

## 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<sub>1</sub> or A<sub>3</sub> 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<sub>1</sub> and A<sub>2A</sub> and human cloned A<sub>3</sub> adenosine receptors showed that (i) the substituent on the appended 2-phenyl ring could be used to modulate A<sub>1</sub> and A<sub>3</sub> affinity, (ii) the 4-amino group was necessary for A<sub>1</sub> and A<sub>2A</sub> binding activity, and (iii) a nuclear or extranuclear C=O proton acceptor at position 4 yielded potent and selective A<sub>3</sub> 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<sub>3</sub> nanomolar affinity of **1–8**, **31–33**, and **35** confirms the hypothesis of the presence in the N-6 region of the adenosine A<sub>3</sub> subtype of a proton donor able to bind to a C=O proton acceptor at position 4.

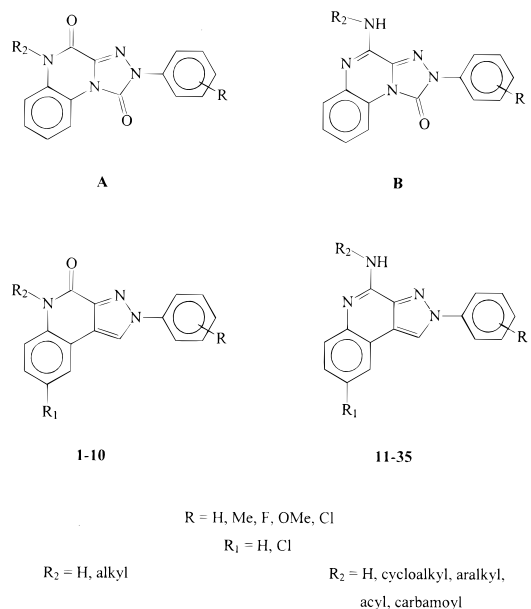
### Introduction

Adenosine is thought to mediate a wide variety of effects by interacting with four subtypes of adenosine receptors (AR): A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>. All four AR subtypes are coupled via a G-protein to the enzyme adenylyl cyclase in either an inhibitory (A<sub>1</sub> and A<sub>3</sub> subtypes) or stimulatory manner (A<sub>2A</sub> and A<sub>2B</sub> subtypes).<sup>1</sup> For A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> AR, selective agonists and antagonists are now available,<sup>2–7</sup> while the A<sub>2B</sub> subtype still lacks selective ligands.

In recent years, the potential therapeutic use of selective AR subtype antagonists as renal protective,<sup>8,9</sup> anti-Parkinson,<sup>10</sup> antiinflammatory, antiasthmatic, and antiischemic agents<sup>11–14</sup> 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.<sup>15–19</sup>

In a recent paper<sup>20</sup> we reported the synthesis and binding activity at bovine A<sub>1</sub> and A<sub>2A</sub> AR and at human cloned A<sub>3</sub> 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<sub>1</sub> or A<sub>3</sub> 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<sub>2</sub> proton donor group was essential for A<sub>1</sub> and A<sub>2A</sub> receptor–ligand interaction while it was not necessary for A<sub>3</sub> 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

**Chart 1.** Previously and Hereby Reported Adenosine Receptor Antagonists

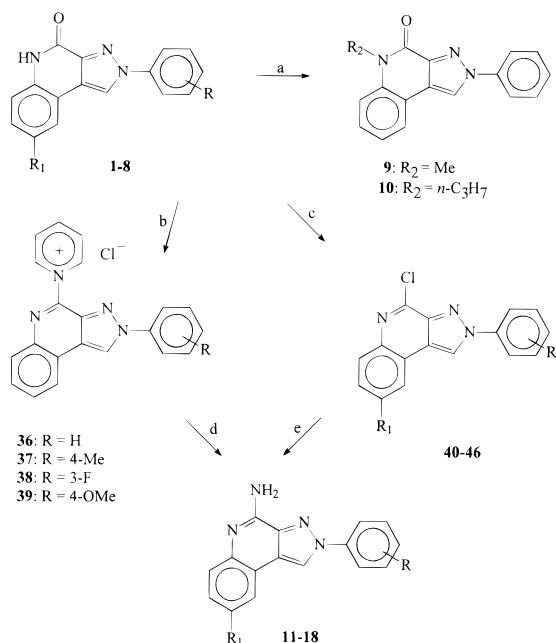


some potent and selective A<sub>3</sub> receptor antagonists. Moreover, in both series the nature and the position of the substituent on the 2-phenyl ring could be used to modulate the A<sub>1</sub>/A<sub>3</sub> selectivity. To verify whether this structural requirement could be applied to tricyclic systems of similar size and shape, we report in this paper the synthesis and A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> 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.

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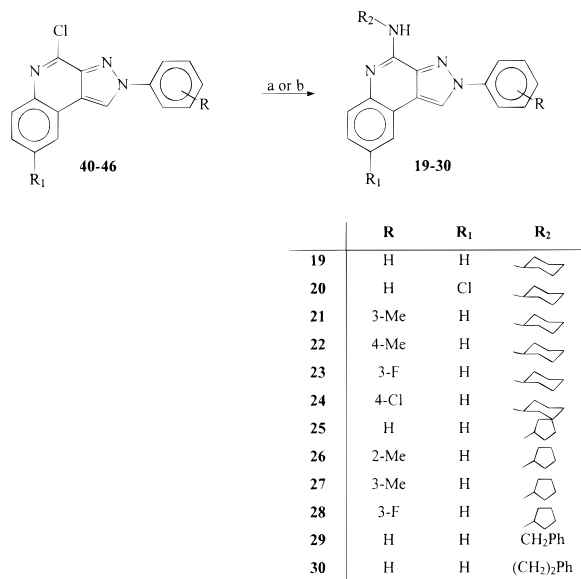
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Scheme 1<sup>a</sup>

	R	R <sub>1</sub>		R	R <sub>1</sub>
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

<sup>a</sup> (a) NaH, R<sub>1</sub>X, DMF; (b) PCl<sub>5</sub>/POCl<sub>3</sub>, pyridine; (c) PCl<sub>5</sub>/POCl<sub>3</sub>; (d) method A: cyclohexylamine; (e) method B: NH<sub>3</sub>(g), absolute EtOH.

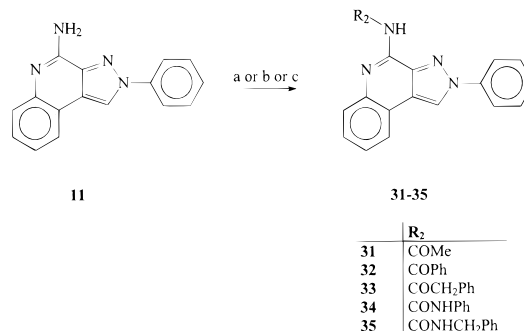
Scheme 2<sup>a</sup>

<sup>a</sup> (a) Method A: excess of R<sub>2</sub>NH<sub>2</sub>; (b) method B: R<sub>2</sub>NH<sub>2</sub>, Et<sub>3</sub>N, 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.<sup>21</sup> The 2-(2-methylphenyl) derivative 3 and its 2-(4-chlorophenyl) analogue 8 were obtained by reacting the 3-ethoxalylindole<sup>22</sup> with arylhydrazine hydrochlorides as described to prepare 1, 2,

Scheme 3<sup>a</sup>

<sup>a</sup> (a) RCOCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>; (b) PhCH<sub>2</sub>COOH, 1-hydroxybenzotriazole, Et<sub>3</sub>N, 4-(dimethylamino)pyridine, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, DMF; (c) RNCO, THF.

and 4–7.<sup>21</sup> The 5-*N*-*n*-propyl derivative 10 ensued by the reaction of 1 with *n*-propyl bromide following the procedure described to prepare 9.<sup>21</sup> Reaction of 1 and 5–7 with a mixture of PCl<sub>5</sub>/POCl<sub>3</sub> 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<sub>5</sub>/POCl<sub>3</sub> 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).

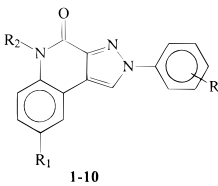
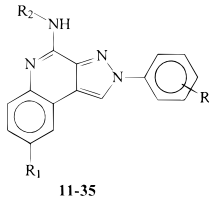










Allowing the 4-chloro intermediates 40–46 to react with suitable amines gave the 4-*N*-cycloalkylamines 19–28 and 4-*N*-aralkylamines 29 and 30 (Scheme 2).

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.

## Biochemistry

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

**Table 1.** Adenosine Receptor Binding Activity

	 1-10			 11-35		
	R	R <sub>1</sub>	R <sub>2</sub>	K <sub>i</sub> (nM) or % inhibition <sup>a</sup>		
				A <sub>1</sub> <sup>b</sup>	A <sub>2A</sub> <sup>c</sup>	A <sub>3</sub> <sup>d</sup>
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	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	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 <sub>2</sub> Ph	132 ± 11	5400 ± 380	35.8 ± 2.8
30	H	H	(CH <sub>2</sub> ) <sub>2</sub> Ph	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 <sub>2</sub> Ph	107 ± 9.3	13%	9.9 ± 0.8
34	H	H	CONHPh	1500 ± 138	32%	108 ± 9.6
35	H	H	CONHCH <sub>2</sub> Ph	186 ± 15	5%	8.3 ± 0.7
Theophylline				3800 ± 340	21000 ± 1800	86000 ± 7800
DPCPX				0.5 ± 0.03	337 ± 28	1300 ± 125

<sup>a</sup> The K<sub>i</sub> values are means ± SEM of four separate assays, each performed in triplicate. <sup>b</sup> Displacement of specific [<sup>3</sup>H]CHA binding in bovine brain membranes or percentage of inhibition (I%) of specific binding at 20 μM concentration. <sup>c</sup> Displacement of specific [<sup>3</sup>H]CGS 21680 in bovine striatal membranes or percentage of inhibition (I%) of specific binding at 20 μM concentration. <sup>d</sup> Displacement of specific [<sup>125</sup>I]AB-MECA binding at human A<sub>3</sub> 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 theophylline and DPCPX included as antagonist reference compounds, are shown in Table 1.

## Results and Discussion

The A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> 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<sub>1</sub> (**19**, **25**, **28**) and A<sub>3</sub> (**4**, **5**, **7**, **8**, **32**) antagonists and only one compound (**16**) showed good A<sub>2A</sub> affinity.

The A<sub>3</sub> 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<sub>1</sub> affinity, were inactive at the A<sub>2A</sub>, and displayed nanomolar adenosine A<sub>3</sub> receptor affinity. Alkylation at position 5 of the parent compound **1** afforded compounds **9** and **10** which showed slightly increased A<sub>1</sub> and decreased A<sub>3</sub> 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<sup>5,17,29–31</sup> 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<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> 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<sub>1</sub> 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<sub>1</sub> and A<sub>2A</sub> potency, while the A<sub>3</sub> 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<sub>2A</sub> affinity of the 4-*N*-cyclopentylamino derivative **26** (K<sub>i</sub> = 329.6 nM) which was 2.5-fold more active than its corresponding 4-*N*-cyclopentyl-2-phenyl parent compound **25** (K<sub>i</sub> = 849 nM).

The electron-donating 3-methyl substituent (compounds **4**, **14**, **21**, **27**) increased the A<sub>3</sub> affinity, while it decreased the A<sub>1</sub> and A<sub>2A</sub> ones, with the only exception being compound **4** which was 2.5-fold more active at the A<sub>1</sub> 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<sub>1</sub> affinity of **6**, the A<sub>2A</sub> of **16**, and the A<sub>3</sub> 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<sub>2A</sub> activity ( $K_i = 49.5$  nM). We should also like to point out the A<sub>1</sub> 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<sub>1</sub>/A<sub>3</sub>-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<sub>3</sub> 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<sub>2A</sub> subtype. A similar negative effect of the *p*-phenyl substituent can be observed in the A<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>3</sub> 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<sub>1</sub> and A<sub>2A</sub> receptor affinities than the corresponding 4-one derivatives **1–8**. These latter, on the contrary, were more active than **11–18** on the A<sub>3</sub> subtype. These SAR are in accordance with those of the previously reported series **A** and **B**<sup>20</sup> and confirm the importance of the 4-amino proton donor group in A<sub>1</sub> and A<sub>2A</sub><sup>17,20</sup> and the 4-carbonyl group in A<sub>3</sub><sup>20</sup> 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<sub>2A</sub> affinity. The aralkyl derivatives **29** and **30** displayed nanomolar A<sub>1</sub> and A<sub>3</sub> affinities and, as a consequence, were not A<sub>1</sub>/A<sub>3</sub>-selective antagonists. Among the 4-amido derivatives **31–33**, the 4-acetamido **31** was an A<sub>1</sub>/A<sub>3</sub> nonselective ligand while the 4-benzoylamido **32** was the most potent and selective A<sub>3</sub> antagonist among those tested in this study. Homologation of **32** gave the 4-phenylacetamido **33**, which although still A<sub>3</sub> potent ( $K_i = 9.9$  nM) was also active at the A<sub>1</sub> subtype ( $K_i = 107.2$  nM) and as a consequence was less A<sub>3</sub>/A<sub>1</sub>-selective than **32**. The importance of the C=O amide group at position 4 in A<sub>3</sub> receptor–ligand interaction is shown by comparing the A<sub>3</sub> 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<sub>1</sub> and A<sub>2A</sub> receptors while they were more active at the A<sub>3</sub> subtype than **11**, further confirming the importance of the C=O group at position 4 for A<sub>3</sub> affinity.

In conclusion, the SAR of compounds **1–35** were in accordance with those of the previously reported series **A** and **B**<sup>20</sup> 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<sub>2A</sub> receptor and the substituent on the 2-phenyl ring affected differently the A<sub>1</sub> and A<sub>3</sub> affinities. The comparison of A<sub>1</sub> and A<sub>2A</sub> 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<sub>1</sub> and A<sub>2A</sub> receptor–ligand interaction.<sup>20</sup> The nanomolar A<sub>3</sub> affinity of compounds **1–8**, **31–33**, and **35** stressed the importance for A<sub>3</sub> 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.<sup>20</sup> 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,<sup>20</sup> 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<sub>3</sub> nanomolar affinity of **1–8**, **31–33**, and **35** could be due to the presence in the N-6 region of the adenosine A<sub>3</sub> subtype of a proton donor able to bind to the C=O proton acceptor, which should explain the A<sub>3</sub> activity of **1–8**, **31–33**, and **35** and of the other A<sub>3</sub> antagonists reported in the literature.<sup>5–7,32</sup>

## Experimental Section

**(A) Chemistry.** Silica gel plates (Merck F<sub>254</sub>) 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  $\pm 0.4\%$  of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm<sup>-1</sup>. The <sup>1</sup>H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in  $\delta$  (ppm) and are relative to the central peak of the solvent that was always DMSO-*d*<sub>6</sub>. 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-ethoxalyldindole<sup>22</sup> (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:** <sup>1</sup>H NMR 2.27 (s, 3H, CH<sub>3</sub>), 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:** <sup>1</sup>H 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 <sub>1</sub>	R <sub>2</sub>	mp, °C	sol <sup>a</sup>	% yield
3	2-Me	H	H	> 300	A	60
8	4-Cl	H	H	> 300	B	43
10	H	H	<i>n</i> -C <sub>5</sub> H <sub>7</sub>	140–143	C	62
11	H	H	H	197–198	D	56 <sup>b</sup> , 90 <sup>c</sup>
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 <sub>2</sub> Ph	128–130	E	45
30	H	H	(CH <sub>2</sub> ) <sub>2</sub> Ph	125–127	G	60
31	H	H	COMe	231–233	E	98
32	H	H	COPh	223–225	J	98
33	H	H	COCH <sub>2</sub> Ph	240–242	K	62
34	H	H	CONHPh	255–257	B	47
35	H	H	CONHCH <sub>2</sub> Ph	242–243	B	70

<sup>a</sup> 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.

<sup>b</sup> From pyridinium chloride **36**. <sup>c</sup> From 4-chloro derivative **40**.

displayed the following <sup>1</sup>H NMR data: 0.99 (t, 3H, CH<sub>3</sub>, *J* = 7.2 Hz), 1.65–1.80 (m, 2H, CH<sub>2</sub>), 4.30 (t, 2H, N–CH<sub>2</sub>, *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-2H-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 <sup>1</sup>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<sub>3</sub>), 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<sub>3</sub>), 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-2H-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 <sup>1</sup>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<sub>3</sub>), 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<sub>3</sub>), 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<sub>3</sub>), 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-2H-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<sub>2</sub>SO<sub>4</sub>). 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:** <sup>1</sup>H NMR 6.93 (br s, 2H, NH<sub>2</sub>), 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:** <sup>1</sup>H NMR 7.08 (br s, 2H, NH<sub>2</sub>), 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:** <sup>1</sup>H NMR 2.26 (s, 3H, CH<sub>3</sub>), 6.92 (br s, 2H, NH<sub>2</sub>), 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:** <sup>1</sup>H NMR 2.46 (s, 3H, CH<sub>3</sub>), 6.95 (br s, 2H, NH<sub>2</sub>), 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:** <sup>1</sup>H NMR 2.39 (s, 3H, CH<sub>3</sub>), 6.93 (br s, 2H, NH<sub>2</sub>), 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:** <sup>1</sup>H NMR 6.99 (br s, 2H, NH<sub>2</sub>), 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:** <sup>1</sup>H NMR 3.85 (s, 3H, OCH<sub>3</sub>), 6.93 (br s, 2H, NH<sub>2</sub>), 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:**  $^1\text{H}$  NMR 6.97 (br s, 2H,  $\text{NH}_2$ ), 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:**  $^1\text{H}$  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:**  $^1\text{H}$  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:**  $^1\text{H}$  NMR 1.10–1.85 (m, 8H, cyclohexyl protons), 2.00–2.08 (m, 2H, cyclohexyl protons), 2.45 (s, 3H,  $\text{CH}_3$ ), 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:**  $^1\text{H}$  NMR 1.10–1.83 (m, 8H, cyclohexyl protons), 2.00–2.10 (m, 2H, cyclohexyl protons), 2.42 (s, 3H,  $\text{CH}_3$ ), 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:**  $^1\text{H}$  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:**  $^1\text{H}$  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:**  $^1\text{H}$  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:**  $^1\text{H}$  NMR 1.50–1.88 (m, 6H, cyclopentyl protons), 1.92–2.16 (m, 2H, cyclopentyl protons), 2.26 (s, 3H,  $\text{CH}_3$ ), 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:**  $^1\text{H}$  NMR 1.56–1.78 (m, 6H, cyclopentyl protons), 2.00–2.09 (m, 2H, cyclopentyl protons), 2.51 (s, 3H,  $\text{CH}_3$ ), 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:**  $^1\text{H}$  NMR 1.55–1.79 (m, 6H, cyclopentyl protons), 2.05–2.09 (m, 2H, cyclopentyl protons), 4.50–4.70 (m, 1H, cyclo-

pentyl 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,  $\text{CH}_2$ ,  $J = 6.2$  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:**  $^1\text{H}$  NMR 3.07 (t, 2H,  $\text{CH}_2$ ,  $J = 7.7$  Hz), 3.80–3.87 (m, 2H,  $\text{CH}_2$ ), 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:**  $^1\text{H}$  NMR 2.39 (s, 3H,  $\text{CH}_3$ ), 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:**  $^1\text{H}$  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:  $^1\text{H}$  NMR 4.06 (s, 2H,  $\text{CH}_2$ ), 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  $^1\text{H}$  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,  $\text{CH}_2$ ,  $J = 5.8$  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.  $\text{A}_1$  and  $\text{A}_{2\text{A}}$  Receptor binding.** Displacement of [ $^3\text{H}$ ]CHA from  $\text{A}_1$  AR in bovine cortical membranes and [ $^3\text{H}$ ]CGS 21680 from  $\text{A}_{2\text{A}}$  AR in bovine striatal membranes was performed as described.<sup>33</sup>

**$\text{A}_3$  Receptor binding.** The displacement of [ $^{125}\text{I}$ ]AB-MECA in membranes prepared from CHO cells stably expressing the human  $\text{A}_3$  receptor was performed as described.<sup>34</sup> The assay medium consisted of a buffer containing 50 mM Tris-HCl, 10 mM  $\text{MgCl}_2$ , and 1 mM EDTA at pH 8.12. The glass incubation tubes, containing 20  $\mu\text{L}$  of the membrane suspension (0.2 mg of protein/mL, stored at  $-80$  °C in the same buffer), 20  $\mu\text{L}$  of [ $^{125}\text{I}$ ]AB-MECA (final concentration 0.2 nM), and 10  $\mu\text{L}$  of the tested ligand, were incubated for 60 min at 25 °C in a total volume of 100  $\mu\text{L}$ . 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  $\mu\text{M}$  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,<sup>35</sup> 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 [<sup>3</sup>H]CHA, [<sup>3</sup>H]CGS 21680 or [<sup>125</sup>I]-AB-MECA binding (IC<sub>50</sub>) was calculated using a nonlinear regression method implemented in the InPlot program (Graph-Pad, San Diego, CA) with five concentrations of displacer, each performed in triplicate. Inhibition constants (K<sub>i</sub>) were calculated according to the Cheng–Prusoff equation.<sup>36</sup> The dissociation constants (K<sub>d</sub>) of [<sup>3</sup>H]CHA, [<sup>3</sup>H]CGS 21680, and [<sup>125</sup>I]AB-MECA are 1.2, 14, and 1.4 nM, respectively.

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