1*H*-Imidazo[4,5-*c*]quinolin-4-amines: Novel Non-Xanthine Adenosine Antagonists

Philip J. M. van Galen,[†,‡] Peter Nissen,[§] Ineke van Wijngaarden,[§] Adriaan P. IJzerman,*[,†] and Willem Soudijn[†]

Division of Medicinal Chemistry, Center for Bio-Pharmaceutical Sciences, P.O.B. 9502, 2300 RA Leiden, The Netherlands, and Duphar B.V., Postbus 900, 1380 DA Weesp, The Netherlands. *Received July 9, 1990*

On the basis of a model we recently developed for the antagonist binding site of the adenosine A_1 receptor (*J. Med. Chem.* **1990**, *33*, 1708-1713), it was predicted that 1*H*-imidazo[4,5-*c*]quinolin-4-amines would be antagonists of the A_1 receptor. Furthermore, it was expected that certain hydrophobic substitutions at the 2- and 4-positions would enhance affinity. Here, we report on the synthesis and the adenosine A_1 and A_2 receptor affinity of substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amines. Some of these compounds have nanomolar affinity for the A_1 receptor. The structure-activity relationships (SAR) of these compounds are discussed in relation to SAR for other adenosine receptor ligands. The 1*H*-imidazo[4,5-*c*]quinolin-4-amines constitute a novel class of non-xanthine adenosine antagonists.

Introduction

Adenosine exerts a host of physiological effects, which are mediated via ubiquitous membrane-bound adenosine receptors. These include effects on among others the cardiovascular, the nervous, and the immune system. Both adenosine receptor agonists and compounds that enhance the physiological actions of adenosine—by interfering with its intracellular uptake or by modulating its binding to the receptor—as well as adenosine receptor antagonists may have considerable therapeutic potential.[1] Adenosine receptors are conventionally divided into two classes, A_1 and A_2, respectively, either on the basis of different SAR profiles for a series of agonists or on differences in coupling to adenylate cyclase: A_1 receptors inhibit adenylate cyclase, whereas A_2 receptors are stimulatory to this enzyme.[2]

The best known antagonists of the adenosine receptor are the xanthines, including caffeine and theophylline, but in the last few years many other classes of antagonists have also been described. Recently, we have developed a model for the antagonist binding site on the adenosine A_1 receptor.[3] It is based on common steric, electrostatic and hydrophobic properties of a variety of adenosine antagonists, including xanthines, triazolo[1,5-*c*]quinazolines,[4,5] triazolo[4,3-*a*]quinoxalines,[6,7] and pyrazolo[4,3-*d*]pyrimidines.[8] Figure 1 shows a schematic representation of this model. Briefly, a flat π-electron-rich fused heterocycle with a well-defined molecular electrostatic potential (MEP) pattern appeared to be common to all antagonists studied. A large Y-shaped area of negative electrostatic potential (EP) could be discerned, as well as two areas of positive EP. A nitrogen atom at position 7—probably acting as a hydrogen-bond acceptor—appeared to be essential. Furthermore, two areas where hydrophobic substitution may increase A_1 affinity were identified, adjacent to positions 6 and 8. An essential feature of the model is that xanthine antagonists bind to the receptor in a so-called flipped orientation relative to adenosine-derived agonists (Figure 2).

In the present study, we aimed to design a novel class of non-xanthine adenosine antagonists on the basis of this model, in order to test its predictive value. Of several likely candidates, 1*H*-imidazo[4,5-*c*]quinolin-4-amine (8a, Figure 3) was chosen as a lead for the development of novel adenosine A_1 antagonists. The basic ring structure of 8a bears a structural resemblance to the [1,2,4]triazolo[1,5-*c*]quinazolin-4-amines[4,5] and the [1,2,4]triazolo[4,3-*a*]-quinoxalin-4-amines,[6,7] known to act as potent adenosine antagonists, but it has a different arrangement of the nitrogen atoms. It complies with the required MEP pattern (Figure 3) and it contains a nitrogen atom, available as an hydrogen bond acceptor, at position 3. Furthermore, the positions 2 and 4 are available for substitutions that might enhance affinity. Note that these numbers refer to the positions corresponding to 7, 8, and 6 according to the conventional numbering scheme of adenine-like compounds, as used in the model depicted in Figure 1.

Three series of 1*H*-imidazo[4,5-*c*]quinolines were synthesized. The N-substituted 4-amines (Compounds **8a-e**) were made to test the hypothesis that hydrophobic substituents at the exocyclic amino group enhance affinity for A_1 receptors. Compounds **12a-c** and **16a-c** contain an additional 2-cyclopentyl or a 2-phenyl substituent, respectively. According to the antagonist model, such a substitution should enhance affinity as well.

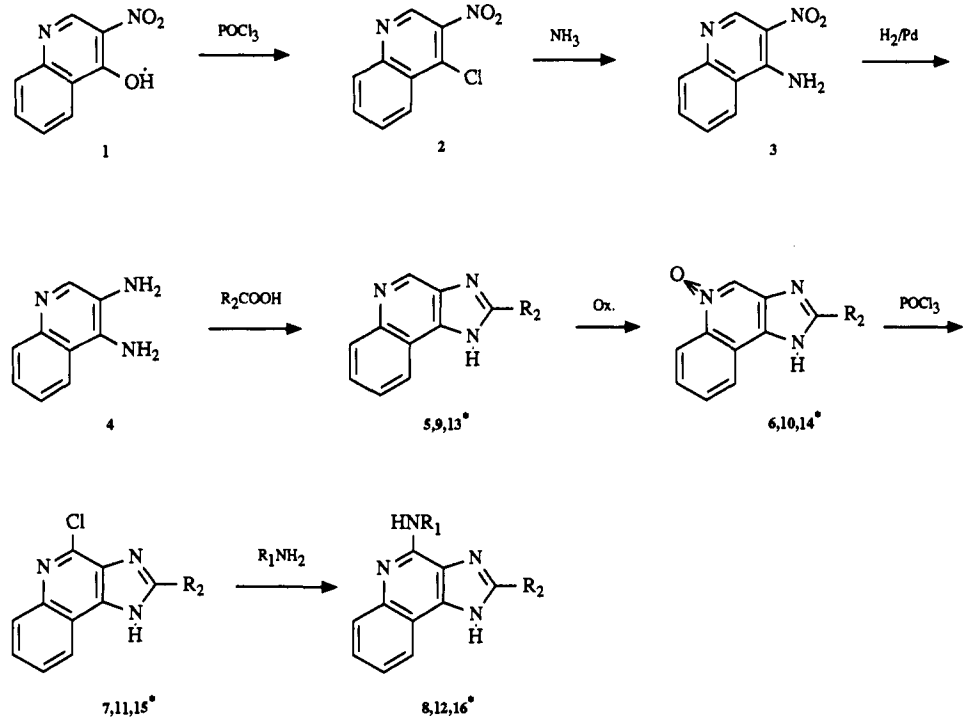
The novel substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amines were synthesized applying the route described by Gerster,[9] as indicated in Scheme I. 3-Nitro-4-hydroxyquinoline (**1**) is treated with phosphorus oxychloride to afford 3-nitro-4-chloroquinoline (**2**). This is converted to 3-nitro-4-aminoquinoline (**3**) with NH_3, which is subsequently reduced by catalytic hydrogenation to 3,4-diaminoquinoline (**4**), with 5% palladium on charcoal as catalyst. The next step involves ring-closure with formic acid, cyclopentyl carboxylic acid, or benzoic acid to yield 1*H*-imidazo[4,5-*c*]quinoline (**5**), 2-cyclopentyl-1*H*-imidazo[4,5-*c*]quinoline (**9**), and 2-phenyl-1*H*-imidazo-[4,5-*c*]quinoline (**13**), respectively. Oxidation with 3-chloroperbenzoic acid affords the respective 5-oxides (**6**, **10**, **14**), which subsequently can be converted with phosphorus oxychloride into the 4-chlorides (**7**, **11**, **15**). Finally, reaction with the appropriate amines affords N-substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amines (**8a-e**), 2-cyclopentyl-1*H*-imidazo[4,5-*c*]quinolin-4-amines (**12a-c**), and 2-phenyl-1*H*-imidazo[4,5-*c*]quinolin-4-amines (**16a-c**), respectively.

[†] Center for Bio-Pharmaceutical Sciences.
[‡] Present address: NIH, NIDDK, Laboratory of Chemistry, Building 8A, Room B1-17, Bethesda, MD 20892.
[§] Duphar B.V.

(1) Williams, M. *Neurochem. Int.* **1989**, *14*, 249-264.
(2) Stone, T. W. In *Purines, Pharmacology and Physiological Roles*; Stone, T. W., Ed.; VCH: Weinheim, 1985; pp 1-4.
(3) Van Galen, P. J. M.; Van Vlijmen, H. W. T.; IJzerman, A. P.; Soudijn, W. *J. Med. Chem.* **1990**, *33*, 1708-1713.
(4) Williams, M.; Francis, J.; Ghai, G.; Braunwalder, A.; Psychoyos, S.; Stone, G. A.; Cash, W. D. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 415-420.
(5) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; Stone, G. A.; Desai, M.; Williams, M. *J. Med. Chem.* **1988**, *31*, 1014-1020.
(6) Trivedi, B. K.; Bruns, R. F. *J. Med. Chem.* **1988**, *31*, 1011-1014.
(7) Sarges, R.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Koe, B. K. *J. Med. Chem.* **1990**, *33*, 2240-2254.
(8) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Bristol, J. A. *J. Med. Chem.* **1987**, *30*, 91-96.
(9) Gerster, J. F. U.S. Patent 4,689,338, August 25, 1987.

0022-2623/91/1834-1202$02.50/0

Scheme I. Synthesis of 1*H*-Imidazo[4,5-c]quinolin-4-amines



*For compounds **5–8**, R_2 = H; compounds **9–12**, R_2 = cyclopentyl; compounds **13–16**, R_2 = phenyl.

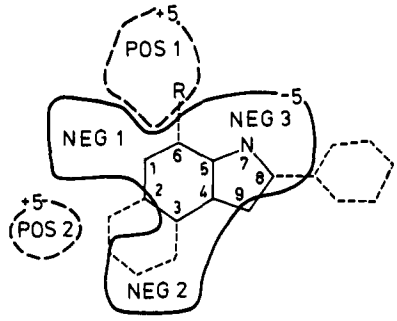


Figure 1. Model of the antagonist binding site of the adenosine A_1 receptor, based on steric, electrostatic, and hydrophobic properties, as described previously.[3]

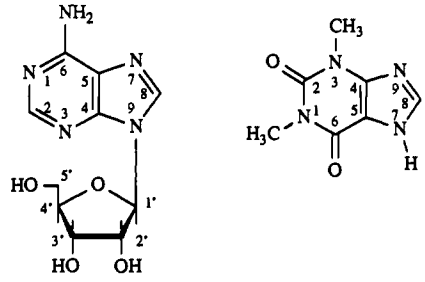


Figure 2. Structure and ring numbering of adenosine and theophylline, with the latter in a flipped orientation.

A_1 affinities were determined in receptor binding experiments with [^{3}H]CPX (8-cyclopentyl-1,3-dipropylxanthine) as the radioligand in both calf and rat brain cortical membranes. A_2 receptor affinities were determined in rat striatal membranes with [^{3}H]NECA (*N*-ethyladenosin-5′-uronamide) as the radioligand, with the addition of 50 nM cyclopentyladenosine (CPA), in order to inhibit binding to A_1 receptors.

Results

The affinities of 1*H*-imidazo[4,5-c]quinolin-4-amines are presented in Table I. pseudo-Hill coefficients approximated unity in all cases.

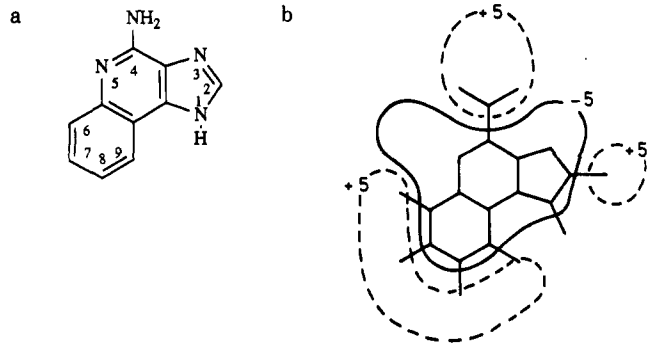


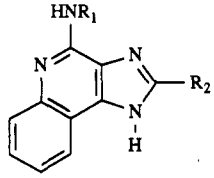
Figure 3. Structure (a) and MEP (b) of 1*H*-imidazo[4,5-c]-quinolin-4-amine (**8a**). Electrostatic potential contours are shown at 5 kcal/mol in the plane of the heterocycle.

As predicted, the unsubstituted compound 8a has affinity for A_1 receptors from rat brain, albeit moderate (K_i 1600 nM). It has virtually the same affinity for A_1 receptors from calf brain (K_i 1700 nM), as well as for rat striatal A_2 receptors (K_i 1400 nM). Thus it is not selective for A_1 or A_2 receptors from rat brain, nor does it distinguish in affinity between A_1 receptors from rat or calf brain.

Hydrophobic substituents at the exocyclic amino group may greatly enhance affinity at A_1 receptors. A cyclopentyl substituent (**8c**) results in the largest increase in this series, 170- and 37-fold for calf and rat brain A_1 receptors, respectively. The *R* and *S* enantiomers of the 1-phenyl-2-propyl-substituted compounds, **8d** and **8e**, show stereoselectivity: **8d** is 5–6-fold more potent than **8e**. In contrast, the affinity-enhancing effect of substitution at this position is much less at A_2 receptors: 5-fold at the most (**8c**), and with a phenyl substituent (**8b**), affinity is even 7.5-fold reduced. Thus, N substitution generates moderately A_1-selective compounds.

A cyclopentyl substituent at the 2-position (**12a**) also enhances the affinity of the nonsubstituted parent compound 8a: 6-fold at A_1 receptors (in both rat and calf brain) and 2-fold at A_2 receptors. There appears to be lack of additivity of the affinity-enhancing effects of substitu-

Table I. A_1 and A_2 Adenosine Receptor Affinities of Novel 1*H*-Imidazo[4,5-*c*]quinolin-4-amines and Some Reference Xanthines



compd	R_1	R_2	A_1 K_i,[a] nM[e] calf	A_1 K_i,[b] nM[e] rat	A_2 K_i,[c] nM[e] rat	A_2/A_1[d] rat
8a	H	H	1700 ± 250	1600 ± 170	1400 ± 220	0.9
8b	phenyl	H	490 ± 60	2100 ± 100	10500 ± 900	5.0
8c	cyclopentyl	H	10 ± 2	43 ± 2	290 ± 100	6.7
8d	(*R*)-1-methyl-2-phenylethyl	H	74 ± 5	310 ± 40	810 ± 130	2.6
8e	(*S*)-1-methyl-2-phenylethyl	H	460 ± 50	1500 ± 100	2800 ± 440	1.9
12a	H	cyclopentyl	290 ± 30	270 ± 50	740 ± 100	3.0
12b	phenyl	cyclopentyl	23 ± 2	230 ± 20	6% (1 μM)[f]	>4
12c	cyclopentyl	cyclopentyl	9.1 ± 0.7	39 ± 2	450 ± 60	11
16a	H	phenyl	17 ± 2	34 ± 8	290 ± 30	8.5
16b	phenyl	phenyl	50 ± 6	460 ± 20	9% (1 μM)[f]	>2
16c	cyclopentyl	phenyl	1.5 ± 0.2	10 ± 1	450 ± 60	45
theophylline			6000 ± 300	9200 ± 800	25000 ± 2000[g]	2.7
8-phenyltheophylline			3.1 ± 0.4	37 ± 4	850 ± 120[g]	23

[a] [^{3}H]CPX binding to calf brain cortical membranes. [b] [^{3}H]CPX binding to rat brain cortical membranes. [c] [^{3}H]NECA binding to rat striatal membranes. [d] Ratio A_2 vs A_1 affinity in the rat. [e] Values are means of three independent experiments ± SEM. [f] Percentage of displacement at 1 μM. [g] Values taken from ref 19.

tions at positions 2 and the exocyclic amino group: the dicyclopentyl substituted derivative **12c** is equal in potency to the *N*-cyclopentyl-substituted compound **8c** at A_1 receptors of calf and rat brain. On the other hand, in calf brain compound **12b**, which combines an *N*-phenyl substituent with a cyclopentyl substituent at position 2, is much more potent than could be expected from the contributions of the individual substituents. This does not hold for rat brain: in rat brain, **12b** is equipotent to **12a**, which lacks the *N*-phenyl substituent.

A dramatic increase in A_1 affinity is seen with a phenyl substituent in the 2-position: compound **16a** has 100- (calf) or 47-fold (rat) higher affinity for A_1 receptors than the parent compound **8a**. The A_2 affinity increases only 5-fold, and thus **16a** is moderately A_1 selective. The A_1 affinity is further increased with a cyclopentyl substituent at the exocyclic amino group, analogous to compounds **8c** and **12c**: **16c** is the most potent 1*H*-imidazo[4,5-*c*]quinoline synthesized so far, with A_1 affinities of 1.5 nM (calf) and 10 nM (rat), respectively. The A_2 affinity is somewhat reduced, and thus **16c** is also the most A_1-selective compound (45-fold). Unexpectedly, an *N*-phenyl substituent (**16b**) reduces affinity at A_1 receptors in this series, again stressing the lack of additivity of substitutions at the 2-position and the exocyclic amino group.

Discussion

As predicted by our model, the 1*H*-imidazo[4,5-*c*]-quinolin-4-amines indeed have affinity for the adenosine A_1 receptor. Some compounds in this series display affinities in the lower nanomolar range. Distinct similarities between the SAR of the 1*H*-imidazo[4,5-*c*]quinolin-4-amines and related antagonists as the triazolo[1,5-*c*]-quinazolines[4,5] and the triazolo[4,3-*a*]quinoxalines,[6,7] as well as the 9-methyladenines,[10] are evident. In all cases the basic structure, not substituted at the exocyclic amino group, is either slightly A_2 selective or not selective. Monosubstitution at the exocyclic amino group with hydrophobic substituents may greatly enhance A_1 affinity, but this effect is usually much smaller for A_2 receptors. In many cases, the A_2 affinity actually decreases considerably. Thus, compounds which initially were A_2 selective become A_1 selective because of this N substitution. In the case of the imidazoquinolines, the triazoloquinoxalines, and the 9-methyladenines, the highest A_1 affinity and selectivity is invariably found with a cycloalkyl group, either cyclopentyl or cyclohexyl. Furthermore, for these classes the 1-phenyl-2-propyl-substituted derivatives show stereoselectivity: the *R* isomers are 3–6-fold more potent than the corresponding *S* isomers.[7,10] Unfortunately, no data are available for analogously substituted triazoloquinazolines. Nevertheless, from the data for the few *N*-alkyl-substituted triazoloquinazolines that have been reported in the literature,[5] some similarities with the other antagonists as well as the corresponding N^6 region of adenosine agonists are evident, as previously discussed.[3]

The increase in affinity seen with a cyclopentyl or a phenyl substituent at position 2 also has a parallel in other classes of adenosine antagonists. Aryl and cycloalkyl substituents at this site (which corresponds to position 8 in the model of Figure 1) have been shown to be important for high affinity in xanthines,[11] pyrazolo[4,3-*d*]pyrimidines,[8] and triazolo[1,5-*c*]quinazolines.[5] In the triazolo-[4,3-*a*]quinoxaline series, analogous substitution is not feasible, since it would involve quaternization of a nitrogen atom. This would lead to a very different charge distribution in the heterocyclic system and a different orientation of the substituent, both incompatible with our model.

The lack of additivity of the effects of N and 2 substitution has a precedent in other ligands for the adenosine receptor. The affinity for the A_1 receptor of N^6,C5′-disubstituted adenosine derivatives is determined primarily by the N^6 substituent.[12,13] When substituents that individually induce A_2 selectivity and enhance A_2 affinity are simultaneously introduced at N^6 and C2 of adenosine, the A_2 selectivity is retained, but the affinity drops sharply.[14]

(10) Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W.; Daly, D. T.; Olsson, R. A. *FEBS Lett.* **1987**, *215*, 203–208.

(11) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1989**, *32*, 1231–1237.

(12) Olsson, R. A.; Kusachi, S.; Thompson, R. D.; Ukena, D.; Padgett, W.; Daly, J. W. *J. Med. Chem.* **1986**, *29*, 1683–1689.

(13) Paton, D. M.; Lockwood, P. A.; Martlew, D. L.; Olsson, R. A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 301–304.

Table II. Analytical Data for the Synthesis of Novel 1H-Imidazo[4,5-c]quinolin-4-amines

compd[a]	salt[b]	yield, %	mp, °C	bruto formula	anal.
8a	HCl	66	>350 dec	$C_{10}H_8N_4 \cdot HCl \cdot H_2O$	C,H,N,Cl
8b	HCl	82	298–303 dec	$C_{16}H_{12}N_4 \cdot HCl \cdot H_2O$	C,H,N,Cl
8c	2HCl	77	260–262	$C_{15}H_{16}N_4 \cdot 2HCl$	C,H,N,Cl
8d	HCl	70	176–178 dec	$C_{19}H_{18}N_4 \cdot 2HCl \cdot 0.5H_2O$	C,H,Cl;N[c]
8e	HCl	78	169–172 dec	$C_{19}H_{18}N_4 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl
12a	FB	72	126–128	$C_{15}H_{16}N_4 \cdot H_2O$	C,H,N
12b	FB	55	156–158	$C_{21}H_{20}N_4 \cdot H_2O$	C,H,N
12c	FB	62	181–183	$C_{20}H_{24}N_4 \cdot H_2O$	C,H,N
16a	FB	67	145–147	$C_{16}H_{12}N_4 \cdot 0.2H_2O$	C,H,N
16b	FB	61	231–233	$C_{22}H_{16}N_4$	C,H,N
16c	1.6HCl	71	302–304	$C_{21}H_{20}N_4 \cdot 1.6HCl$	C,H,N,Cl

[a] Please refer to Table I for structures. [b] FB = free base. [c] Calcd 14.48, found 13.88.

In xanthine antagonists it has been shown that the substantial A_1 selectivity of 1-isoamyl-3-isobutylxanthine is annihilated when an additional 8-*p*-sulfophenyl group is introduced, whereas 8-(*p*-sulfophenyl)theophylline in itself is slightly A_1 selective.[15] Furthermore, the very large affinity-enhancing effect of an 8-phenyl or an 8-cyclopentyl substituent in 1,3-dipropylxanthine is completely abolished by an additional methyl substituent at position 7. Adding a 7-methyl group to 1,3-dipropylxanthine on the other hand has only a minor effect.[11] Thus it is evident that for both adenosine receptor agonists and antagonists, interaction at one site can be markedly influenced by the absence or presence of a substituent at another—often distal—site.

At least three different explanations can be conceived. An obvious one is the direct interaction between two nearby groups. It has been argued by Trivedi and Bruns[14] that N^6 and C2 substituents may (partly) occupy the same subsite of the receptor, and in this way they can mutually influence each other. A similar explanation is also the most likely one for the influence of 7-methyl substitution on the affinity of 8-substituted xanthines:[11] the 7-substituent may sterically hinder the orientation of the 8-substituent necessary for optimal interaction with the receptor. A second possibility is that binding of one group to the receptor induces a conformational change, either in the receptor or in the ligand, thereby altering the attachment site for the second group. A third possibility might be the explanation for the interdependence of the effects of N and 2 substitution in the 1*H*-imidazo[4,5-*c*]quinolin-4-amines. For this third mode of mutual interdependence, it must be assumed that the heterocyclic core, present in every known adenosine receptor ligand, binds rather loosely to the receptor. A substituent at the appropriate site would then serve as an anchor, securing the position of the heterocyclic core and hampering optimal interaction of the receptor with a second substituent, which would presumably need a somewhat different position of the heterocycle. The assumption of a loose fit of the core is consistent with the large array of structurally diverse heterocycles accepted by the receptor.

The results with these compounds again stress the differences between A_1 receptors from calf and rat brain. Whereas the compounds with unsubstituted exocyclic amino groups (**8a**, **12a**, **16a**) do not differ greatly in affinity in both species, N substitution enhances affinity for calf brain A_1 receptors considerably more (approximately 4-fold) than for rat brain. With an additional 2-cyclopentyl or 2-phenyl group, the effect becomes even larger, and thus compound **12b** is 10-fold more potent in calf than in rat brain. Similar or even larger interspecies differences have also been observed for N^6-substituted adenosines (4–18-fold more potent in bovine than in rat brain) and a series of 1,3,8-substituted xanthines (11–49-fold more potent in bovine brain).[16] In the case of calf brain, comparatively large hydrophobic substituents appear to be accomodated better by the receptor site where the N substituent binds—which corresponds to the N^6 region of adenosine according to our model. This is consistent with earlier findings,[17,18] where it was shown that within a series of N^6-*n*-alkyl-substituted adenosine derivatives, optimal affinity in calf brain is found with larger chain lengths than in rat brain.

Finally, it has been suggested that the N^6 region of adenosine receptor agonists and the C8 region of xanthine antagonists might bind to the same part of the receptor.[19] The suggestion was made on the basis of similarities in SAR profiles for N^6 and C8 substitution, respectively, for a very limited number of compounds. For instance, a cyclopentyl substituent enhances A_1 affinity and selectivity in both N^6-substituted adenosines and C8-substituted xanthines. This view is clearly not in accordance with our antagonist model, in particular because of huge discrepancies in MEP patterns when both classes are superimposed with the N^6 and C8 regions coinciding. The results with the 1*H*-imidazo[4,5-*c*]quinolin-4-amines appear to bear this out: cyclopentyl substituents at *both* the exocyclic amino group *and* position 2 increase A_1 affinity and selectivity. These are obviously distinct sites and would correspond, according to our model, with the N^6 and C8 regions, respectively.

In conclusion, our model of the antagonist binding site of the adenosine A_1 receptor has successfully predicted the 1*H*-imidazo[4,5-*c*]quinolin-4-amines as a novel class of non-xanthine adenosine antagonists. The most potent compounds that have been synthesized so far in this series have nanomolar affinity for the A_1 receptor.

Experimental Section

Synthesis. All reagents were of analytical grade and were obtained from standard local sources. 3-Nitro-4-hydroxyquinoline (1) was prepared as described.[20] The synthesis of *N*-phenyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (**8b**) starting from 1 is described here. The other compounds were synthesized in a similar

(14) Trivedi, B. K.; Bruns, R. F. *J. Med. Chem.* **1989**, *32*, 1667–1673.
(15) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. *J. Med. Chem.* **1985**, *28*, 487–492.
(16) Ferkany, J. W.; Valentine, H. L.; Stone, G. A.; Williams, M. *Drug Dev. Res.* **1986**, *9*, 85–93.
(17) Van Galen, P. J. M.; IJzerman, A. P.; Soudijn, W. *FEBS Lett.* **1987**, *223*, 197–201.
(18) Pirovano, I. M.; IJzerman, A. P.; Van Galen, P. J. M.; Soudijn, W. *Eur. J. Pharmacol.—Mol. Pharmacol. Sect.* **1989**, *172*, 185–193.
(19) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331–346.
(20) Bachman, G. B.; Welton, D. E.; Jenkins, G. L.; Christian, J. E. *J. Am. Chem. Soc.* **1947**, *69*, 365–371.

manner. Melting points were determined in open tubes in a Büchi-Tottoli melting point apparatus and are uncorrected. ^{13}C NMR spectra were recorded on a Varian VXR 400 S spectrometer. All NMR spectra are consistent with the assigned structures. Elemental analyses were performed by TNO, Zeist, The Netherlands. Analytical data are given in Table II.

3-Nitro-4-chloroquinoline (2). To 120 mL (1.3 mol) of phosphorus oxychloride, was added 40.0 g (0.21 mol) of **1** with stirring. The mixture was refluxed for 30 min. After cooling the solvent was evaporated in vacuo and the resulting syrup was poured over crushed ice while stirring. After 1 h the solid that was formed was filtered off, washed with cold water, and dissolved in methylene chloride and some methanol. The solution was extracted once with ice-cold NaOH (1 N) and dried over $MgSO_4/Na_2SO_4$, with the addition of activated charcoal. The solution was filtered over Hyflo, and the solvent was evaporated. The residue was triturated with isopropyl ether and subsequently dried in vacuo: yield 39.0 g (89%); mp 120–121 °C.

3-Nitro-4-aminoquinoline (3). NH_3 was passed for 5 h, while stirring, through a solution of 39.0 g (0.19 mol) of **2** in 320 mL of toluene and 80 mL of 2-propanol. During the course of these 5 h, the temperature was gradually raised and maintained at 70 °C during the last half hour. After cooling, the solid was separated by filtration and washed successively with toluene/2-propanol (70:30), ether, and cold water until Cl^- could no longer be detected. The solid was filtered off and dried at 80 °C: yield 34.3 g (97%); mp 268–270 °C.

3,4-Diaminoquinoline (4). To a mixture of 22.0 g (0.12 mol) of **3** in 265 mL of ethanol was added 0.88 g of 5% palladium on charcoal (moistened). The mixture was hydrogenated for 2.5 h under atmospheric pressure at room temperature and subsequently filtered over Hyflo. The filtrate was evaporated, and the residue was successively coevaporated with methanol and with ether. The residue gradually solidified and was dried in vacuo at 50 °C: yield 18.9 g (theoretically 18.5 g) of a yellow solid; mp 171–173 °C.

1*H*-Imidazo[4,5-*c*]quinoline (5). An 18.9-g (0.12 mol) sample of **4** was heated under stirring with 330 mL of trimethyl orthoformate. To the clear solution was cautiously added formic acid (6 mL) whereupon a solid precipitated. The mixture was refluxed for 1 h. After cooling to 40 °C, 40 mL of ether and 5 mL of absolute ethanol were added and the mixture was cooled on ice for 1 h. The solid that was formed was filtered off, washed with ether, and subsequently with ethyl acetate and crystallized from ethanol; yield 17.0 g in two crops (87%); mp 285–288 °C.

1*H*-Imidazo[4,5-*c*]quinolin-5-oxide (6). A 2.8-g (16.6 mmol) sample of **5** was almost completely dissolved (with heating) in 30 mL of chloroform, 30 mL of methylene chloride, and 6 mL of methanol. 3-Chloroperoxybenzoic acid (5 g, 55%) was added, and the solution was refluxed. Within a few minutes, a solid started to precipitate. After 30 min, 0.5 g of Na_2CO_3 was added and the mixture was refluxed for one more hour. The reaction mixture was cooled in an ice bath; the solid that precipitated was filtered off and was subsequently washed with methylene chloride (2×), ether (2×), water (2×), and ether/absolute ethanol (50:10) (3×). The product was dried in vacuo at 40 °C: yield 2.7 g (88%); mp 287–290 °C dec.

4-Chloro-1*H*-imidazo[4,5-*c*]quinoline (7). A mixture of 14 mL of toluene and 28 mL of dimethylformamide was cooled in an ice bath, and 5.3 mL of phosphorus oxychloride (58 mmol) was added. After 10 min, 5.68 g (31 mmol) of **6** was added and the solution was stirred at ambient temperature for 10 min. Subsequently, the solution was heated on a steam bath for 30 min. Upon cooling, the solvent was evaporated and the resulting syrup was poured over crushed ice while stirring. The mixture was then warmed to room temperature and carefully adjusted to pH 6–7 with solid $NaHCO_3$. After 2 h, the solid that was formed was filtered off, washed with water and with isopropyl ether/isopropyl alcohol (80:20), and subsequently dried in vacuo: yield 5.23 g (84%); mp > 252 °C dec.

***N*-Phenyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (8b).** 1.30 g (6.4 mmol) of **7** was dissolved (with heating) in 4 mL of aniline and 8 mL of dimethylformamide. The solution was heated for 3 h at 105 °C under nitrogen. After cooling, the solvent was concentrated in vacuo as far as possible. The residue was dissolved in toluene and chromatographed on a silica column with methylene chloride/ethanol (98.5:1.5) as the eluent. Separation was monitored on TLC (Merck Silica GF 254; methylene chloride 85/methanol 15/ammonia 0.6). Combination of the appropriate fractions and evaporation of the solvent yielded 1.60 g of product, which was converted to the hydrochloride salt. Yield 1.64 g (82%) of the hydrochloride salt, mp 298–303 °C dec.

Receptor Binding. [^{3}H]CPX (specific activity 106 Ci/mmol) and [^{3}H]NECA (specific activity 40 Ci/mmol) were from amersham, Buckinghamshire, UK. Adenosine deaminase, *R*-PIA, and CPA were from Boehringer, Mannheim, FRG. Other reagents were of analytical grade and obtained from standard local sources.

Preparation of Membranes. Calf brain cortical membranes were prepared as described previously.[17] Rat brain cortical membranes were prepared according to Lohse et al.,[21] but before workup striata were dissected and used for the preparation of striatal membranes. Fresh striata (ca. 1.6 g) of 20 male Wistar rats (weighing ca. 120–150 g) were taken up in 15 mL of buffer A (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.7), disrupted with an Ultraturrax homogenizer, and centrifuged at 50000*g* for 10 min. The supernatant was discarded and the pellet was suspended in 12 mL of buffer A, homogenized in a Potter tube with a tight-fitting teflon pestle and centriguted at 50000*g* for 10 min. This procedure was repeated once. The pellet was resuspended in 12 mL of buffer A, homogenized, and incubated with 2 IU/mL of adenosine deaminase for 30 min at 37 °C. The preparation was stored in liquid nitrogen until further use. Protein was assayed according to the procedure of Lowry et al.[22]

Binding Assays. [^{3}H]CPX binding to calf or rat brain cortical membranes was assayed in 50 mM Tris-HCl, pH 7.4 (Buffer B) in a final volume of 400 μL. Assays contained ca. 15 μg of membrane protein and 0.1 nM of [^{3}H]CPX (calf brain) or ca. 25 μg of membrane protein and 0.3 nM of [^{3}H]CPX (rat brain). Incubations were carried out in duplicate for 1 h at 25 °C and were terminated by diluting the samples with 1 mL of ice-cold buffer B, followed by rapid filtration over Whatman GF/B glass fiber filters. Subsequently, tubes were rinsed with 1 mL of buffer B and filters were washed three times with 3 mL of buffer B. Filters were dried for 45 min at 60 °C, 3.5 mL of scintillation cocktail (Packard Emulsifier Safe) was added, and radioactivity was determined in a LKB 1214 Rackbeta liquid scintillation counter. Solutions of compounds tested were made in DMSO and were diluted with buffer to a final DMSO concentration of 1% at the most, which had no influence on specific binding. Nonspecific binding was determined in the presence of 10 μM *R*-PIA.

[^{3}H]NECA binding to rat brain striatal membranes was performed similarly, with the following modifications. Buffer A was used throughout. Approximately 50 μg of membrane protein was used and 4 nM of [^{3}H]NECA. In each assay 50 nM CPA was included to prevent [^{3}H]NECA binding to A_1 receptors. Nonspecific binding was determined in the presence of 100 μM CPA. All K_i values are means of three independent experiments. Each concentration of inhibitor was tested in duplicate.

Data Analysis. IC_{50} values were determined from pseudo-Hill plots of the displacement curves and transformed into K_i values according to the Cheng-Prusoff equation:[23] $K_i = IC_{50}/(1 + [L^*]/K_d)$. L* denotes the concentration and K_d the dissociation constant of the radioligand. K_d values of 0.085 nM and 0.28 nM were used for [^{3}H]CPX binding to calf and rat brain membranes, respectively (Van Galen and Pirovano, unpublished results). For [^{3}H]NECA binding, the modified Cheng–Prusoff equation $K_i = IC_{50}/\{1 + ([L^*]/15.3) + (50/685)\}$ was used, in which 15.3 (nM) represents the K_d value of [^{3}H]NECA binding to rat striatal membranes, 50 (nM) the concentration of CPA added, and 685 (nM) the affinity of CPA for striatal A_2 receptors.[19]

Acknowledgment. Use of the services of the Dutch CAOS/CAMM Center, under grant numbers SON-11-20-700 and STW-NCH-44.0703, is gratefully acknowledged.

(21) Lohse, M. J.; Lenschow, V.; Schwabe, U. *Mol. Pharmacol.* **1984**, *26*, 1–9.
(22) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265–275.
(23) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.