Synthesis and Structure−Activity Relationships of a New Set of 2-Arylpyrazolo[3,4-*c*]quinoline Derivatives as Adenosine Receptor Antagonists

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In a recent paper (Colotta et al. *J. Med. Chem.* **2000**, *43*, 1158−1164) we reported the synthesis and adenosine receptor binding activity of two sets of 2-aryl-1,2,4-triazolo[4,3-*a*]quinoxalines (**A** and **B**) some of which were potent and selective A_1 or A_3 antagonists. In this paper the synthesis of a set of 2-arylpyrazolo[3,4-*c*]quinolin-4-ones **1**−**10**, 4-amines **11**−**18**, and 4-amino-substituted derivatives **19**−**35** are reported. The binding activity at bovine A_1 and A_{2A} and human cloned A_3 adenosine receptors showed that (i) the substituent on the appended 2-phenyl ring could be used to modulate A_1 and A_3 affinity, (ii) the 4-amino group was necessary for A_1 and A_{2A} binding activity, and (iii) a nuclear or extranuclear C═O proton acceptor at position 4 yielded potent and selective A_3 antagonists. These results are in agreement with those of the previously reported series **A** and **B** suggesting a similar adenosine receptor binding mode. In particular, the A_3 nanomolar affinity of **1**−**8**, **31**−**33**, and **35** confirms the hypothesis of the presence in the N-6 region of the adenosine A_3 subtype of a proton donor able to bind to a C═O proton acceptor at position 4.

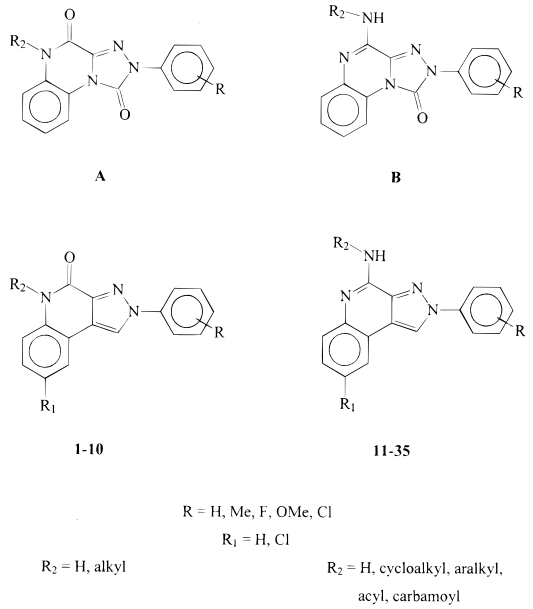
Introduction

Adenosine is thought to mediate a wide variety of effects by interacting with four subtypes of adenosine receptors (AR): A_1, A_{2A}, A_{2B}, and A_3. All four AR subtypes are coupled via a G-protein to the enzyme adenylcyclase in either an inhibitory (A_1 and A_3 subtypes) or stimulatory manner (A_{2A} and A_{2B} subtypes).[1] For A_1, A_{2A}, and A_3 AR, selective agonists and antagonists are now available,[2−7] while the A_{2B} subtype still lacks selective ligands.

In recent years, the potential therapeutic use of selective AR subtype antagonists as renal protective,[8,9] anti-Parkinson,[10] antiinflammatory, antiasthmatic, and antiischemic agents[11−14] has attracted great attention. Since most of the AR antagonists are nitrogen-containing heterocyclic compounds, some research in our laboratory has been directed toward the synthesis of tricyclic heteroaromatic systems as AR antagonists.[15−19]

In a recent paper[20] we reported the synthesis and binding activity at bovine A_1 and A_{2A} AR and at human cloned A_3 AR of two sets of 2-aryl-1,2,4-triazolo[4,3-*a*]-quinoxalines (**A** and **B**). Some of these **A** and **B** compounds were potent and selective A_1 or A_3 antagonists. A structure−activity relationship (SAR) study on the 1,4-dione and 4-amino-1-one series (see **A** and **B**, respectively, Chart 1) showed that the 4-NH_2 proton donor group was essential for A_1 and A_{2A} receptor−ligand interaction while it was not necessary for A_3 recognition. The binding results indicated also that the presence of a 4-oxo function (series **A**) or of an acyl substituent on the 4-amino group (series **B**) afforded some potent and selective A_3 receptor antagonists. Moreover, in both series the nature and the position of the substituent on the 2-phenyl ring could be used to modulate the A_1/A_3 selectivity. To verify whether this structural requirement could be applied to tricyclic systems of similar size and shape, we report in this paper the synthesis and A_1, A_{2A}, and A_3 AR binding activity of some 2-arylpyrazolo[3,4-*c*]quinolin-4-ones **1**−**10** and of their corresponding 4-amines **11**−**18** and 4-amino-substituted derivatives **19**−**35**, which can be considered the 1-decarbonyl analogues of series **A** and **B**, respectively.

Chart 1. Previously and Hereby Reported Adenosine Receptor Antagonists



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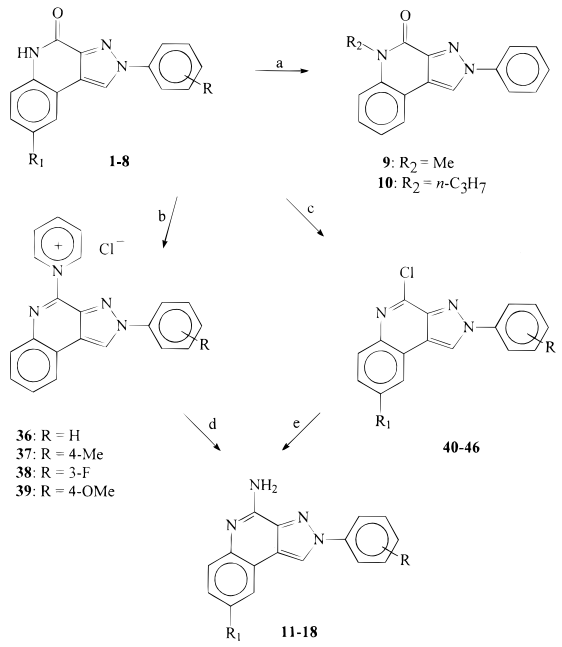
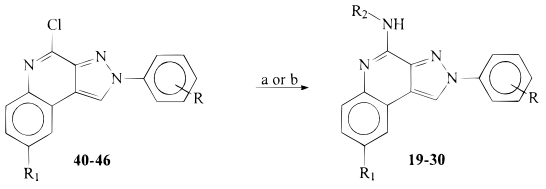
Scheme 1[a]



	R	R1		R	R1
1, 11, 40	H	H	5, 15, 44	4-Me	H
2, 12, 41	H	Cl	6, 16, 45	3-F	H
3, 13, 42	2-Me	H	7, 17	4-OMe	H
4, 14, 43	3-Me	H	8, 18, 46	4-Cl	H

[a] (a) NaH, R_1X, DMF; (b) $PCl_5/POCl_3$, pyridine; (c) $PCl_5/POCl_3$; (d) method A: cyclohexylamine; (e) method B: NH_3(g), absolute EtOH.

Scheme 2[a]



	R	R1	R2
19	H	H	
20	H	Cl	
21	3-Me	H	
22	4-Me	H	
23	3-F	H	
24	4-Cl	H	
25	H	H	
26	2-Me	H	
27	3-Me	H	
28	3-F	H	
29	H	H	CH_2Ph
30	H	H	$(CH_2)_2Ph$

[a] (a) Method A: excess of R_2NH_2; (b) method B: R_2NH_2, Et_3N, absolute EtOH.

Chemistry

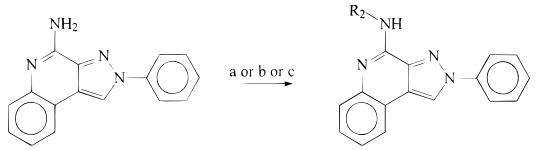
The synthetic pathways which yielded compounds **1**−**37** are illustrated in Schemes 1−3.

The synthesis of **1**, **2**, **4**−**7**, and **9**, which were originally prepared as benzodiazepine receptor ligands, has already been reported.[21] The 2-(2-methylphenyl) derivative **3** and its 2-(4-chlorophenyl) analogue **8** were obtained by reacting the 3-ethoxalylindole[22] with arylhydrazine hydrochlorides as described to prepare **1**, **2**, and **4**−**7**.[21] The 5-*N*-*n*-propyl derivative **10** ensued by the reaction of **1** with *n*-propyl bromide following the procedure described to prepare **9**.[21] Reaction of **1** and **5**−**7** with a mixture of $PCl_5/POCl_3$ and pyridine afforded the 1-(2-aryl-2*H*-pyrazolo[3,4-*c*]quinolin-4-yl)pyridinium chlorides **36**−**39**, while the reaction of **1**−**6** and **8** with a neat mixture of $PCl_5/POCl_3$ gave the 2-aryl-4-chloro-2*H*-pyrazolo[3,4-*c*]quinolines **40**−**46**. It must be noted that both the pyridinium salts **36**−**39** and the 4-chloro derivatives **40**−**46** were unstable; nevertheless they were pure enough to be spectroscopically characterized and used without further purification. Refluxing **36**−**39** with an excess of cyclohexylamine gave the 2-aryl-2*H*-pyrazolo[3,4-*c*]quinolin-4-amines **11** and **15**−**17**. Compound **11** was also obtained with more satisfactory yields from its corresponding 4-chloro derivative **40** and ammonia. Thus, the other 4-amino derivatives **12**−**14** and **18** were prepared following this pathway, i.e., from the corresponding 4-chloro intermediates **41**−**43** and **46** and ammonia (Scheme 1).

Allowing the 4-chloro intermediates **40**−**46** to react with suitable amines gave the 4-*N*-cycloalkylamines **19**−**28** and 4-*N*-aralkylamines **29** and **30** (Scheme 2).

Finally, Scheme 3 depicts the reaction of 2-phenyl-2*H*-pyrazolo[3,4-*c*]quinolin-4-amine **11** with suitable acyl chlorides or phenylacetic acid, or with suitable isocyanates, to afford the 4-amido **31**−**33** and 4-ureido derivatives **34** and **35**, respectively.

Scheme 3[a]



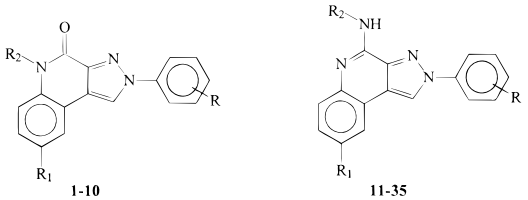
	R2
31	COMe
32	COPh
33	$COCH_2Ph$
34	CONHPh
35	$CONHCH_2Ph$

[a] (a) RCOCl, pyridine, CH_2Cl_2; (b) $PhCH_2COOH$, 1-hydroxybenzotriazole, Et_3N, 4-(dimethylamino)pyridine, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, DMF; (c) RNCO, THF.

Biochemistry

Compounds **1**−**35** were tested for their ability to displace [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) from A_1 AR in bovine cerebral cortical membranes, [^{3}H]-2-[[4-(2-carboxyethyl)phenethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680) from A_{2A} AR in bovine striatal membranes, and [^{125}I]N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine ([^{125}I]AB-MECA) from human cloned A_3 AR stably expressed in CHO cells. In fact, due to the species differences in A_3 primary amino acid sequence, new A_3 AR ligands have to be tested on cloned human A_3 receptors.[23−25] On the contrary, for A_1 and A_{2A} AR subtypes there is a good amino acid sequence homology,[1] since standard antagonists, as theophylline and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), showed an affinity at bovine A_1 and A_{2A} receptors comparable to those reported at the cloned human ones.[26−28]

Table 1. Adenosine Receptor Binding Activity



				K_i (nM) or % inhibition[a]		
	R	R_1	R_2	A_1[b]	A_{2A}[c]	A_3[d]
1	H	H	H	2100 ± 170	8600 ± 710	30.8 ± 2.6
2	H	Cl	H	284 ± 23	52%	44.1 ± 3.2
3	2-Me	H	H	3200 ± 260	0%	79.2 ± 6.3
4	3-Me	H	H	830 ± 75	17%	5.0 ± 0.4
5	4-Me	H	H	3900 ± 290	22%	3.2 ± 0.2
6	3-F	H	H	583 ± 49	0%	45.3 ± 3.9
7	4-OMe	H	H	1078 ± 29	18%	3.2 ± 0.2
8	4-Cl	H	H	464 ± 39	35%	2.9 ± 0.1
9	H	H	Me	1230 ± 110	47%	118 ± 10
10	H	H	*n*-C_3H_7	950 ± 86	50%	68.4 ± 5.3
11	H	H	H	69.2 ± 4.8	331 ± 26	551 ± 34
12	H	Cl	H	86.1 ± 6.3	5750 ± 480	309 ± 23
13	2-Me	H	H	520 ± 38	1600 ± 140	3600 ± 280
14	3-Me	H	H	129 ± 9.5	3750 ± 310	99.3 ± 7.8
15	4-Me	H	H	215 ± 14	21%	188 ± 15
16	3-F	H	H	110 ± 8.9	49.5 ± 3.2	788 ± 69
17	4-OMe	H	H	238 ± 20	3700 ± 310	90.2 ± 7.3
18	4-Cl	H	H	2290 ± 180	11200 ± 960	150 ± 12
19	H	H		8.6 ± 0.6	3800 ± 320	707 ± 53
20	H	Cl		27.1 ± 2.2	4140 ± 380	2128 ± 170
21	3-Me	H		22.2 ± 1.8	11400 ± 980	44.1 ± 3.7
22	4-Me	H		71.9 ± 5.6	13%	115 ± 9.1
23	3-F	H		54.7 ± 4.1	5750 ± 510	155 ± 12
24	4-Cl	H		478 ± 39	8%	56.5 ± 4.5
25	H	H		3.2 ± 0.24	849 ± 75	60.5 ± 4.8
26	2-Me	H		148 ± 13	329 ± 26	152 ± 13
27	3-Me	H		40.7 ± 31	1200 ± 94	22.3 ± 1.6
28	3-F	H		9.3 ± 0.8	2210 ± 180	34%
29	H	H	CH_2Ph	132 ± 11	5400 ± 380	35.8 ± 2.8
30	H	H	$(CH_2)_2Ph$	60.1 ± 5.2	5051 ± 460	32.9 ± 2.3
31	H	H	COMe	75.9 ± 6.4	2400 ± 190	48.2 ± 3.7
32	H	H	COPh	42%	3%	2.1 ± 0.1
33	H	H	$COCH_2Ph$	107 ± 9.3	13%	9.9 ± 0.8
34	H	H	CONHPh	1500 ± 138	32%	108 ± 9.6
35	H	H	$CONHCH_2Ph$	186 ± 15	5%	8.3 ± 0.7
Theophylline				3800 ± 340	21000 ± 1800	86000 ± 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 in bovine striatal membranes or percentage of inhibition (*I*%) of specific binding at 20 μM concentration. [d] Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in CHO cells or percentage of inhibition (*I*%) of specific binding at 1 μM concentration.

The binding results of **1**−**35**, together with those of theophilline and DPCPX included as antagonist reference compounds, are shown in Table 1.

Results and Discussion

The A_1, A_{2A}, and A_3 binding results of compounds **1**−**35** displayed in Table 1 show that the syntheses of these 2-arylpyrazolo[3,4-*c*]quinolines have produced some potent and selective A_1 (**19**, **25**, **28**) and A_3 (**4**, **5**, **7**, **8**, **32**) antagonists and only one compound (**16**) showed good A_{2A} affinity.

The A_3 affinity and selectivity of the 2-arylpyrazolo-[3,4-*c*]quinolin-4-ones **1**−**10** is noteworthy. In fact, compounds **1**−**8** showed low A_1 affinity, were inactive at the A_{2A}, and displayed nanomolar adenosine A_3 receptor affinity. Alkylation at position 5 of the parent compound **1** afforded compounds **9** and **10** which showed slightly increased A_1 and decreased A_3 affinity with respect to **1**.

The influence of the 8-chloro substituent on the benzo-fused moiety and that of simple substituents at different positions of the 2-phenyl ring was investigated in the 4-ones **1**−**8**, 4-amines **11**−**18**, and 4-*N*-cycloalkylamino derivatives **19**−**28**. It is well-known[5,17,29−31] that the AR affinity of ligands of similar size and shape can be enhanced by the presence of chlorine atom on the benzo-fused moiety in a position corresponding to our 8 position. However, in our pyrazoloquinolines a chlorine atom at position 8 (compounds **2**, **12**, **20**) did not elicit the expected beneficial effect on either AR subtypes. In fact, the A_1, A_{2A}, and A_3 affinities of **2**, **12**, and **20** were either unaffected or decreased by the presence of the 8-chloro substituents with the exception of the lone 8-chloro-2-phenylpyrazoloquinolin-4-one **2** which showed an A_1 affinity 7-fold higher than that of the 8-unsubstituted compound **1**.

The presence of a substituent on the 2-phenyl ring affected AR subtype affinities differently. In general, the presence of substituent on the appended 2-phenyl ring negatively affected A_1 and A_{2A} potency, while the A_3 affinity was dependent on the nature and position of the substituent.

A methyl group in the *ortho*-position (compounds **3**, **13**, **26**) decreased the affinity at all three AR subtypes, with the only exception being the A_{2A} affinity of the 4-*N*-cyclopentylamino derivative **26** (K_i = 329.6 nM) which was 2.5-fold more active than its corresponding 4-*N*-cyclopentyl-2-phenyl parent compound **25** (K_i = 849 nM).

The electron-donating 3-methyl substituent (compounds **4**, **14**, **21**, **27**) increased the A_3 affinity, while it decreased the A_1 and A_{2A} ones, with the only exception being compound **4** which was 2.5-fold more active at the A_1 receptor than its parent compound **1**. The electron-withdrawing fluorine atom at the *meta*-position of the 2-phenyl ring (compounds **6**, **16**, **23**, **28**) had contrasting effects on the affinity at all three AR subtypes. In fact, these 2-(3-fluorophenyl) derivatives showed a decreased affinity at all three AR subtypes with respect to those of the corresponding 2-phenyl-unsubstituted compounds (**1**, **11**, **19**, **25**). There were three exceptions: the A_1 affinity of **6**, the A_{2A} of **16**, and the A_3 of **23** which were on the contrary enhanced. It must be noted that

compound **16** is the only one reported in this paper that showed A_{2A} activity (K_i = 49.5 nM). We should also like to point out the A_1 affinity of the 4-*N*-cyclopentyl-2-(3-fluorophenyl) **28** (K_i = 9.3 nM) which, although less active at this subtype than its corresponding parent compound **25** (K_i = 3.2 nM), is a highly A_1/A_3-selective antagonist.

The electron-donating 4-methyl group (compounds **5**, **15**, **22**) and 4-methoxy group (compounds **7**, **17**) and the electron-withdrawing 4-chloro substituent (compounds **8**, **18**, **24**) on the appended 2-phenyl moiety enhanced the A_3 affinity in particular in the 4-one series (see **5**, **7**, **8** vs **1**). On the contrary, all the 2-(4-phenyl-substituted) derivatives were inactive at the A_{2A} subtype. A similar negative effect of the *p*-phenyl substituent can be observed in the A_1 affinity, with the only exception being the *para*-substituted 4-one derivatives **7** and **8** which showed a 2- and 4.5-fold enhancement, respectively, in A_1 affinity with respect to that of the parent compound **1**.

We would like to highlight the contrasting effect of the substituent on the 2-phenyl ring toward A_1 affinity in the 4-one series **1**−**8** and 4-amino series **11**−**28**: in compounds **4** and **6**−**8** the effect was positive while in amines **11**−**28** the effect was always negative. Instead, the A_3 affinity in both the 4-one (**1**−**8**) and 4-amino (**11**−**28**) series was enhanced by the presence of a *para*-substituent, of whatever nature, or by the electron-donating 3-methyl substituent on the appended 2-phenyl ring.

Replacement of the 4-oxo function with the 4-amino group yielded nonselective AR ligands. In fact, the pyrazoloquinolin-4-amines **11**−**18**, as a whole, displayed higher A_1 and A_{2A} receptor affinities than the corresponding 4-one derivatives **1**−**8**. These latter, on the contrary, were more active than **11**−**18** on the A_3 subtype. These SAR are in accordance with those of the previously reported series **A** and **B**[20] and confirm the importance of the 4-amino proton donor group in A_1 and A_{2A}[17,20] and the 4-carbonyl group in A_3[20] receptor−ligand interactions.

Finally, a hydrogen atom of the 4-amino group of the parent 2-phenyl-2*H*-pyrazolo[3,4-*c*]quinolin-4-amine **11** was replaced by an aralkyl (compounds **29**, **30**), an acyl (compounds **31**−**33**), and a carbamoyl residue (compounds **34**, **35**). All these 4-N-substituted compounds **29**−**35** displayed low or null A_{2A} affinity. The aralkyl derivatives **29** and **30** displayed nanomolar A_1 and A_3 affinities and, as a consequence, were not A_1/A_3-selective antagonists. Among the 4-amido derivatives **31**−**33**, the 4-acetamido **31** was an A_1/A_3 nonselective ligand while the 4-benzoylamido **32** was the most potent and selective A_3 antagonist among those tested in this study. Homologation of **32** gave the 4-phenylacetamido **33**, which although still A_3 potent (K_i = 9.9 nM) was also active at the A_1 subtype (K_i = 107.2 nM) and as a consequence was less A_3/A_1-selective than **32**. The importance of the C═O amide group at position 4 in A_3 receptor−ligand interaction is shown by comparing the A_3 affinity of the 4-*N*-benzoylamido **32** (K_i = 2.1 nM) vs the 4-*N*-benzylamino **29** (K_i = 35.8 nM) and the 4-*N*-phenylacetamido **33** (K_i = 9.9 nM) vs the 4-*N*-phenethylamino **30** (K_i = 32.9 nM). By replacing one hydrogen atom of the 4-amino group of **11** with a carbamoyl residue the ureido derivatives **34** and **35** were prepared. Compounds **34** and **35** were both less active at the A_1 and A_{2A} receptors while they were more active at the A_3 subtype than **11**, further confirming the importance of the C═O group at position 4 for A_3 affinity.

In conclusion, the SAR of compounds **1**−**35** were in accordance with those of the previously reported series **A** and **B**[20] and confirmed some different structural requirements of each AR subtype recognition site. Similarly as in the **A** and **B** series compounds **1**−**35** were little active at the A_{2A} receptor and the substituent on the 2-phenyl ring affected differently the A_1 and A_3 affinities. The comparison of A_1 and A_{2A} affinities of the 4-amino derivatives **11**−**18** with those of the 4-ones **1**−**8** confirmed that in these tricyclic ligands the 4-amino group is essential for A_1 and A_{2A} receptor−ligand interaction.[20] The nanomolar A_3 affinity of compounds **1**−**8**, **31**−**33,** and **35** stressed the importance for A_3 receptor recognition of the presence at position 4 of either a nuclear (as in 4-ones **1**−**8**) or extranuclear (as in amides **32** and **33** or ureide **35**) carbonyl group.[20] Finally, the similarity of the SAR of **1**−**35**, **A**, and **B** suggests a similar AR binding mode. Since we have hypothesized that in compounds **B** the N-4 region corresponds to that of the N-6 of adenosine,[20] we may hypothesize that in compounds **11**−**35** as well the N-4 region corresponds to the adenosine N-6 one. It follows that the A_3 nanomolar affinity of **1**−**8**, **31**−**33**, and **35** could be due to the presence in the N-6 region of the adenosine A_3 subtype of a proton donor able to bind to the C═O proton acceptor, which should explain the A_3 activity of **1**−**8**, **31**−**33**, and **35** and of the other A_3 antagonists reported in the literature.[5−7,32]

Experimental Section

(A) 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 values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm^{-1}. The ^1H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent that was always DMSO-d_6. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, and ar = aromatic protons. Physical data of the newly synthesized compounds are listed in Table 2.

General Procedure for the Synthesis of 4,5-Dihydro-2-(2-methylphenyl)-2*H*-pyrazolo[3,4-*c*]quinolin-4-one (3) and 2-(4-Chlorophenyl)-4,5-dihydro-2*H*-pyrazolo[3,4-*c*]-quinolin-4-one (8). The title compounds were obtained from 3-ethoxalylindole[22] (1.0 g, 4.6 mmol) and the suitable arylhydrazine hydrochloride (10.1 mmol) as described in ref 21 to prepare **1, 2**, and **4**−**7**. The title compounds displayed the following spectral data.

3: ^1H NMR 2.27 (s, 3H, CH_3), 7.23−7.57 (m, 7H, ar), 7.98 (d, 1H, ar, J = 7.6 Hz), 9.04 (s, 1H, H-1), 11.48 (s, 1H, NH); IR 3140, 1670.

8: ^1H NMR 7.24−7.40 (m, 3H, ar), 7.72 (d, 2H, ar, J = 8.9 Hz), 7.95 (d, 1H, ar, J = 7.7 Hz), 8.07 (d, 2H, ar, J = 8.9 Hz), 9.51 (s, 1H, H-1), 11.51 (s, 1H, NH); IR 3160, 1680.

4,5-Dihydro-2-phenyl-5-*N*-*n*-propyl-2*H*-pyrazolo[3,4-*c*]-quinolin-4-one (10). The title compound was obtained from **1** (0.250 g, 0.96 mmol) and *n*-propyl bromide (0.124 mL, 1.44 mmol) as described in ref 21 to prepare **9**. The title compound

Table 2. Physical Data of Newly Synthesized Compounds

	R	R_1	R_2	mp, °C	solv[a]	% yield
3	2-Me	H	H	> 300	A	60
8	4-Cl	H	H	> 300	B	43
10	H	H	*n*-C_3H_7	140-143	C	62
11	H	H	H	197-198	D	56[b], 90[c]
12	H	Cl	H	235-237	E	80
13	2-Me	H	H	186-188	F	50
14	3-Me	H	H	218-220	E	60
15	4-Me	H	H	235-237	D	35
16	3-F	H	H	217-220	F	45
17	4-OMe	H	H	193-194	E	40
18	4-Cl	H	H	243-246	F	64
19	H	H		138-140	G	58
20	H	Cl		165-168	E	57
21	3-Me	H		146-148	E	35
22	4-Me	H		147-149	F	50
23	3-F	H		152-155	E	88
24	4-Cl	H		180-183	F	83
25	H	H		104-106	H	35
26	2-Me	H		100-102	I	40
27	3-Me	H		96-98	I	45
28	3-F	H		143-145	G	77
29	H	H	CH_2Ph	128-130	E	45
30	H	H	$(CH_2)_2Ph$	125-127	G	60
31	H	H	COMe	231-233	E	98
32	H	H	COPh	223-225	J	98
33	H	H	$COCH_2Ph$	240-242	K	62
34	H	H	CONHPh	255-257	B	47
35	H	H	$CONHCH_2Ph$	242-243	B	70

[a] Recrystallization solvents: A = 1-ethoxyethanol; B = glacial acetic acid; C = cyclohexane/ethyl acetate; D = ethyl acetate; E = ethanol; F = methanol; G = cyclohexane; H = *n*-hexane; I = cyclohexane/petroleum ether; J = acetonitrile; K = 2-butanone. [b] From pyridinium chloride **36**. [c] From 4-chloro derivative **40**.

displayed the following ^{1}H NMR data: 0.99 (t, 3H, CH_3, J = 7.2 Hz), 1.65−1.80 (m, 2H, CH_2), 4.30 (t, 2H, N−CH_2, J = 7.5 Hz), 7.29−7.68 (m, 6H, ar), 8.03−8.07 (m, 3H, ar), 9.51 (s, 1H, H-1).

General Procedure for the Synthesis of 1-(2-Aryl-2*H*-pyrazolo[3,4-*c*]quinolin-4-yl)pyridinium Chlorides 36−39. A mixture of **1**, **5**−**7** (1.38 mmol) and phosphorus pentachloride (0.085 g, 0.41 mmol) in phosphorus oxychloride (8 mL) and anhydrous pyridine (0.3 mL, 3.71 mmol) was heated at reflux for 30 min. The cooled mixture was quenched with ice/water (about 50 mL) to yield a solid which was collected and washed with water. These pyridinium chlorides were very unstable; nevertheless they were pure enough to be characterized and used without further purification. The ^{1}H NMR data of the title compounds are as follows.

36: 7.56−7.77 (m, 3H, ar), 7.82−7.98 (m, 2H, pyridinium H-3 and H-5), 8.24−8.30 (m, 3H, ar), 8.50−8.57 (m, 3H, ar), 9.02 (t, 1H, pyridinium H-4, J = 7.7 Hz), 10.19−10.23 (m, 3H, H-1 + pyridinium H-2 and H-6).

37: 2.44 (s, 3H, CH_3), 7.53 (d, 2H, ar, J = 8.6 Hz), 7.82−7.98 (m, 2H, pyridinium H-3 and H-5), 8.16 (d, 2H, ar, J = 8.6 Hz), 8.27 (d, 1H, ar, J = 8.2 Hz), 8.49−8.56 (m, 3H, ar), 9.02 (t, 1H, pyridinium H-4, J = 7.6 Hz), 10.12 (s, 1H, H-1), 10.21 (d, 2H, pyridinium H-2 and H-6, J = 6.8 Hz).

38: 7.40−7.96 (m, 4H, 2 ar + pyridinium H-3 and H-5), 8.15−8.29 (m, 3H, ar), 8.45−8.57 (m, 3H, ar), 9.02 (t, 1H, pyridinium H-4, J = 7.6 Hz), 10.21−10.30 (m, 3H, H-1 + pyridinium H-2 and H-6).

39: 3.90 (s, 3H, OCH_3), 7.26 (d, 2H, ar, J = 8.9 Hz), 7.82−7.94 (m, 2H, pyridinium H-3 and H-5), 8.17−8.29 (m, 3H, ar), 8.49−8.56 (m, 3H, ar), 9.01 (t, 1H, pyridinium H-4, J = 7.9 Hz), 10.09 (s, 1H, H-1), 10.21 (d, 2H, pyridinium H-2 and H-6, J = 6.7 Hz).

General Procedure for the Synthesis of 2-Aryl-4-chloro-2*H*-pyrazolo[3,4-*c*]quinolines 40−46. A mixture of **1**−**6**, **8** (5.35 mmol) and phosphorus pentachloride (0.335 g, 1.61 mmol) in phosphorus oxychloride (50 mL) was heated at reflux for 2 h. Evaporation at reduced pressure of the excess of phosphorus oxychloride yielded a residue which was treated with cold water (50 mL) and quickly collected. These 4-chloro derivatives were very unstable; nevertheless they were pure enough to be characterized and used without further purification. The ^{1}H NMR data of the title compounds are as follows.

40: 7.54−7.80 (m, 5H, ar), 8.01 (d, 1H, ar, J = 7.0 Hz), 8.14 (d, 2H, ar, J = 7.3 Hz), 8.33 (d, 1H, ar, J = 7.7 Hz), 9.86 (s, 1H, H-1).

41: 7.56−7.75 (m, 4H, ar), 8.00−8.12 (m, 3H, ar), 8.46 (d, 1H, H-9, J = 2.4 Hz), 9.89 (s, 1H, H-1).

42: 2.23 (s, 3H, CH_3), 7.47−7.70 (m, 6H, ar), 7.97−8.02 (m, 1H, ar), 8.30−8.34 (m, 1H, ar), 9.36 (s, 1H, H-1).

43: 2.49 (s, 3H, CH_3), 7.38 (d, 1H, ar, J = 8.1 Hz), 7.55 (t, 1H, ar, J = 7.9 Hz), 7.60−7.77 (m, 3H, ar), 8.33 (d, 1H, ar, J = 8.9 Hz), 9.84 (s, 1H, H-1).

44: 2.42 (s, 3H, CH_3), 7.48 (d, 2H, ar, J = 8.0 Hz), 7.68−7.74 (m, 2H, ar), 8.01−8.04 (m, 3H, ar), 8.32 (d, 1H, ar, J = 7.4 Hz), 9.81 (s, 1H, H-1).

45: 7.37−7.45 (m, 1H, ar), 7.62−7.80 (m, 3H, ar), 7.98−8.05 (m, 3H, ar), 8.29 (dd, 1H, ar, J = 7.6, 2.3 Hz), 9.89 (s, 1H, H-1).

46: 7.70−7.79 (m, 4H, ar), 8.02 (d, 1H, ar, J = 7.7 Hz), 8.19 (d, 2H, ar, J = 8.9 Hz), 8.31 (d, 1H, ar, J = 8.3 Hz), 9.89 (s, 1H, H-1).

General Procedure for the Synthesis of 2-Aryl-2*H*-pyrazolo[3,4-*c*]quinolin-4-amines 11−18. Method A (11, 15−17). A mixture of **36**−**39** (0.75 mmol) in cyclohexylamine (0.85 mL) was heated at reflux for 30 min. The resulting oil was treated with water (200 mL) and extracted with diethyl ether (200 mL). The organic phase was washed with water (2 × 100 mL) and dried (Na_2SO_4). Evaporation at reduced pressure of the solvent yielded a residue which was treated with diethyl ether and collected. Compounds **11** and **16** were directly recrystallized, while compounds **15** and **17**, before recrystallization, were further purified by column chromatography, eluting system chloroform/methanol (9:1).

Method B (11−14, 18). A mixture of **40**−**43**, **46** (2 mmol) in absolute ethanol (20 mL) saturated with ammonia was heated overnight at 120 °C in a sealed tube. Upon cooling, the mixture yielded solid crude **11**−**14** and **18**, which were collected, washed with water and recrystallized. The spectral data of the title compounds are as follows.

11: ^{1}H NMR 6.93 (br s, 2H, NH_2), 7.19−7.61 (m, 6H, ar), 8.01 (d, 1H, ar, J = 7.8 Hz), 8.12 (d, 2H, ar, J = 8.0 Hz), 9.50 (s, 1H, H-1); IR 3500, 3300, 3100, 1690.

12: ^{1}H NMR 7.08 (br s, 2H, NH_2), 7.41−7.70 (m, 5H, ar), 8.06−8.10 (m, 3H, ar), 9.57 (s, 1H, H-1); IR 3490, 3310, 1660.

13: ^{1}H NMR 2.26 (s, 3H, CH_3), 6.92 (br s, 2H, NH_2), 7.25 (t, 1H, ar, J = 7.8 Hz), 7.35−7.59 (m, 6H, ar), 8.00 (d, 1H, ar, J = 7.9 Hz), 9.03 (s, 1H, H-1); IR 3360, 3220, 3180, 1650.

14: ^{1}H NMR 2.46 (s, 3H, CH_3), 6.95 (br s, 2H, NH_2), 7.20−7.56 (m, 5H, ar), 7.88−8.02 (m, 3H, ar), 9.48 (s, 1H, H-1); IR 3490, 3310, 1650.

15: ^{1}H NMR 2.39 (s, 3H, CH_3), 6.93 (br s, 2H, NH_2), 7.22−7.53 (m, 5H, ar), 7.97−8.01 (m, 3H, ar), 9.44 (s, 1H, H-1); IR 3470, 3300, 1650.

16: ^{1}H NMR 6.99 (br s, 2H, NH_2), 7.24−7.72 (m, 5H, ar), 7.95−8.02 (m, 3H, ar), 9.56 (s, 1H, H-1); IR 3460, 3290, 1640.

17: ^{1}H NMR 3.85 (s, 3H, OCH_3), 6.93 (br s, 2H, NH_2), 7.15−7.25 (m, 3H, ar), 7.38−7.52 (m, 2H, ar), 7.98−8.02 (m, 3H, ar), 9.37 (s, 1H, H-1); IR 3450, 3300, 1640.

18: ^{1}H NMR 6.97 (br s, 2H, NH_2), 7.24−7.50 (m, 3H, ar), 7.78 (d, 2H, ar, J = 9.0 Hz), 8.00 (d, 1H, ar, J = 7.4 Hz), 8.16 (d, 2H, ar, J = 8.9 Hz), 9.54 (s, 1H, H-1); IR 3470, 3310, 1650.

General Procedure for the Synthesis of 4-*N*-Amino-substituted-2-aryl-2*H*-pyrazolo[3,4-*c*]quinolines 19−30. Method A (19, 20, 23, 25, 28−30). A mixture of **40**−**41**, **45** (2 mmol) and the suitable cycloalkyl- or aralkylamine (2 mL) was heated overnight at 120 °C in a sealed tube. The cooled mixture was treated with diethyl ether (20 mL) affording a solid which was eliminated. Evaporation at reduced pressure of the clear solution gave a solid which was collected and treated with cyclohexane to yield the crude title compounds. All crude compounds but one (**25**) were directly recrystallized from suitable solvent. Compound **25**, before recrystallization, was purified by column chromatography, eluting system chloroform/ethyl acetate (9:1). Evaporation of the central eluates gave an oil which became solid by treatment with petroleum ether.

Method B (21, 22, 24, 26, 27). A mixture of **42**−**44**, **46** (2 mmol), the suitable cycloalkylamine (2.4 mmol) and triethylamine (0.55 mL, 4 mmol) in absolute ethanol (20 mL) was heated overnight at 120 °C in a sealed tube. Evaporation of the solvent at reduced pressure yielded a solid which was treated with petroleum ether, collected and washed with water. Crude compounds **22** and **24** were directly recrystallized, while crude compounds **21** and **26**−**27**, before recrystallization, were purified by column chromatography, eluting system chloroform/ethyl acetate (9:1). Evaporation of the central eluates gave an oil which became solid by treatment with petroleum ether. The spectral data of the title compounds are as follows.

19: ^{1}H NMR 1.19−2.06 (m, 10H, cyclohexyl protons), 4.23−4.38 (m, 1H, cyclohexyl proton), 6.98 (d, 1H, NH, J = 8.7 Hz), 7.17−7.69 (m, 6H, ar), 8.00 (d, 1H, ar, J = 7.7 Hz), 8.13 (d, 2H, ar, J = 7.2 Hz), 9.48 (s, 1H, H-1); IR 3430.

20: ^{1}H NMR 1.07−2.09 (m, 10H, cyclohexyl protons), 4.15−4.35 (m, 1H cyclohexyl proton), 7.18 (d, 1H, NH, J = 8.3 Hz), 7.37 (dd, 1H, ar, J = 8.7, 2.4 Hz), 7.46−7.58 (m, 2H, ar), 7.66 (t, 2H, ar, J = 7.3 Hz), 8.07−8.17 (m, 3H, ar), 9.60 (s, 1H, H-1); IR 3430.

21: ^{1}H NMR 1.10−1.85 (m, 8H, cyclohexyl protons), 2.00−2.08 (m, 2H, cyclohexyl protons), 2.45 (s, 3H, CH_3), 4.18−4.35 (m, 1H, cyclohexyl proton), 6.97 (d, 1H, NH, J = 8.1 Hz), 7.16−7.56 (m, 5H, ar), 7.88−7.99 (m, 3H, ar), 9.45 (s, 1H, H-1); IR 3440.

22: ^{1}H NMR 1.10−1.83 (m, 8H, cyclohexyl protons), 2.00−2.10 (m, 2H, cyclohexyl protons), 2.42 (s, 3H, CH_3), 4.18−4.48 (m, 1H, cyclohexyl proton), 6.94 (d, 1H, NH, J = 7.9 Hz), 7.21 (t, 1H, ar, J = 7.4 Hz), 7.33−7.56 (m, 4H, ar), 7.96−8.04 (m, 3H, ar), 9.45 (s, 1H, H-1); IR 3440.

23: ^{1}H NMR 1.10−2.02 (m, 10H, cyclohexyl protons), 4.22−4.34 (m, 1H, cyclohexyl proton), 7.06 (d, 1H, NH, J = 8.6 Hz), 7.17−7.45 (m, 3H, ar), 7.52−7.75 (m, 2H, ar), 7.92−8.09 (m, 3H, ar), 9.55 (s, 1H, H-1); IR 3440.

24: ^{1}H NMR 1.10−1.81 (m, 8H, cyclohexyl protons), 2.00−2.01 (m, 2H, cyclohexyl protons), 4.20−4.29 (m, 1H, cyclohexyl proton), 7.01 (d, 1H, NH, J = 8.9 Hz), 7.17−7.55 (m, 3H, ar), 7.72 (d, 2H, ar, J = 8.9 Hz), 7.95 (d, 1H, ar, J = 7.0 Hz), 8.16 (d, 2H, ar, J = 8.9 Hz), 9.50 (s, 1H, H-1); IR 3440.

25: ^{1}H NMR 1.60−1.74 (m, 6H, cyclopentyl protons), 2.02−2.10 (m, 2H, cyclopentyl protons), 4.68−4.71 (m, 1H, cyclopentyl proton), 7.16−7.25 (m, 2H, 1 ar + NH), 7.35−7.68 (m, 5H, ar), 7.99 (d, 1H, ar, J = 7.7 Hz), 8.13 (d, 2H, ar, J = 7.5 Hz), 9.49 (s, 1H, H-1); IR 3430.

26: ^{1}H NMR 1.50−1.88 (m, 6H, cyclopentyl protons), 1.92−2.16 (m, 2H, cyclopentyl protons), 2.26 (s, 3H, CH_3), 4.60−4.79 (m, 1H, cyclopentyl proton), 7.16−7.59 (m, 8H, 7 ar + NH), 7.98 (d, 1H, ar, J = 7.6 Hz), 9.02 (s, 1H, H-1); IR 3360.

27: ^{1}H NMR 1.56−1.78 (m, 6H, cyclopentyl protons), 2.00−2.09 (m, 2H, cyclopentyl protons), 2.51 (s, 3H, CH_3), 4.58−4.76 (m, 1H, cyclopentyl proton), 7.13−7.57 (m, 6H, 5 ar + NH), 7.85−7.98 (m, 3H, ar), 9.45 (s, 1H, H-1); IR 3420.

28: ^{1}H NMR 1.55−1.79 (m, 6H, cyclopentyl protons), 2.05−2.09 (m, 2H, cyclopentyl protons), 4.50−4.70 (m, 1H, cyclopentyl proton), 7.18−7.43 (m, 4H, 3 ar + NH), 7.53−7.74 (m, 2H, ar), 7.93−8.09 (m, 3H, ar), 9.54 (s, 1H, H-1); IR 3440.

29: 4.84 (d, 2H, CH_2, J = 6.2 Hz), 7.22−7.67 (m, 11H, ar), 7.98−8.02 (m, 4H, 3 ar + NH), 9.50 (s, 1H, H-1); IR 3430.

30: ^{1}H NMR 3.07 (t, 2H, CH_2, J = 7.7 Hz), 3.80−3.87 (m, 2H, CH_2), 7.21−7.69 (m, 12H, 11 ar + NH), 8.01 (d, 1H, ar, J = 7.6 Hz), 8.11 (d, 2H, ar, J = 7.6 Hz), 9.50 (s, 1H, H-1); IR 3410.

General Procedure for the Synthesis of 4-*N*-Acetamido-2-phenyl-2*H*-pyrazolo[3,4-*c*]quinoline (31) and 4-*N*-Benzamido-2-phenyl-2*H*-pyrazolo[3,4-*c*]quinoline (32). A solution of acetyl chloride or benzoyl chloride (4 mmol) in anhydrous dichloromethane (3 mL) was slowly added at 0 °C to a suspension of **11** (0.520 g, 2 mmol) in anhydrous dichloromethane (10 mL) and anhydrous pyridine (1.61 mL, 20 mmol). The mixture was stirred at room temperature for 24 h. Evaporation at reduced pressure of the solvent yielded a residue which was treated with ethanol/water (10 mL), collected and recrystallized. The title compounds displayed the following spectral data.

31: ^{1}H NMR 2.39 (s, 3H, CH_3), 7.51−7.70 (m, 5H, ar), 7.84−7.89 (m, 1H, ar), 8.13−8.24 (m, 3H, ar), 9.70 (s, 1H, H-1), 10.31 (br s, 1H, NH); IR 3400, 3140, 1680.

32: ^{1}H NMR 7.42−7.90 (m, 12H, 11 ar + NH), 8.03 (d, 2H, ar, J = 7.5 Hz), 8.29 (d, 1H, ar, J = 7.5 Hz), 9.80 (s, 1H, H-1); IR 3160, 1695.

4-*N*-Phenylacetamido-2-phenyl-2*H*-pyrazolo[3,4-*c*]quinoline (33). A mixture of **11** (0.260 g, 1 mmol), phenylacetic acid (0.816 g, 6 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1.150 g, 6 mmol), 1-hydroxybenzotriazole (0.810 g, 6 mmol), triethylamine (2.08 mL, 15 mmol) and 4-(dimethylamino)pyridine (0.012 g, 0.1 mmol) in anhydrous DMF (5 mL) was stirred at room temperature for 2 h. The resulting solid was collected, washed with water (10 mL) and recrystallized. The title compound displayed the following spectral data: ^{1}H NMR 4.06 (s, 2H, CH_2), 7.27−7.71 (m, 10H, ar), 7.85−7.90 (m, 1H, ar), 8.12−8.23 (m, 3H, ar), 9.71 (s, 1H, H-1), 10.57 (br s, 1H, NH); IR 3380, 3140, 1680.

4-Phenylureido-2-phenyl-2*H*-pyrazolo[3,4-*c*]quinoline (34) and 4-Benzylureido-2-phenyl-2*H*-pyrazolo[3,4-*c*]quinoline (35). The suitable isocyanate (1.7 mmol) was added to a suspension of **11** (0.300 g, 1.15 mmol) in anhydrous THF (20 mL). The mixture was refluxed for 2 h under nitrogen atmosphere. The resulting solid was collected and recrystallized. The title compounds displayed the following ^{1}H NMR data.

34: 7.12 (t, 1H, ar, J = 8.4 Hz), 7.36−7.98 (m, 9H, ar), 8.00 (d, 1H, ar, J = 6.2 Hz), 8.17−8.24 (m, 3H, ar), 9.68−9.73 (m, 2H, H-1 + NH), 12.73 (s, 1H, NH).

35: 4.60 (d, 2H, CH_2, J = 5.8 Hz), 7.34−7.78 (m, 11H, ar), 8.13−8.22 (m, 3H, ar), 9.22 (br s, 1H, NH), 9.68 (s, 1H, H-1), 10.48 (t, 1H, NH, J = 5.8 Hz).

(B) Biochemistry. A_1 and A_{2A} Receptor binding. Displacement of [^{3}H]CHA from A_1 AR in bovine cortical membranes and [^{3}H]CGS 21680 from A_{2A} AR in bovine striatal membranes was performed as described.[33]

A_3 Receptor binding. The displacement of [^{125}I]AB-MECA in membranes prepared from CHO cells stably expressing the human A_3 receptor was performed as described.[34] The assay medium consisted of a buffer containing 50 mM Tris-HCl, 10 mM $MgCl_2$, and 1 mM EDTA at pH 8.12. The glass incubation tubes, containing 20 μL of the membrane suspension (0.2 mg of protein/mL, stored at −80 °C in the same buffer), 20 μL of [^{125}I]AB-MECA (final concentration 0.2 nM), and 10 μL of the tested ligand, were incubated for 60 min at 25 °C in a total volume of 100 μL. After incubation, the samples were filtered on Whatman GF/C filters presoaked for 1 h in 0.5% poly(ethylenimine) followed by three washes with 5 mL of ice-cold incubation buffer. Nonspecific binding was determined in the presence of 200 μM NECA. Specific binding was obtained by subtracting nonspecific binding from total binding.

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

Protein estimation was based on a reported method,[35] after solubilization with 0.75 N sodium hydroxide, using bovine serum albumine as standard.

The concentration of the tested compound that produced 50% inhibition of specific [^{3}H]CHA, [^{3}H]CGS 21680 or [^{125}I]-AB-MECA binding (IC_{50}) was calculated using a nonlinear regression method implemented in the InPlot program (GraphPad, San Diego, CA) with five concentrations of displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the Cheng−Prusoff equation.[36] The dissociation constants (K_d) of [^{3}H]CHA, [^{3}H]CGS 21680, and [^{125}I]AB-MECA are 1.2, 14, and 1.4 nM, respectively.

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