.13	3.28 ± 0.23
24	NH	H	H	H	3.30 ± 0.28	3.70 ± 0.34
25	NH	H	OMe	H	0.25 ± 0.021	0.71 ± 0.056
26	NH	H	NO_2	H	0.47 ± 0.038	>20
27	NH	H	NO_2	Cl	>20	>20
28	NH	H	OH	H	2.23 ± 0.17	1.5 ± 0.12
29	NH	H	NH_2	H	18 ± 1.3	5.16 ± 0.38
30	NH	H	NH_2	Cl	4.47 ± 0.37	4.46 ± 0.32
31			OCH_3		5.1 ± 0.34	18.2 ± 1.3
32			NO_2		0.24 ± 0.016	2.96 ± 0.17
33			OH		2.63 ± 0.18	>20

[a] The tests were carried out dissolving the tested compounds in DMSO (DMSO/buffer, 2%). [b] The K_i values are means ± SEM of four separate assays, each performed in triplicate. [c] A_1 binding was measured as displacement of [^{3}H]CHA binding. [d] A_{2a} binding was measured as displacement of [^{3}H]CGS 21680 binding. [e] Reference 7. [f] Percentage of inhibition (*I*%) of specific radioligand at the compound concentration shown in parentheses. Due to the compound insolubility, this concentration is the highest possible.

following spectral data. IR: 2800–2000, 1630, 1550, 1350. ^{1}H NMR (DMSO-d_6): 2.22 (s, 3H, CH_3), 6.5–7.2 (br s + m, 4H, 2H, ar + 2OH), 7.33–7.45 (m, 1H, ar), 7.56–7.65 (m, 1H, ar), 7.85 (d, 1H, ar, J = 8.9 Hz), 8.14–8.20 (m, 1H, ar), 8.57 (s, 1H, ar).

1-Aryl-1,4-dihydro-3-methyl[1]benzopyrano[2,3-*c*]pyrazol-4-ones 2–8. A suspension of **45–51** (3.1 mmol) in $POCl_3$ (10 mL) was heated in an oil bath at 80 °C for 15 min. Evaporation at reduced pressure of the excess of $POCl_3$ yielded an oily residue which was treated with chloroform (80 mL) and water (40 mL). The organic layer was washed three times with water (30 mL each time), dried (Na_2SO_4), and evaporated at reduced pressure to yield a residue which was recrystallized. Compound **2** displayed the following spectral data. IR: 1670, 1540, 1370. ^{1}H NMR ($CDCl_3$): 2.71 (s, 3H, CH_3), 7.45–7.75 (m, 4H, ar), 8.16–8.20 (m, 1H, ar), 8.35–8.40 (m, 1H, ar), 8.59 (s, 1H, ar).

1-Aryl-1,4-dihydro-7(or 6)-hydroxy-3-methyl[1]benzopyrano[2,3-*c*]pyrazol-4-ones 9–12. To a suspension of the 7- or 6-methoxy derivative **3–6** (3.3 mmol) in glacial acetic acid (10 mL) was added hydrobromic acid (48%, 25 mL). The mixture was heated at reflux for 12 h and then cooled. The resulting precipitate was collected by filtration, washed with water, and recrystallized. Compound **9** displayed the following spectral data. IR: 3145, 1650, 1620, 1540. ^{1}H NMR (DMSO-d_6): 2.57 (s, 3H, CH_3), 6.92–6.99 (m, 2H, ar), 7.40–7.50 (m, 1H, ar), 7.58–7.68 (m, 2H, ar), 7.90–7.94 (m, 2H, ar), 8.0–8.05 (m, 1H, ar), 10.88 (s, 1H, OH).

7-(Alkyloxy)-1,4-dihydro-3-methyl-1-(3-nitrophenyl)[1]-benzopyrano[2,3-*c*]pyrazol-4-ones 13 and 14. To a suspension of **10** (4.4 mmol) in 2-butanone (60 mL) were added K_2CO_3 (13.2 mmol) and phenethyl bromide (13.2 mmol) or *n*-propyl bromide (22.2 mmol). The mixture was heated at reflux for 10 h. Evaporation of the solvent at reduced pressure yielded a residue which was treated with water (20 mL) to eliminate the excess of carbonate, filtered, and recrystallized. Compound **13** displayed the following spectral data. IR: 1670, 1630, 1540, 1350. ^{1}H NMR (DMSO-d_6): 2.58 (s, 3H, CH_3), 3.13 (t, 2H, CH_2, J = 7.3 Hz), 4.86 (t, 2H, OCH_2, J = 7.3 Hz), 7.05–7.15 (m, 1H, ar), 7.25–7.40 (m, 6H, ar), 7.82–7.92 (m, 1H, ar), 8.05 (d, 1H, ar, J = 8.9 Hz), 8.25–8.30 (m, 1H, ar), 8.45–8.50 (m, 1H, ar), 8.65 (t, 1H, ar, J = 2.2 Hz).

7-(Benzyloxy)-1,4-dihydro-3-methyl-1-(3-nitrophenyl)-[1]benzopyrano[2,3-*c*]pyrazol-4-one (15). A mixture of equimolar amounts (1.04 mmol) of **10**, sodium ethoxide, and benzyl bromide in ethanol (10 mL) was heated at reflux for

Table 2. Physical Data of the Newly Synthesized Compounds

compd	mp, °C (solvent)[a]	% yield	compd	mp, °C (solvent)[a]	% yield
2	230–232 (A)	67	**28**	293–296 (J)	28
3	178–179 (B)	80	**29**	>300 (K)	94
4	228–231 (A)	75	**30**	265–268 (A)	88
5	160–163 (B)	91	31	197–198 (L)	90
6	229–232 (A)	86	**32**	290–291 (E)	80
7	160–162 (C)	55	33	280–282 (M)	87
8	204–205 (A)	72	**38**	233–234 (A)	98
9	>300 (D)	77	**39**	211–212 (B)	90
10	>300 (E)	90	**40**	241–243 (C)	98
11	275–278 (D)	74	**41**	193–196 (B)	78
12	>300 (E)	80	**42**	210–213 (E)	67
13	>300 (C)	70	**44**	238–239 (A)	57
14	212–216 (F)	70	**45**	220–221 (A)	75
15	193–195 (D)	82	**46**	234–236 (A)	90
16	196–199 (G)	47	**47**	225–226 (A)	75
17	169–171 (B)	70	**48**	165–168 (A)	74
18	185–188 (B)	46	**49**	218–221 (A)	89
19	183–186 (B)	62	**50**	203–204 (B)	93
20	>300 (E)	70	**51**	250–251 (A)	80
21	140–143 (C)	54	**52**[b]	212–214 (C)	83
22	190–194 (B)	65	**53**	155–159 (H)	40
23	141–143 (B)	15	**54**	216–218 (C)	64
24	275–277 (B)	56	**55**	243–245 (B)	64
25	214–218 (H)	20	**56**	190–192 (C)	75
26	>300 (B)	32	**57**	>300 (B)	90
27	>300 (I)	63	**58**	273–275 (N + B)	60

[a] Recrystallization solvents: A = glacial acetic acid; B = ethanol; C = ethyl acetate; D = dioxane; E = dimethylformamide; F = nitromethane; G = ethyl acetate/glacial acetic acid; H = cyclohexane/ethyl acetate; I = ethanol/glacial acetic acid; J = ethyl acetate ethanol; K = product was washed with a saturated solution of $NaHCO_3$ and then with acetone; L = acetone; M = ethanol/dimethylformamide; N = column chromatography, eluting system chloroform/methanol, 9:1. [b] Reference 17; mp 214–216 °C from ethanol.

2.5 h. The solid resulting from the cooled mixture was collected, washed with water, and recrystallized. IR: 1675, 1630, 1540, 1355. ^{1}H NMR ($CDCl_3$): 2.70 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 7.09–7.15 (m, 2H, ar), 7.35–7.50 (m, 5H, ar), 7.68–7.80 (m, 1H, ar), 8.20–8.36 (m, 3H, ar), 8.83–8.90 (m, 1H, ar).

1-(3-Aminoaryl)-1,4-dihydro-3-methyl[1]benzopyrano[2,3-*c*]pyrazol-4-ones 16–22. Method A. To a solution of the nitro derivative (1.2 mmol) in glacial acetic acid (150 mL) was added 40%, w/w, Pd/C (10%). The mixture was hydrogenated in a Parr apparatus at 30 psi for 20 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a residue which was recrystallized. Following this method compounds, **16–19** were prepared from **2**, **4**, **6**, and **8**, respectively.

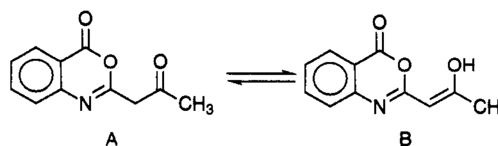
Method B. To a solution of the nitro derivative (1.4 mmol) in ethyl acetate (200 mL) was added 40% Pd/C (10%). The mixture was hydrogenated in a Parr apparatus at 20 psi for 16 h. Elimination of the catalyst and and evaporation at reduced pressure of the solvent yielded an oily residue which was worked up with cyclohexane (20 mL) to yield a solid. The crude product was collected by filtration and recrystallized. Following this method, compound **21** was prepared from **13**.

Method C. To a solution of the nitro derivative (0.6 mmol) in dimethylformamide (40 mL) was added 60%, w/w, Pd/C (10%). The mixture was hydrogenated in a Parr apparatus at 30 psi for 16 h. The catalyst was filtered off, and the reaction was quenched with water (200 mL). The resultant precipitate was collected by filtration, washed thoroughly with diethyl ether, and recrystallized. Following this method, compounds **20** and **22** were prepared from **10** and **14**, respectively.

Compound **16** displayed the following spectral data. IR: 3480, 3350, 3220, 1675, 1535. ^{1}H NMR ($CDCl_3$): 2.70 (s, 3H, CH_3), 4.1 (br s, 2H, NH_2), 7.20–7.56 (m, 5H, ar), 7.65–7.71 (m, 1H, ar), 8.35–8.39 (m, 1H, ar).

1-[3-(Benzylamino)phenyl]-1,4-dihydro-3-methyl[1]benzopyrano[2,3-*c*]pyrazol-4-one (23). A mixture of 1 (1.7 mmol), benzyl chloride (5.2 mmol), and K_2CO_3 (2.6 mmol) in anhydrous dimethylformamide (6 mL) was heated under stirring in an oil bath at 80 °C for 15 h. The reaction was quenched with water (10 mL), and the resultant oil was extracted three times with chloroform (15 mL each time). The combined organic extracts were dried (Na_2SO_4), and the solvent was evaporated at reduced pressure to yield an oil which was purified by column chromatography, eluting system cyclohexane/ethyl acetate (6:4). Evaporation at reduced pressure of the solvents of the middle eluates afforded an oily residue which yielded a solid when worked up with ethanol. IR: 3400, 3380, 1660, 1620, 1540. ^{1}H NMR ($CDCl_3$): 2.70 (s, 3H, CH_3), 4.4 (br s, 1H, NH), 4.43 (s, 2H, CH_2), 6.64–6.68 (m, 1H, ar), 7.10–7.48 (m, 10H, ar), 7.64–7.74 (m, 1H, ar), 8.35 (d, 1H, ar, J = 7.8 Hz).

2-Acetonyl-4*H*-3,1-benzoxazin-4-one.[12] Equimolar amounts (29 mmol) of anthranilic acid and 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (diketene acetone adduct) in xylene (6 mL) were heated at 100 °C for 4 h while distilling off acetone. Xilene was then distilled off at reduced pressure. The resulting solid was suspended in CCl_4 (12 mL) and acetic anhydride (5 mL) and heated at 80 °C for 5 h. The cooled mixture yielded a solid which was collected and recrystallized from acetonitrile. Mp: 120–122 °C (lit.[12] mp 121–122 °C). Yield: 40%. IR: 1770, 1650. ^{1}H NMR analysis indicated that in solution (DMSO-d_6) the product was a mixture of the tautomeric keto (A) and enol (B) forms in a ratio of approximately 1.3:1, respectively: (A) 2.29 (s, CH_3), 4.01 (s, CH_2); (B) 2.10 (s, CH_3), 5.36 (s, =CH), 10.7 (s, OH), 7.05–8.15 (m, 4H, ar).



***N*-(1-Aryl-3-methylpyrazol-5-yl)anthranilic Acids 52–55.** 2-Acetonyl-4*H*-3,1-benzoxazin-4-one[12] (5 mmol) was added to a solution of equimolar amount of hydrazine free base (when noncommercialy available, the free base was obtained from the corresponding hydrochloride and triethylamine in diethyl ether or by treating the hydrochloride with sodium acetate in water) in ethanol (20 mL) and heated at reflux for 30 min. The solid was isolated by filtration, washed with acetonitrile, and recrystallized. Compound **52** displayed the following spectral data. IR: 3300–2000, 1685. ^{1}H NMR (DMSO-d_6): 2.25 (s, 3H, CH_3), 6.25 (s, 1H, H-4 pyrazole), 6.75–6.85 (m,

1H, ar), 7.04 (d, 1H, ar, J = 8.3 Hz), 7.30–7.60 (m, 6H, ar), 7.88 (d, 1H, ar, J = 7.9 Hz), 9.89 (s, 1H, NH), 13.2 (br s, 1H, COOH).

1-Aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones 24–27. A mixture of **52–55** (4 mmol) in poly(phosphoric acid) (about 10 g) and P_2O_5 (5 g) was heated under stirring in an oil bath at 90 °C for 6 h. The reaction was quenched with ice and water, and the resulting solid was collected and recrystallized. Compound **24** displayed the following spectral data. IR: 3460, 3240, 1650, 1600. ^{1}H NMR (DMSO-d_6): 2.58 (s, 3H, CH_3), 7.25–7.36 (m, 1H, ar), 7.52–7.75 (m, 7H, ar), 8.21 (d, 1H, ar, J = 7.7 Hz), 11.75 (s, 1H, NH).

4,9-Dihydro-1-(3-hydroxyphenyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-one (28). Hydrobromic acid (48%, 20 mL) was added to a solution of **25** (2.6 mmol) in glacial acetic acid (7 mL). The mixture was heated at reflux for 10 h. The reaction was quenched with water to yield a solid which was collected and recrystallized. IR: 3500–2000, 1630, 1600. ^{1}H NMR (DMSO-d_6): 2.58 (s, 3H, CH_3), 6.93 (m, 1H, ar), 7.06–7.13 (m, 2H, ar), 7.22–7.48 (m, 2H, ar), 7.62–7.78 (m, 2H, ar), 8.21 (d, 1H, ar, J = 7.9 Hz), 10.0 (br s, 1H, OH), 11.77 (s, 1H, NH).

1-(3-Aminophenyl)-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-one (29). To a solution of **26** (2.2 mmol) in ethanol (150 mL) was added Pd/C (10%, 0.28 g). The mixture was hydrogenated in a Parr apparatus at 20 psi for 16 h. Elimination of the catalyst and evaporation of the solvent at reduced pressure yielded a residue which did not bear heat and thus could not be recrystallized. IR: 3400, 3260, 1640, 1600. ^{1}H NMR (DMSO-d_6): 2.55 (s, 3H, CH_3), 5.5 (br s, 2H, NH_2), 6.60–6.85 (m, 3H, ar), 7.20–7.31 (m, 2H, ar), 7.55–7.76 (m, 2H, ar), 8.20 (d, 1H, ar, J = 7.8 Hz), 11.6 (br s, 1H, NH).

1-(3-Amino-4-chlorophenyl)-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-one (30). To a solution of **27** (2.3 mmol) in glacial acetic acid (100 mL) was added Pd/C (10%, 0.32 g). The mixture was hydrogenated in a Parr apparatus at 20 psi for 16 h. Elimination of the catalyst and evaporation of the solvent at reduced pressure yielded a residue which was recrystallized. IR: 3440, 3360, 1640, 1595. ^{1}H NMR (DMSO-d_6): 2.55 (s, 3H, CH_3), 5.7 (br s, 2H, NH_2), 6.80–6.86 (m, 1H, ar), 7.06–7.08 (m, 1H, ar), 7.28–7.31 (m, 1H, ar), 7.40–7.45 (m, 1H, ar), 7.60–7.80 (m, 2H, ar), 8.20 (d, 1H, ar, J = 7.8 Hz), 11.73 (s, 1H, NH).

3-(Arylamino)quinoxalin-2-amines 56–58. A mixture of 3-chloroquinoxalin-2-amine[13] (2.8 mmol) and the suitable aniline (3.1 mmol) was heated in a sublimation apparatus at 170 °C. The reaction was monitored by TLC (chloroform/methanol, 9:1), and the heating was carried on until the starting chloroquinoxalinamine had disappeared. Upon cooling the solution precipitated the hydrochloride of the title compound which was worked up with diethyl ether/acetone (1:1, 5 mL), collected by filtration, and recrystallized from ethanol. To a suspension of the hydrochloride (1.0 mmol) in hot water (30 mL), an equimolar amount of $NaHCO_3$ was carefully added. The free base precipitated and was collected by filtration, washed with water, and recrystallized. Compound **56** displayed the following spectral data. IR: 3450, 3180, 1625. ^{1}H NMR (DMSO-d_6): 3.81 (s, 3H, OCH_3), 6.64 (dd, 1H, ar, J = 10.67, 2.49 Hz), 7.0 (br s, 2H, NH_2), 7.24–7.33 (m, 3H, ar), 7.41–7.56 (m, 3H, ar), 7.79–7.81 (m, 1H, ar), 9.65 (s, 1H, NH).

1-Aryl-1*H*-imidazo[4,5-*b*]quinoxalines (31–33). A mixture of **56–58** (1.0 mmol) in an excess of triethyl orthoformate (1.8 mL) was heated at 120 °C. The reaction was monitored by TLC (chloroform/methanol, 9:1), and the heating was carried on until the starting material had disappeared. The residue was worked up with a small amount of diethyl ether, collected by filtration, and recrystallized. Compound **31** displayed the following spectral data. IR: 3070, 1610, 1595. ^{1}H NMR (DMSO-d_6): 3.90 (s, 3H, OCH_3), 7.12 (dd, 1H, ar, J = 10.38, 1.75 Hz), 7.56–7.71 (m, 3H, ar), 7.84–7.90 (m, 2H, ar), 8.18–8.28 (m, 2H, ar), 9.62 (s, 1H, H-2).

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 ultraturrax homogenizer. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant again centrifuged at 48000*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). Then it was homogenized and centrifuged at 48000*g* for 15 min at 4 °C.

The pellet was dispersed in 40 volumes of fresh T_1 buffer, incubated with adenosine deaminase (1 IU/mL) at 37 °C for 60 min, and then recentrifuged at 48000*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 the A_1 binding assay was performed in triplicate by incubating at 25 °C for 45 min in 0.5 mL of T_1 buffer containing 1.3 nM [^{3}H]-CHA in the absence or presence of unlabeled 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 of ice-cold Tris buffer. The filters were placed in scintillation vials, and 4 mL of Beckman Ready-Protein solvent scintillation fluid was added. The radioactivity was counted with a LS 1800 scintillation counter. Specific binding was obtained by subtracting nonspecific binding from total binding and 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 48000*g* for 10 min at 4 °C, the pellet then being suspended in 20 volumes of Tris buffer(T_2) containing adenosine deaminase (1 IU/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.

The A_{2a} binding assay was performed in triplicate, by incubating aliquots of the membrane fraction (0.2–0.3 mg of 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. Nonspecific 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 nonspecific 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 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,[18] after solubilization with 0.75 N sodium hydroxide, using bovine serum albumine as standard.

The concentration of tested compound that produced 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 to the equation:[19] $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ligand concentration and K_d is its dissociation constant. K_d of [^{3}H]CHA binding to cortex membranes was 1.6 nM, and the K_d of [^{3}H]CGS 21680 binding to striatal membranes was 15 nM.[7]

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