Isoquinoline and Quinazoline Urea Analogues as Antagonists for the Human Adenosine A_3 Receptor

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Isoquinoline and quinazoline urea derivatives were found to bind to human adenosine A_3 receptors. Series of *N*-phenyl-*N*′-quinazolin-4-ylurea derivatives and *N*-phenyl-*N*′-isoquinolin-1-ylurea derivatives were synthesized and tested in radioligand binding assays on their adenosine receptor affinities. A structure−affinity analysis indicated that on the 2-position of the quinazoline ring or the equivalent 3-position of the isoquinoline ring a phenyl or heteroaryl substituent increased the adenosine A_3 receptor affinity in comparison to unsubstituted or aliphatic derivatives. Furthermore, the structure−affinity relationship of substituted phenylurea analogues was investigated. Substituents such as electron-withdrawing or electron-donating groups were introduced at different positions of the benzene ring to probe electronic and positional effects of substitution. Substitution on the 3- or 4-position of the phenyl ring decreased the adenosine A_3 receptor affinity. Substitution at position 2 with an electron-donating substituent, such as methyl or methoxy, increased human adenosine A_3 receptor affinity, whereas substitution on the 2-position with an electron-withdrawing substituent did not influence affinity. Combination of the optimal substituents in the two series had an additive effect, which led to the potent human adenosine A_3 receptor antagonist *N*-(2-methoxyphenyl)-*N*′-(2-(3-pyridyl)quinazolin-4-yl)urea (VUF5574, **10a**) showing a K_i value of 4 nM and being at least 2500-fold selective vs A_1 and A_{2A} receptors. Compound **10a** competitively antagonized the effect of an agonist in a functional A_3 receptor assay, i.e., inhibition of cAMP production in cells expressing the human adenosine A_3 receptor; a pA_2 value of 8.1 was derived from a Schild plot. In conclusion, compound **10a** is a potent and selective human adenosine A_3 receptor antagonist and might be a useful tool in further characterization of the human A_3 receptor.

Introduction

Extracellular adenosine exerts its physiological effects by activation of cell membrane-spanning receptors called P_1-purinoceptors. The P_1-purinoceptors are divided into three subtypes: A_1, A_2, and A_3, with A_2 further subdivided into A_{2A} and A_{2B}. All four receptors are coupled via a G protein to the adenylate cyclase-cAMP signal transduction pathway. Activated adenosine A_1 and A_3 receptors inhibit adenylate cyclase, whereas A_{2A} and A_{2B} receptors stimulate this enzyme. The target receptor in the present study, the human adenosine A_3 receptor, is mainly expressed in lung, liver, kidney, and heart but is also found in the CNS, testes, and immune system.[1−3]

Selective adenosine A_3 antagonists are putative antiinflammatory, antiasthmatic, or antiischemic agents.[4−9] Xanthines, although versatile leads for antagonists of the adenosine A_1 and A_{2A} receptors, are much less potent at the adenosine A_3 receptor.[10] For this reason library screening was used to search for novel leads. Triazolonaphthpyridine,[11,12] 1,4-dihydropyridines[13−15] and pyridines,[16] triazoloquinazolines,[17,18] isoquinolines and quinazolines,[19,20] and flavonoids[21] were identified as adenosine A_3 receptor ligands this way, and chemical optimization of the leads yielded selective adenosine A_3 receptor antagonists.

Recently we have reported on a series of isoquinoline and quinazoline analogues as adenosine A_3 receptor ligands.[19,20] From these studies we concluded that higher adenosine A_3 receptor affinity resulted from spacer-coupled aromatic groups on the 1-position of the isoquinoline ring (Figure 1). By altering the aromatic substitution pattern, selectivity for the adenosine A_3 receptor was obtained. We have since extended the scope of our investigations into this class of compounds and found that a urea moiety as spacer also provided an increase in binding affinity compared to a directly coupled aromatic group on the 1-position of the isoquinoline ring. In this new series, the influence on adenosine A_3 receptor affinity of substituents at the 2-position of phenylurea quinazolines was investigated first. Subsequently, the influence of substitution of the phenylurea moiety was analyzed. Finally, computer-aided visualization of common elements within the isoquinoline and quinazoline series was used to derive clues for their high affinity.

Chemistry

The preparation of compounds **5a**−**k** was performed following the general synthetic strategy depicted in Scheme 1. The intermediates **4a**−**k** were synthesized

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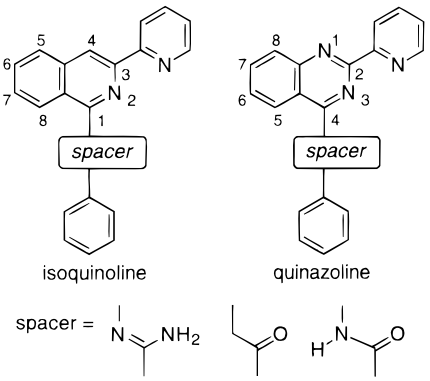


Figure 1. Isoquinolines and quinazolines as human adenosine A_3 receptor ligands.

initially based on a method described by Linschoten et al.[22] with some modifications. Treatment of 2-aminobenzonitrile **(1)** with strong base yielded a relatively stable anion as intermediate. This nucleophile reacted with nitriles **3a**−**k,** and after hydrolysis the quinazoline derivatives **4a**−**k** were obtained. The yield of the derivatives was dependent on the bulkiness of substituents R_1 of nitriles **3a**−**k** due to steric hindrance in the nucleophilic attack of the anion. In such a case the anion could react with the starting nitrile **1** which, after hydrolysis, yielded the dimeric side product 2-(2-aminophenyl)-4-quinazolineamine (**2**). This agrees with the findings of Taylor and Borror[23] who suggested that in the case of different aminobenzonitriles the critical factor determining dimerization was the ability of the nitrile group to undergo nucleophilic attack rather than the basicity of the attacking amino group. The course of the condensation reaction's dependence on the accessibility of the participating nitrile group to undergo nucleophilic attack was clearly demonstrated by a mixture of **1** and trimethylacetonitrile **(3c)**, which yielded only dimer **2**. The method of Smyrl and Smithwick[24] with sodium hydroxide as catalyst also failed with reactants **1** and **3c**. However, method B favored the formation of **4c** over **2**. In method B sodium hydride was used as base, so the formation of the anion was completed before nitrile **3** was added. With method A, an equilibrium could exist between **1** and the anion, which can compete with nitrile **3**, resulting in the formation of **2**. Good yields were also reached with method B in the synthesis of **4e**, although the nitrogen in **3e** on the meta position is not able to delocalize the negative charge originating from the nucleophilic attack of the anion of **1**. Shishoo et al.[25] have described a hydrogen chloride-catalyzed reaction of 2-aminobenzonitrile with acetonitrile yielding 63% of 4-amino-2-methylquinazoline. However, an examination of this method using trimethylacetonitrile (**3c**) revealed that neither desired product nor side product was formed. These authors have described that the electron-withdrawing ability of the substituent on the nitrile did not affect the course of the reaction, and from our results we suggest that the steric factor plays a dominant role, both in the base-catalyzed reaction and in the acid-catalyzed reaction.

Finally, derivatives **5a**−**k** were prepared by reaction of phenyl isocyanate **(7i)** with 4-aminoquinazolines **4**. The substituted phenylurea derivatives **9a**−**h** as well as compounds **8**, **10**, and **11** were prepared as depicted in Scheme 2. Reaction of quinazolines **4** and isoquinolines **6** in dry acetonitrile at 30−50 °C with the appropriate isocyanate **7a**−**i** afforded the products **8**−**11** in good overall yields. The low solubility of the products in acetonitrile simplified the isolation and purification. For this reason, we favor this method[26] above reaction in refluxing dry THF.[27] The yields, method used, and analytical data of amines **4** and **6** and products **5** and **8**−**11** are summarized in Table 1.

Results and Discussion

Binding Studies. All synthesized compounds were tested in radioligand binding assays to determine their affinities at adenosine A_3, A_1, and A_{2A} receptors. The affinities at adenosine A_1 and A_{2A} receptors were determined on rat brain cortex and rat striatum with [^{3}H]DPCPX and [^{3}H]CGS 21680 as radioligands, respectively.[28,29] The affinity at adenosine A_3 receptors was determined on membranes from HEK 293 cells, stably expressing the human A_3 receptor, using [^{125}I]-AB-MECA.[30,31] The results are shown in Tables 2−4.

Preliminary SAR. In previous studies we have described isoquinoline and quinazoline derivatives as a novel class of ligands for the adenosine A_3 receptor.[19,20] An aromatic ring, coupled by a conjugated spacer to the 1-position of the isoquinoline ring, proved beneficial for high adenosine A_3 receptor affinity. All spacers were three bond lengths long (Figure 1). In the present study we prolonged the spacer with one extra atom, thus introducing a urea moiety as spacer.

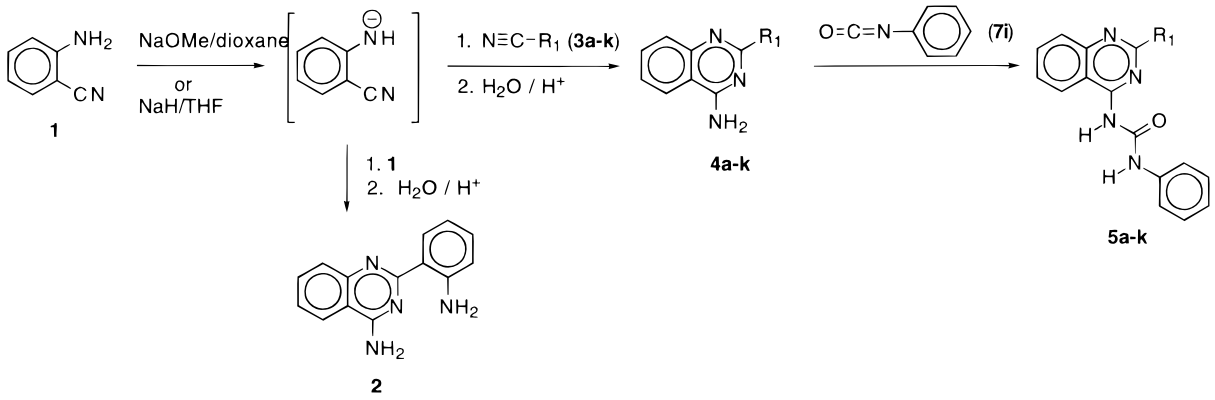
Comparison of the affinities of compounds containing the four different spacers (Table 2) revealed that compound **12**, bearing an amidine group as spacer, and compound **13**, containing a ketone spacer, showed moderate affinity at the adenosine A_3 receptor, whereas compounds with an amide and especially those with a urea moiety had higher adenosine A_3 receptor affinities. Compound **8b** with a urea spacer showed the highest A_3 selectivity. These data led us to explore the urea derivatives further.

In our previous studies[20] it had been shown that isoquinolines differ only slightly from quinazolines in their affinities at adenosine receptors. To check whether this also held in the urea series, we synthesized and determined the affinity of quinazolines **5a**,**b**,**d** and isoquinolines **8a**,**b**,**d** (Table 3). The results proved to be somewhat ambiguous. Quinazolines within the unsubstituted derivatives **5a** and **8a** and the phenyl derivatives **5d** and **8d** had increased adenosine A_3 receptor affinities, whereas 2-pyridyl derivatives **5b** and **8b** showed the opposite effect. In view of the better accessibility of 4-aminoquinazolines compared to the 1-aminoisoquinolines, we decided to focus on the quinazoline derivatives only.

Optimization of the Substituent on the 2-Position. All isoquinolines and quinazolines described so far as ligands for the adenosine A_3 receptor have a 2-pyridyl group on the 3-position of the isoquinoline ring or the equivalent 2-position of the quinazoline ring, respectively. In the present study, we investigated the influence of other substituents on this position, and therefore we synthesized compounds **5a**−**k** (Table 3).

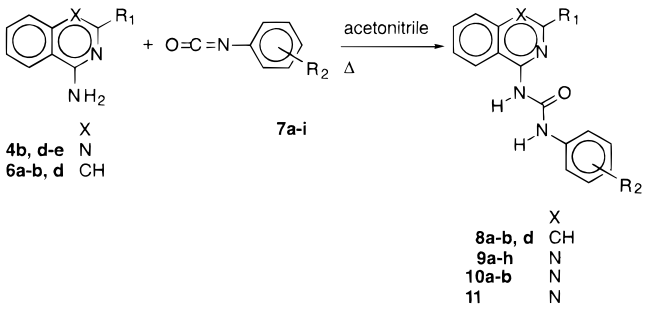
Quinazoline **5a**, being unsubstituted at position 2, had low adenosine A_3 receptor affinity. Substitution with a bulky *tert*-butyl group at position 2 was unfavorable

Scheme 1[a]



[a] R_1 is defined in Table 1.

Scheme 2[a]



[a] R_1 and R_2 are defined in Table 1.

(**5c**). However, substitution with an aromatic group resulted in a largely increased adenosine A_3 receptor affinity (compounds **5b**,**d**−**f**). Within this group of compounds, some differences in adenosine A_3 receptor affinity were observed. The phenyl group of **5d** and the 2-pyridyl and 4-pyridyl groups of compounds **5b**,**f**, respectively, contributed almost equally to adenosine A_3 receptor affinity, whereas the 3-pyridyl group (derivative **5e**) increased the affinity several times.

o-Methyl substitution of the 2-pyridyl group as in **5g** was allowed. However, a 4,6-dimethylpyrimidinyl substituent yielded modestly active **5h**. The furyl analogue (**5i**) showed affinity and selectivity at adenosine receptors very similar to those of the phenyl and 2- and 4-pyridyl derivatives. Next, 2-substitution of the quinazoline ring with an amine functionality was investigated. The pyrrolidine derivative **5k** and the diethylamine derivative **5j** both possessed relatively high adenosine A_3 receptor affinity. This relatively high adenosine A_3 receptor affinity of the amine-substituted compounds **5j**,**k** could not be caused by their high lipophilicity, because compound **5c**, bearing the most lipophilic substituent within this series (*tert*-butyl), was inactive at the adenosine A_3 receptor. The high adenosine A_3 receptor affinity of compounds **5j**,**k** may be due to the lone pair of the nitrogen atom, which may have a similar electrostatic interaction with the receptor as the π-electrons of an aromatic ring. This might explain the low affinity of the unsubstituted compound **5a** and the aliphatic derivative **5c**. Interestingly, compound **5j** showed also high adenosine A_1 receptor affinity, even being slightly A_1 selective.

In conclusion, substituents at position 2 of the quinazoline ring that are relatively small and possess a high electron density imposed moderate to high adenosine A_3 receptor affinity in this series of quinazolines.

Substituent Effect of Phenylurea. In an approach according to Topliss for aromatic compounds,[32] a series of phenylurea-substituted *N*-aryl-*N*′-(2-phenylquinazol-4-yl)urea derivatives were synthesized. All compounds were tested on their adenosine receptor affinities (Table 4). The substituted compounds **9a**−**d** showed highly decreased adenosine A_3 receptor affinity compared to the unsubstituted derivative **5d**.

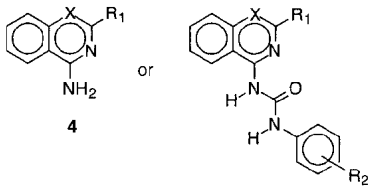
The potent and subtype-selective adenosine A_3 receptor antagonist VUF8504 (**15**; Figure 2) bears a 4-methoxy substituent[20] and differs from the present compound **9d** in the spacer and the heterocyclic ring. Most probably, the length (and thus the substituent direction) of the amide and urea spacer contribute to the observed large difference. Therefore we investigated the 2- and 3-methoxy derivatives (**9f**,**e**, respectively) and compared them with the 4-methoxy derivative (**9d**).

The 3-methoxy derivative **9e** showed adenosine A_3 receptor affinity with a K_i value in the low micromolar range, whereas the 2-methoxy analogue VUF5386 (**9f**) had high adenosine A_3 receptor affinity with a K_i value of 87 nM (Table 4). The large difference in adenosine receptor affinities between **9d**,**f** is very remarkable. Since the electronic influence of ortho and para substitution is comparable, the steric aspects may be responsible for the observed difference.

Next, we examined the influence of another electron-donating substituent at the ortho position (Me, **9h**) as well as an electron-withdrawing substituent (Cl, **9g**) at this position. The affinities of compounds **9f**−**h** are in agreement with the electronic effect of the 4-substituted 3-(2-pyridyl)isoquinolin-1-ylbenzamides described before:[20] i.e., electron-withdrawing substituents did not influence adenosine A_3 receptor affinity compared to the unsubstituted phenyl derivative, while electron-donating substituents increased adenosine A_3 receptor affinity. This suggests a comparable type of interaction between the adenosine A_3 receptor and the substituents on both positions. We tried to visualize this by molecular modeling studies (next section).

Finally, the substituents of the most potent compounds of Tables 3 and 4 were combined in derivatives **10** in order to design active adenosine A_3 receptor antagonists. The substituent effects were found to be additive in compounds **10a**,**b**. Both compounds were very active, and again, the methoxy derivative showed

Table 1. Yields and Analytical Data of Isoquinoline and Quinazoline Analogues



4 5, 8-11

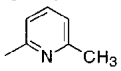
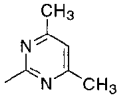
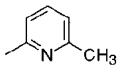
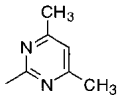
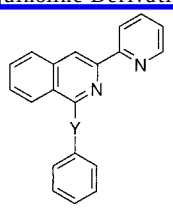
Compd	X	R_1	R_2	yield (%)	Method	mp (°C)	purification [a]	formula	anal.
4a	N	H	-	74	A	274-275[b]	column EA	$C_8H_7N_3$	-
4c	N	$C(CH_3)_3$	-	16	B	178	sublimation	$C_{12}H_{15}N_3$	-
4d	N	Ph	-	54	A	146[c]	MeOH/PE	$C_{14}H_{11}N_3$	-
4e	N	3-pyridyl	-	86	B	185-188	MeOH	$C_{13}H_{10}N_4$	-
4f	N	4-pyridyl	-	68	A	292-294	MeOH/EA	$C_{13}H_{10}N_4$	-
4g	N		-	36	B	200-202	MeOH/Hex	$C_{14}H_{12}N_4$	-
4h	N		-	29	B	219	MeOH/PE	$C_{14}H_{13}N_5$	-
4i	N		-	51	A	232-233	MeOH	$C_{12}H_9N_3O$	-
4j	N		-	23	A	247-249	MeOH	$C_{12}H_{16}N_4$	-
4k	N		-	34	A	325[d]	MeOH	$C_{12}H_{14}N_4$	-
5a	N	H	H	79	-	238-239	DMF/MeOH	$C_{15}H_{12}N_4O$	C, H, N
5b	N	2-pyridyl	H	85	-	260-262	DMF/MeOH	$C_{20}H_{15}N_5O$	C, H, N
5c	N	$C(CH_3)_3$	H	73	-	260-261[e]	DMF/MeOH	$C_{19}H_{20}N_4O \cdot 0.3\ CH_3OH$	C, H, N
5d	N	Ph	H	81	-	251-253	DMF/MeOH	$C_{21}H_{16}N_4O$	C, H, N
5e	N	3-pyridyl	H	76	-	247	DMF/MeOH	$C_{20}H_{15}N_5O \cdot 0.2\ CH_3OH$	C, H, N
5f	N	4-pyridyl	H	74	-	251	DMF/MeOH	$C_{20}H_{15}N_5O \cdot 0.2\ CH_3OH$	C, H, N
5g	N		H	69	-	243[d]	DMF/MeOH	$C_{21}H_{17}N_3O$	C, H, N
5h	N		H	78	-	260	DMF/MeOH	$C_{21}H_{18}N_6O$	C, H, N
5i	N		H	74	-	230[d]	DMF/MeOH	$C_{19}H_{14}N_4O_2 \cdot 0.1\ CH_3OH$	C, H, N
5j	N		H	77	-	211	DMF/MeOH	$C_{19}H_{21}N_5O$	C, H, N
5k	N		H	76	-	238[d]	DMF/MeOH	$C_{19}H_{19}N_5O$	C, H, N
8a	CH	H	H	99	-	221-223	DMF/EtOH	$C_{16}H_{13}N_3O$	C, H, N
8b	CH		H	98	-	228	DMF/MeOH	$C_{21}H_{16}N_4O \cdot 0.3\ CH_3OH$	C, H, N
8d	CH	Ph	H	98	-	250-251	DMF/MeOH	$C_{22}H_{17}N_3O \cdot 0.2\ CH_3OH$	C, H, N
9a	N	Ph	4-Cl	88	-	291-292	DMSO/MeOH	$C_{21}H_{15}ClN_4O$	C, H, N
9b	N	Ph	4-Me	66	-	258-260	NMP/MeOH	$C_{22}H_{18}N_4O \cdot 0.2\ CH_3OH$	C, H, N
9c	N	Ph	3,4-diCl	81	-	282-284	NMP/MeOH	$C_{21}H_{14}Cl_2N_4O \cdot 0.3\ CH_3OH$	C, H, N
9d	N	Ph	4-OMe	72	-	259-261	NMP/MeOH	$C_{22}H_{18}N_4O_2 \cdot 0.3\ CH_3OH$	C, H, N
9e	N	Ph	3-OMe	57	-	255-258	NMP/MeOH	$C_{22}H_{18}N_4O_2 \cdot 0.2\ H_2O$	C, H, N

Table 1 (Continued)

Compd	X	R_1	R_2	yield (%)	Method	mp (°C)	purification [a]	formula	anal.
9f	N	Ph	2-OMe	32	-	227-229	NMP/MeOH	$C_{22}H_{18}N_4O_2 \cdot 0.2\ H_2O$	C, H, N
9g	N	Ph	2-Cl	69	-	233-234	DMF/MeOH	$C_{21}H_{15}ClN_4O \cdot 0.2\ CH_3OH$	C, H, N
9h	N	Ph	2-Me	45	-	120-121	DMF/MeOH	$C_{22}H_{18}N_4O \cdot 0.2\ CH_3OH$	C, H, N
10a	N	3-pyridyl	2-OMe	77	-	257-258	DMF/MeOH	$C_{21}H_{17}N_5O_2$	C, H, N
10b	N	3-pyridyl	2-Me	50	-	227-228	DMF/EtOH	$C_{21}H_{17}N_5O$	C, H, N
11	N	2-pyridyl	2-OMe	68	-	190	DMF/EtOH	$C_{21}H_{17}N_5O_2$	C. H. N

Table 2. Adenosine Receptor Subtype Affinities of Isoquinoline Derivatives with Different Spacers



Compound	Y	A_3[a] K_i (μM)	A_1[b] K_i (μM)	A_{2A}[c]	A_1/A_3	Ref.
8b		0.076 ± 0.001	21 %	9 %	> 100	
12		0.73 ± 0.2	34 %	4 %	> 10	19
13		0.66 ± 0.2	0.24 ± 0.1	22 %	0.36	19
14		0.20 ± 0.04	3.2 ± 0.3	0 %	16	20

[a] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as K_i ± SEM in nM ($n = 3-5$). [b] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM ($n = 2-3$) or K_i ± SEM in μM ($n = 3$). [c] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM ($n = 2-3$).

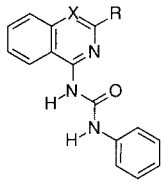
the highest affinity. This led to VUF5574 (**10a**), a potent human adenosine A_3 receptor antagonist with a K_i value of 4 nM and at least 2500-fold selectivity over A_1 and A_{2A} receptors.

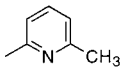
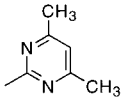
We also synthesized compound **11**, since isoquinolines and quinazolines described earlier also contained a 2-pyridyl group. Compound **11** is also a high-affinity human adenosine A_3 receptor antagonist but showed a 7-fold decrease in affinity compared to **10a**. This agrees with the earlier finding (derivatives **5b**,**e**) that in these quinazoline urea series a 3-pyridyl group is preferred over the 2-pyridyl group.

QSAR. We attempted some quantitative approaches to elucidate the relationships between the substituents at the 2-position of the quinazoline ring and the affinities. A similar approach was followed for substituents at the 2-position of the phenyl. We performed correlation analyses using various substituent parameters describing steric, electronic, and lipophilic properties. However, no correlation was found in the first series of compounds (compounds **5a**−**k**) and neither between (log P)[2] and the pK_i in contrast to the pyridine derivatives described as antagonists for the adenosine A_3 receptor.[16]

Molecular Modeling. Similar phenyl substituents in the urea and benzamide series led to comparable affinities (comparison of 2- and 4-substituents and 3-substituents).[20] This suggests a common binding site for the benzamide and phenylurea substituents. The structures of 4-methoxy-*N*-[3-(2-pyridyl)isoquinolin-1-yl]benzamide (**15**)[20] and *N*-(2-methoxyphenyl)-*N*′-[2-(3-pyridyl)quinazolin-4-yl]urea (**10a**; Figure 2) were built and minimized. From energy minimizations with molecular mechanics, four conformations emerged, each with the nitrogen of the pyridine ring "upwards", i.e., in the opposite direction of the spacer. In all four conformations the isoquinoline and quinazoline ring showed planarity with the spacer, whereas the pyridyl group was slightly turned out of the plane. The differences between the four conformations are due to two possible orientations of the pyridyl group and two possible orientations of the phenyl ring.

Table 3. Adenosine Receptor Affinities of 3-Substituted *N*-Phenyl-*N′*-(3-R-isoquinolin-1-yl)urea Derivatives and 2-Substituted *N*-Phenyl-*N′*-(2-R-quinazolin-4-yl)urea Derivatives



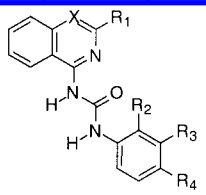
Compound	X	R	A_3[a] K_i (nM)	A_1[b]	A_{2A}[c]
5a	N	-H	1180 ± 50	48 %	42 %
5b	N		495 ± 167	0 %	2 %
5c	N	-C(CH$_3$)$_3$	78700 ± 57000	13 %	19 %
5d	N	-Ph	287 ± 106	36 %	24 %
5e	N		50.9 ± 4.3	170 ± 22	42 %
5f	N		336 ± 58	166 ± 42	27 %
5g	N		264 ± 29	23 %	12 %
5h	N		1260 ± 540	10 %	3 %
5i	N		257 ± 22	27 %	13 %
5j	N		178 ± 55	64.8 ± 13	34 %
5k	N		82.3 ± 15	49 %	11 %
8a	CH	-H	23 %	24 %	31 %
8b	CH		76.0 ± 9.5	21 %	9 %
8d	CH	-Ph	34 %	43 %	9 %

[a] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as K_i ± SEM in nM (n = 3−5) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM (n = 2−3) or K_i ± SEM in nM (n = 3). [c] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM (n = 2−3).

Subsequently, we aimed for matching of both the methoxy group and the pyridyl ring of the two compounds. Of the possible fits no one showed planarity of both the phenylurea and benzamide and the isoquinoline and quinazoline rings. The superimposition of compounds **15** and **10a**, with best possible matching of the nitrogens of the pyridine substituents as well as the methoxy groups, is depicted in Figure 3. This relatively poor fit shows that a comparable conformation of **15** and **10a** in their receptor interaction is not self-evident. The superimposition in Figure 3 shows that both compounds may bind at a common binding site but full overlap is not feasible. This suggests that more space in the receptor pocket exists than is being used, thus giving room for further synthetic efforts.

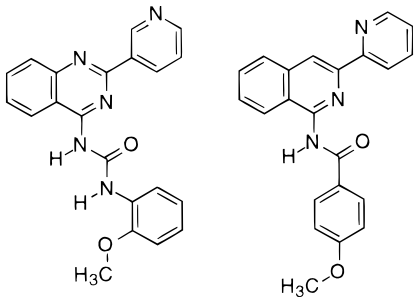
Functional Assay at Adenosine A_3 Receptors. For a functional evaluation intact cells expressing the human adenosine A_3 receptor were used. The inhibition of forskolin-stimulated cAMP production by receptor agonists was used as a read-out. NECA dose−response curves were recorded (n = 4) in the absence and presence of three increasing concentrations of **10a** (Figure 4). Compound **10a** caused a rightward shift of the dose−response curves; a pA_2 value of 8.1 was

Table 4. Adenosine Receptor Affinities of Substituted Quinazol-4-yl- and Isoquin-1-ylurea Derivatives



Compound	X	R_1	R_2	R_3	R_4	A_3[a] K_i (nM)	A_1[b]	A_{2A}[c]
5d	N	phenyl	H	H	H	287 ± 106	36 %	24 %
9a	N	phenyl	H	H	Cl	38 %	26 %	24%
9b	N	phenyl	H	H	CH_3	38 %	5.8 %	3.0 %
9c	N	phenyl	H	Cl	Cl	40 %	3.4 %	10 %
9d	N	phenyl	H	H	OCH_3	40 %	30 %	11 %
9e	N	phenyl	H	OCH_3	H	2040 ± 1890	6.8 %	0 %
9f VUF5386	N	phenyl	OCH_3	H	H	87.3 ± 36.8	3870 ± 510	22 %
9g	N	phenyl	Cl	H	H	220 ± 45	39 %	28 %
9h	N	phenyl	CH_3	H	H	115 ± 31.3	64 %	36 %
10a VUF5574	N	3-pyridyl	OCH_3	H	H	4.03 ± 0.46	52 %	43 %
10b	N	3-pyridyl	CH_3	H	H	23.9 ± 9.4	295 ± 59	12 %
11	N	2-pyridyl	OCH_3	H	H	28.2 ± 7.3	84.5 ± 14	0 %
XAC						108 ± 3.0		
L-249313						172 ± 9.1		
CGS 15943						143 ± 19		

[a] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as K_i ± SEM in nM (n = 3−5) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM (n = 2−3) or K_i ± SEM in nM (n = 3). [c] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM (n = 2−3).



VUF5574 (**10a**) VUF8504 (**15**)

Figure 2. Most potent A_3 ligands of the benzamide and urea series of isoquinolines and quinazolines at the human adenosine A_3 receptor.

calculated from a Schild plot (inset Figure 4). The slope of the Schild plot was not significantly different from unity, suggesting the competitive nature of **10a**. This value is in good agreement with the results from binding studies (pK_i = 8.4, Table 4).

Conclusions

In this study, we report on isoquinoline and quinazoline urea analogues as antagonists of the human adenosine A_3 receptor. From a series of *N*-phenyl-*N*′-quinazolin-4-ylurea derivatives we conclude that an aromatic group or an amine at the 2-position of the quinazoline ring is necessary for adenosine A_3 receptor affinity. Second, the effects of substitution on the phenylurea moiety were investigated. Substitution at the 3- or 4-position of the phenyl ring decreased the

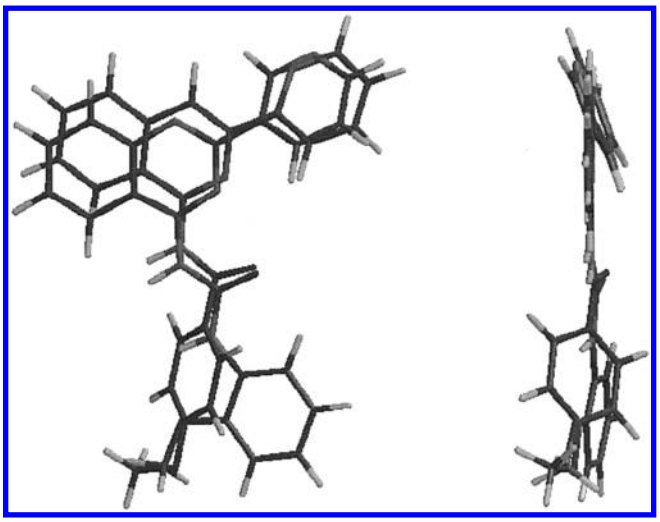


Figure 3. Fit of A_3 antagonists **10a** and **15** (in the plane of the isoquinoline and quinazoline rings (left) or 90° rotated (right)).

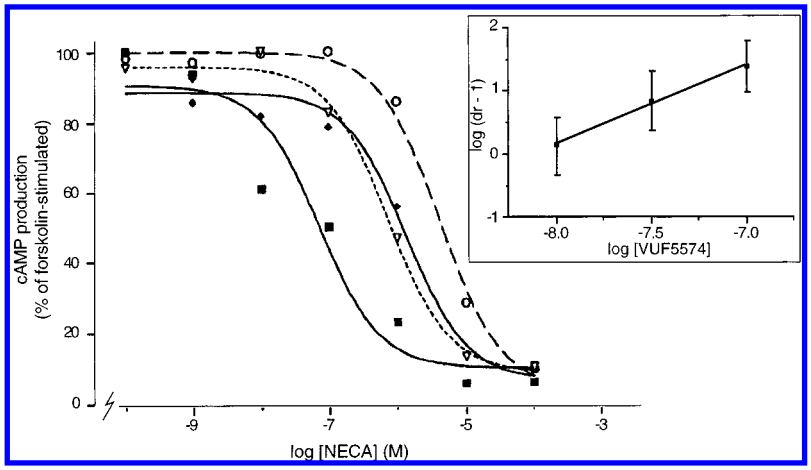


Figure 4. Inhibition of adenylate cyclase in cells stably transfected with human adenosine A_3 receptors. Data were taken from a typical experiment. The assay was carried out as described in the Experimental Section (in the presence of 10 μM forskolin). Dose−response curves of NECA were recorded in the absence and presence of three concentrations of antagonist **10a**. The pA_2 value was determined from a Schild plot (inset). In the Schild plot each data point is shown as mean ± SEM for four determinations. The ligands were: solid squares, NECA; open triangles, NECA + **10a** (10 nM); solid diamonds, NECA + **10a** (30 nM); open circles, NECA + **10a** (100 nM).

affinity, whereas an electron-withdrawing substituent on position 2 did not influence the binding. However, 2-substitution with the electron-donating substituents methyl or methoxy increased human adenosine A_3 receptor affinity.

Combination of the optimal substituents in both series as in **5e** (3-pyridyl) and **9f** (2-methoxyphenylurea) led to **10a**. In binding studies it showed a K_i value of 4 nM.

Functional antagonism was demonstrated in an assay consisting of agonist-induced inhibition of adenylate cyclase. The pA_2 value of compound **10a**, derived from a Schild plot, was 8.1. Thus, VUF5574 (**10a**) is a very active human adenosine A_3 receptor antagonist and is highly selective vs adenosine A_1 and A_{2A} receptors. This potent new human adenosine A_3 receptor antagonist might be a useful tool for pharmacological characterization of the A_3 receptor.

Experimental Section

Abbreviations: APT, attached proton test; CGS15943, 9-fluoro-2-(2-furyl)-5,6-dihydro[1,2,4]triazolo[1,5-*c*]quinazin-5-imine; CI, chemical ionization; COSY, correlated spectroscopy; DEPT, distortionless enhancement by polarization transfer; DMEM, Dulbecco's minimal essential medium; [^{3}H]DPCPX, [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine; [^{3}H]CGS 21680, [^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-(ethylcarbamoyl)adenosine; HEK cells, human embryonic kidney cells; HEPES, 4-(2-hydroxethyl)-1-piperazineethanesulfonic acid; HMPT, hexamethylphosphoric triamide; [^{125}I]AB-MECA, [^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine; K_i, equilibrium inhibition constant; L-249313, 6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl[1,2,4]triazolo[5,1-*a*][2,7]naphth-pyridine; NECA, 5′-(*N*-ethylcarbamoyl)adenosine; SPAP cells, secreted placental alkaline phosphatase; XAC, 8-[4-(((((2-aminoethyl)amino)carbonyl)methoxy)oxy)phenyl]-1,3-dipropylxanthine.

Materials. 6-Methylpyridine-2-carbonitrile was purchased from LONZA Inc. (France). 4,6-Dimethylpyrimidine-2-carbonitrile was commercially available from SALOR (Austria). Acetonitrile was purchased from J.T. Baker (The Netherlands). 1-Aminoisoquinoline (**6a**), 4-chlorophenyl isocyanate, 3,4-dichlorophenyl isocyanate, 2-furonitrile, and *o*-tolyl isocyanate were commercially available from Aldrich (The Netherlands). Benzyl cyanide, 3-cyanopyridine, 4-cyanopyridine, diethylaminoacetonitrile, 2-methoxyphenyl isocyanate, 3-methoxyphenyl isocyanate, 4-methoxyphenyl isocyanate, phenyl isocyanate, 1-pyrrolidinecarbonitrile, *p*-tolyl isocyanate, and trimethylacetonitrile were purchased from ACROS (Belgium). THF was predried over $CaCl_2$ and distilled from $LiAlH_4$. DMF was dried by a passage through a column of Al_2O_3 and acetone was distilled from K_2CO_3. All other solvents used were of analytical grade. 4-Amino-2-(2-pyridinyl)quinazoline (**4b**) was prepared as described by Linschoten et al.[22] 1-Amino-3-(2-pyridinyl)-isoquinoline (**6b**) and 1-amino-3-phenylisoquinoline (**6d**) were available from stock (Vrije Universiteit, Amsterdam).

Synthesis. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)propionate as an internal standard. 2D-NMR (H−H and C−H) COSY techniques were frequently used to support interpretation of 1D spectra. The multiplicity of the carbon signals was determined by DEPT or APT spectra or by a combination of a normal decoupled carbon spectrum and a CH correlation. The symbols used are (p) for primary, (s) for secondary, (t) for tertiary and (q) for quaternary carbon signals. Melting points were measured on a Electrothermal IA9200 apparatus. Elemental analysis were performed by the analytical department of Organic and Molecular Inorganic Chemistry at the University of Groningen (The Netherlands) and are within ±0.4% of theoretical values unless otherwise specified. Reactions were routinely monitored by thin layer chromatography on Merck silica gel F_{254} plates and spots were visualized with UV light at 254 nm or iodine or aqueous potassium permanganate staining.

General Procedure for the Preparation of 4a,c−k. Method A. To a solution of 10.37 g (88.00 mmol) of 2-aminobenzonitrile in anhydrous dioxane (100 mL) was added 1 equiv (4.75 g, 88.0 mmol) of freshly prepared sodium methoxide and the mixture stirred under a nitrogen atmosphere overnight. When all of the sodium methoxide had dissolved a solution of 1 equiv of the appropriate carbonitrile in 30 mL of anhydrous dioxane was added dropwise and refluxed for 16 h. After cooling the reaction mixture was hydrolyzed with 15 mL of water. Two equivalents (300 mL, 0.6 M) of HCl were added and the water layer was extracted with chloroform (3 × 150 mL). After neutralization of the solution with K_2CO_3, the product was extracted with 100 mL of chloroform (5 × 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and evaporated to dryness. The residue was crystalized.

Method B. A solution of 24.0 g (200 mmol) of 2-aminobenzonitril in 70 mL of THF was added dropwise to a suspension of 9.60 g (240 mmol) sodium hydride and 250 mL of anhydrous THF under a nitrogen atmosphere at 0 °C. After slow warming to room temperature 50 mL of the appropriate carbonitrile **3**

(400 mmol) or a solution of carbonitrile in dry THF was added dropwise under stirring and refluxed for 20 h. After cooling the reaction mixture was hydrolyzed with 25 mL of water and 1 equiv of HCl was added. The organic solvent was evaporated and 500 mL of water and 100 mL of chloroform were added to the resulting residue. The mixture was neutralized with 1.5 M NaOH and three times extracted with 100 mL of chloroform or ethyl acetate. The combined organic layers were dried over sodium sulfate and evaporated to dryness. The residue was purified by crystallization or sublimation.

4-Aminoquinazoline (4a). Method A. Extraction was carried out with ethyl acetate and purification by column chromotagraphy using EA as eluent: yield 74%; mp 274−275 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.48 (ddd, $^3J_{65}$ = 7.9 Hz, $^3J_{67}$ = 6.8 Hz, $^4J_{68}$ = 1.2 Hz, 1H, H6), 7.61−7.79 (m, 4H, H7, H8 & NH_2), 8.21 (d, $^3J_{56}$ = 8.0 Hz, 1H, H5), and 8.37 (s, 1H, H2).

2-(1,1-Dimethylethyl)-4-aminoquinazoline (4c). Method B. Extraction with chloroform and purification by sublimation at 0.5 Torr and 145 °C: yield 6.42 g (16%) white needles; mp 178 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 1.34 (s, 9H, CH_3), 7.40 (ddd, $^3J_{65}$ = 8.1 Hz, $^3J_{67}$ = 7.0 Hz, $^4J_{68}$ = 1.6 Hz, 1H, H6), 7.59 (bs, 2H,NH_2), 7.60−7.74 (m, 2H, H7 and H8), and 8.16 (d, $^3J_{56}$ = 8.1 Hz, 1H, H5); ^{13}C NMR (DMSO) 29.33 CH_3 (p) 38.52 $C(CH_3)_3$ (q) 112.4 C10 (q) 123.1 C7 (s) 124.2 C6 (s) 127.2 C8 (s) 132.1 C5 (s) 149.8 C9 (q) 161.5 C2 (q) 172.3 C4 (q).

2-Phenyl-4-aminoquinazoline (4d). Method A. Extraction with ethyl acetate and purification by crystallization from EA/Et_2O: yield 54%; mp 146 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.43−7.51 (m, 4H, ArH & H6), 7.67−7.85 (m, 4H, H7, H8 & NH_2), 8.12−8.37 (m, 1H, H5), and 8.38−8.48 (m, 2H, ArH).

2-(3-Pyridyl)-4-aminoquinazoline (4e). Method B: yield 86%; mp 185−188 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.35−7.46 (m, 2H, H5′ & H6), 7.78−7.83 (m, 2H, H8 & H7), 7.98 (bs, 2H, NH_2), 8.27 (d, $^3J_{56}$ = 8.2 Hz, 1H, H5), 8.66−8.72 (m, 2H, H4′ & H6′), and 9.56 (s, 1H, H2′).

2-(4-Pyridyl)-4-aminoquinazoline (4f). Method A: yield 68%; mp 292−294 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.49−7.60 (m, 1H, H6), 7.81−7.87 (m, 2H, H8 & H7), 8.02 (bs, 2H, NH_2), 8.26−8.31 (m, 3H, H5 & AA′BB′ H3′), 8.73 (dd, $^3J_{AB}$ = 4.5 Hz, $^4J_{AA'}$ = 1.4 Hz, 2H, AA′BB′ H2′).

2-(6-Methyl-2-pyridyl)-4-aminoquinazoline (4g). Method B: yield 36%; mp 200−202 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 2.55 (s, 3H, CH_3), 7.31 (d, $^3J_{5'4'}$ = 7.5 Hz, 1H, H5′), 7.45−7.56 (m, 1H, H6), 7.74−7.83 (m, 3H, H4′, H8 & H7), 7.99 (s, 2H, NH_2), 8.21 (d, $^3J_{3'4'}$ = 7.7 Hz, 1H, H3′), and 8.28 (d, $^3J_{56}$ = 8.2 Hz, 1H, H5).

2-(4,6-Dimethylpyrimidin-2-yl)-4-aminoquinazoline (4h). Method B: yield 29%; mp 219 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 2.50 (s, 6H, CH_3), 7.32 (s, 1H, H5′), 7.50−7.58 (m, 1H, H6), 7.80−7.81 (m, 2H, H7 & H8), 8.00 (s, 2H, NH_2), and 8.27 (d, $^3J_{56}$ = 8.2 Hz, 1H, H5).

2-(2-Furyl)-4-aminoquinazoline (4i). Method A: yield 51%; mp 232−233 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 6.64 (dd, $^3J_{4'5'}$ = 3.4 Hz, $^3J_{4'3'}$ = 1.8 Hz, 1H, H4′) 7.18 (dd, $^3J_{5'4'}$ = 3.3 Hz, $^4J_{5'3'}$ = 0.8 Hz, 1H, H5′), 7.44 (ddd, $^3J_{65}$ = 8.0 Hz, $^3J_{67}$ = 6.5 Hz, $^4J_{68}$ = 1.8 Hz, 1H, H6), 7.66−7.80 (m, 3H, H3′, H8, and H7), 7.84 (bs, 2H, NH_2), 8.20 (d, $^3J_{56}$ = 7.9 Hz, 1H, H5).

2-Diethylamino-4-aminoquinazoline (4j). Method A: yield 23%; mp 247−249 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 1.11 (t, ^3J = 7.0 Hz, 6H, CH_3), 3.60 (q, ^3J = 7.0 Hz, 4H, CH_2), 6.96 (ddd, $^3J_{65}$ = 8.1 Hz, $^3J_{67}$ = 6.9 Hz, $^4J_{68}$ = 1.2 Hz, 1H, H6), 7.19−7.36 (m, 3H, H8 & NH_2), 7.45 (ddd, $^3J_{78}$ = 8.4 Hz, $^3J_{76}$ = 6.9 Hz, $^4J_{75}$ = 1.4 Hz, 1H, H7), and 7.93 (dd, $^3J_{56}$ = 8.1 Hz, $^4J_{57}$ = 1.0 Hz, 1H, H5).

2-(Pyrrolidin-1-yl)-4-aminoquinazoline (4k). Method A: yield 34%; mp 325 °C dec; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 1.88 (t, ^3J = 6.5 Hz, 4H, CH_2), 2.50 (t, ^3J = 6.5 Hz, 4H, CH_2), 6.97 (dd, $^3J_{65}$ = 8.2 Hz, $^3J_{67}$ = 6.7 Hz, 1H, H6), 7.23−7.39 (m, 3H, H8 & NH_2), 7.48 (dd, $^3J_{78}$ = 8.4 Hz, $^3J_{76}$ = 6.8 Hz, 1H, H7), and 7.94 (dd, $^3J_{56}$ = 8.4 Hz, 1H, H5).

General Procedure for the Preparation of 5a−k. One equivalent of quinazolineamine **4** was dissolved in acetonitrile at 20−50 °C (2 mL/mmol) and 1 equiv of phenyl isocyanate was added dropwise. The mixture was stirred at 30−50 °C for 1 h. The precipitate was isolated, dried and recrystallized from DMF/methanol.

***N*-Phenyl-*N*′-(quinazolin-4-yl)urea (5a).** The reaction was carried out at room temperature: yield 79%; mp 238−239 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.10 (t, $^3J_{4'3'}$ = 6.9 Hz, 1H, ArH), 7.37 (t, $^3J_{2'3'}$ = 7.9 Hz. 2H, ArH), 7.68−7.22 (m, 3H, ArH & H6), 7.85−7.96 (m, 2H, H7 & H8), 8.68−8.82 (m, 2H, H5 & H2), 14.08 (bs, 1H, NH). Anal. ($C_{15}H_{21}N_4O$) C, H, N.

***N*-Phenyl-*N*′-[2-(2-pyridyl)quinazolin-4-yl]urea (5b).** The reaction temperature was 50 °C: yield 85%; mp 260−262 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.09 (t, $^3J_{4''3''}$ = 7.3 Hz, 1H, ArH), 7.45 (t, $^3J_{2''3''}$ = 8.0 Hz, 2H, ArH), 7.60−7.94 (m, 5H, ArH, H8, H6 & H7), 8.06 (m, 1H, H4′), 8.24 (ddd, $^3J_{5'4'}$ = 8.8 Hz, $^3J_{5'6'}$ = 8.3 Hz, $^4J_{5'3'}$ = 1.1 Hz, 1H, H5′), 8.65 (dd, $^3J_{56}$ = 7.8 Hz, $^4J_{57}$ = unsolved, 1H, H5), 8.74 (dd, $^3J_{6'5'}$ = 8.4 Hz, $^4J_{6'4'}$ = 1.4 Hz, 1H, H6′), 8.84 (ddd, $^3J_{3'4''}$ = 4.9 Hz, $^4J_{3'5'}$ = 1.0 Hz, 1H, H3′), 10.12 (bs, 1H, NH) and 13.08 (bs, 1H, NH). Anal. ($C_{20}H_{15}N_5O$) C, H, N.

***N*-[2-(1,1-Dimethylethyl)quinazolin-4-yl]-*N*′-phenylurea (5c).** The reaction was carried out at room temperature: yield 73%; mp 260−261 °C dec; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 1.49 (s, 9H, CH_3), 7.08−7.22 (m, 1H, H8), 7.42 (dd, $^3J_{67}$ = unsolved, $^3J_{65}$ = unsolved, 1H, H6), 7.62−7.66 (m, 4H, H7 and ArH), 7.80−7.99 (m, 2H, ArH), 8.71 (d, $^3J_{56}$ = 8.6 Hz, 1H, H5), 10.48 (bs, 1H, NH) and 12.39 (bs, 1H, NH). Anal. ($C_{19}H_{20}N_4O$·0.3MeOH) C, H, N.

***N*-Phenyl-*N*′-[(2-phenyl)quinazolin-4-yl]urea (5d).** The reaction was carried out at room temperature: yield 81%; mp 251−253 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.11−7.23 (m, 2H, ArH), 7.39−7.47 (m, 3H, ArH), 7.61−7.69 (m, 6H, ArH & H8 & H6 & H7), 7.99 (d, $^3J_{56}$ = 8.5 Hz, 1H, H5), 8.30−8.48 (m, 2H, ArH), 10.62 (bs, 1H, NH), and 12.26 (bs, 1H, NH). Anal. ($C_{21}H_{16}N_4O$) C, H, N.

***N*-Phenyl-*N*′-[2-(3-pyridyl)quinazolin-4-yl]urea (5e).** The reaction temperature was 50 °C: yield 76%; mp 247 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.15 (t, $^3J_{4''3''}$ =7.0 Hz, 1H, ArH), 7.43 (t, $^3J_{2''3''}$ = 7.5 Hz, 2H, ArH), 7.65−7.73 (m, 4H, H8, H5′, H6 & H7), 7.98−8.02 (m, 2H, ArH), 8.68−8.79 (m, 3H, H4′, H6′ & H5), 9.54 (bs, 1H, H2′), 10.66 (bs, 1H, NH) and 11.94 (bs, 1H, NH). Anal. ($C_{20}H_{15}N_5O$· 0.2MeOH) C, H, N.

***N*-Phenyl-*N*′-[2-(4-pyridyl)quinazolin-4-yl]urea (5f).** The reaction temperature was 50 °C: yield 74%; mp 251 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 7.15 (t, $^3J_{4''3''}$ = 6.9 Hz, 1H, ArH), 7.44 (t, $^3J_{2''3''}$ = 7.9 Hz, 2H, ArH), 7.66−7.82 (m, 3H, H8, H6 & H7), 8.03−8.06 (m, 2H, ArH), 8.27 (d, $^3J_{AB}$ = 6.0 Hz, 2H, AA′BB′ ArH), 8.77 (d, $^3J_{56}$ = 8.3 Hz, 1H, H5), 8.86 (d, $^3J_{BA}$ = 5.9 Hz, 2H, AA′BB′ ArH), 10.70 (bs, 1H, NH) and 11.90 (bs, 1H, NH). Anal. ($C_{20}H_{15}N_5O$·0.2MeOH) C, H, N.

***N*-[2-(6-Methyl-2-pyridyl)quinazolin-4-yl]-*N*′-phenylurea (5g).** The reaction was carried out at room temperature: yield 69%; mp 243 °C dec; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 2.55 (s, 3H, CH_3), 6.95 (t, $^3J_{4''3''}$ = 7.0 Hz, 1H, ArH), 7.31−7.53 (m, 4H, ArH, H5′ & H7), 7.75−7.92 (m, 3H, H6 & ArH), 8.04−8.18 (m, 3H, H8, H5 & H4′), 8.88 (d, $^3J_{3'4'}$ = 8.0 Hz, 1H, H3′), 10.75 (bs, 1H, NH) and 13.43 (s, 1H, NH). Anal. ($C_{21}H_{17}N_5O$) C, H, N.

***N*-[(4,6-Dimethylpyrimidin-2-yl)quinazolin-4-yl]-*N*′-phenylurea (5h).** The reaction was carried out at room temperature: yield 78%; mp 260 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm) δ 2.55 (s, 6H, CH3), 6.95 (t, $^3J_{4''3''}$ = 7.9 Hz, 1H, H4″), 7.30−7.61 (m, 2H, H3″), 7.63−7.85 (m, 1H, H7), 7.89−8.05 (m, 2H, H2″), 8.20 (m, 1H, H6), 8.45 (d, $^3J_{87}$ = 8.1 Hz, 1H, H8), 8.79 (d, $^3J_{56}$ = 8.3 Hz, 1H, H5), 10.71 (bs, 1H, NH) and 13.59 (bs, 1H, NH). Anal. ($C_{21}H_{18}N_6O$) C, H, N.

***N*-[2-(2-Furyl)quinazolin-4-yl]-*N*′-phenylurea (5i).** The reaction was carried out at room temperature: yield 74%; mp 230 °C dec; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-H = 2.49 ppm)

δ 6.82 (dd, $^3J_{4'5'}$ = 3.4 Hz, $^3J_{4'3'}$ = 1.8 Hz, 1H, H4′), 7.14−7.23 (m, 1H, H4″), 7.41−7.49 (m, 3H, H5′ and H3″), 7.64 (ddd, 1H, H7), 7.78 (d, 2H, H2″), 7.92−7.96 (m, 2H, H8 & H6), 8.14 (sd, $^3J_{3'4'}$ = 1.7 Hz, 1H, H3′), 8.74 (d, $^3J_{56}$ = 8.2 Hz, 1H. H5), 10.65 (bs, 1H, NH) and 12.81 (s, 1H, NH). Anal. ($C_{19}H_{14}N_4O_2$·0.1MeOH) C, H, N.

***N*-(2-Diethylaminoquinazolin-4-yl)-*N*′-phenylurea (5j).** The reaction was carried out at room temperature: yield 77%; mp 211 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 1.20 (t, ^3J = 7.0 Hz, 6H, CH_3), 3.70 (q, ^3J = 7.0 Hz, 4H, CH_2), 7.11−7.16 (m, 3H, H7 & ArH), 7.34−7.42 (m, 2H, H6 & ArH), 7.55−7.66 (m, 3H, H8 & ArH), 8.40 (d, $^3J_{56}$ = 7.8 Hz, 1H, H5), 10.15 (bs, 1H, NH) and 11.59 (s, 1H, NH). Anal. ($C_{19}H_{21}N_5O$) C, H, N.

***N*-Phenyl-*N*′-[(2-pyrrolidin-1-yl)quinazolin-4-yl]urea (5k).** The reaction was carried out at room temperatur: yield 76%; mp 238 °C dec; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 1.88 (bs, 4H, CH_2), 3.53 (bs, 4H, CH_2), 7.05−7.19 (m, 2H, H7 & ArH), 7.29−7.48 (m, 3H, H6 & ArH), 7.52−7.67 (m, 3H, H8 & ArH), 8.51 (d, $^3J_{56}$ = 7.9 Hz, 1H, H5), 10.22 (bs, 1H, NH), and 11.99 (s, 1H, NH). Anal. ($C_{19}H_{19}N_5O$) C, H, N.

General Procedure for the Preparation of 8a−b,d. One equivalent of **6** was dissolved in acetonitrile at 50 °C (5 mL/mmol) and a solution of 1 equiv of phenyl isocyanate **(7i)** in acetonitrile was added (2 mL/mmol) (3-mmol scale). The mixture was stirred at 50 °C for 1 h. The precipitate was isolated, washed several times with subsequently acetonitrile, methanol, and petroleum ether, dried and recrystallized.

***N*-Phenyl-*N*′-(isoquinolin-1-yl)urea (8a).** Recrystallization from DMF/EtOH yielded 99% of white crystals: mp 221−223 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 7.07 (t, $^3J_{4'3'}$ = 7.3 Hz, 1H, ArH), 7.36 (t, $^3J_{3'2'}$ = 7.8 Hz, 2H. ArH), 7.49 (d, $^3J_{3,4}$ = 5.8 Hz, 1H, H3), 7.61−7.73 (m, 3H, H7 & ArH), 7.76−7.84 (m, 1H, H6), 7.94 (d, $^3J_{87}$ = 8.2 Hz, 1H, H8), 8.22 (d, $^3J_{43}$ = 5.8 Hz, 1H, H4), 8.70 (d, $^3J_{56}$ = 8.3 Hz, H5), 9.96 (bs, 1H, NH), and 12.70 (bs, 1H, NH). Anal. ($C_{16}H_{13}N_3O$) C, H, N.

***N*-Phenyl-*N*′-[3-(2-pyridyl)isoquinolin-1-yl]urea (8b).** Recrystallization from DMF/MeOH yielded 98% of yellow/white crystals: mp 228 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 7.40−7.58 (m, 3H, ArH), 7.61 (m, 1H, H4′), 7.67−7.92 (m, 4H, ArH & H6 & H7), 8.02−8.16 (m, 2H, H3′ & H5′), 8.30 (dd, $^3J_{87}$ = 8.0 Hz, $^4J_{86}$ = unsolved, 1H, H8), 8.41 (s, 1H, H4), 8.74 (d, $^3J_{56}$ = 8.6 Hz, 1H, H5), 8.83−8.88 (m, 1H, H6′), 10.15 (bs, 1H, NH), and 13.09 (bs, 1H, NH). Anal. ($C_{21}H_{16}N_4O$·0.3MeOH) C, H, N.

***N*-Phenyl-*N*′-(3-phenylisoquinolin-1-yl)urea (8d).** Recrystallization from DMF/MeOH yielded 98% of yellow/white crystals: mp 250−251 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 7.42−7.62 (m, 3H, ArH), 7.71−7.94 (m, 9H, ArH & H6 & H7), 8.26 (dd, $^3J_{87}$ = 7.9 Hz, $^4J_{86}$ = unsolved, 1H, H8), 8.39 (s, 1H, H4), 8.70 (d, $^3J_{56}$ = 8.4 Hz, 1H, H5), 10.04 (bs, 1H, NH), and 12.89 (bs, 1H, NH). Anal. ($C_{22}H_{17}N_3O$·0.2MeOH) C, H, N.

General Procedure for the Preparation of 9a−h, 10a,b, and 11. One equivalent of 2-phenyl-4-quinazolineamine (**4d**) was dissolved in acetonitrile at 30−50 °C (5 mL/mmol) and a solution of 1 equiv of isocyanate **7** in acetonitrile was added (2 mL/mmol). The mixture was stirred at 30−50 °C for 0.5−4 h. The precipitate was isolated, washed several times with different solvents, dried and recrystallized.

***N*-(4-Chlorophenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9a):** yield 88%; mp 291−292 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 6.51 (d, $^3J_{AB}$ = 8.8 Hz, 2H, AA′BB′ ArH), 7.01 (d, $^3J_{BA}$ = 8.7 Hz, 2H, AA′BB′ ArH), 7.47−7.62 (m, 2H, H6 and H7), 7.62−7.76 (m, 3H, H3′, H4′, and H5′), 8.24 (dd, $^3J_{87}$ = 8.3 Hz, $^4J_{86}$ = unsolved, 1H, H8), 8.43−8.46 (m, 2H, H2′ and H6′), 8.73 (dd, $^3J_{56}$ = unsolved, $^4J_{57}$ = unsolved, 1H, H5), 10.70 (bs, 1H, NH), and 12.29 (bs, 1H, NH). Anal. ($C_{21}H_{15}ClN_4O$) C, H, N.

***N*-(4-Methylphenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9b):** yield 66%; mp 258−260 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 3.61 (s, 3H, CH_3), 6.50 (d, $^3J_{AB}$ = 8.9 Hz, 2H, AA′BB′ ArH), 6.63 (d, $^3J_{BA}$ = 8.9 Hz, 2H, AA′BB′ ArH), 7.51−7.92 (m, 5H, H6, H7, H3′, H4′, and H5′). 8.24 (d, $^3J_{87}$ = 8.0 Hz, 1H, H8), 8.43−8.55 (m, 2H, H2′ & H6′), 8.70 (dd, $^3J_{56}$ = unsolved, $^4J_{57}$ = unsolved, 1H, H5), 10.58 (bs, 1H, NH), and 12.19 (bs, 1H, NH). Anal. ($C_{22}H_{18}N_4O$·0.2MeOH) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9c):** yield 81%; mp 282−284 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 7.16−7.21 (m, 3H, ArH), 7.47−7.50 (m, 2H, H6 and H7), 7.75−7.84 (m, 5H, H2′, H3′, H4′, H5′, and H6′), 8.24 (dd, $^3J_{87}$ = 8.2 Hz, $^4J_{86}$ = unsolved, 1H, H8), 8.45 (dd, $^3J_{56}$ = 8.7 Hz, $^4J_{57}$ = 1.9 Hz, 1H, H5), 10.69 (bs, 1H, NH), and 12.32 (bs, 1H, NH). Anal. ($C_{21}H_{14}Cl_2N_4O$·0.3MeOH) C, H, N.

***N*-(4-Methoxyphenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9d):** yield 72%; mp 259−261 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 3.77 (s, 3H, OCH_3), 7.01 (d, $^3J_{2''3''}$ = 9.0 Hz, 4H, ArH), 7.56−7.60 (m, 5H, H6, H7, H3′ & H4′), 7.97 (dd, $^3J_{87}$ = unsolved, $^4J_{86}$ = unsolved, 1H, H8), 8.37−8.42 (m, 2H, H2′), 8.76 (dd, $^3J_{56}$ = unsolved, $^4J_{57}$ = unsolved, 1H, H5), 10.56 (bs, 1H, NH), and 12.15 (bs, 1H, NH). Anal. ($C_{22}H_{18}N_4O_2$·0.3MeOH) C, H, N.

***N*-(3-Methoxyphenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9e):** yield 57%; mp 255−258 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 3.79 (s, 3H, OCH_3), 6.70−6.74 (m, 1H, H4″), 7.27−7.38 (m, 3H, H2″, H5″, and H6″), 7.62−7.70 (m, 5H, H6, H7, H3′ & H4′), 7.99 (dd, $^3J_{87}$ = unsolved, $^4J_{86}$ = unsolved, 1H, H8), 8.39−8.41 (m, 2H, H2′), 8.75 (dd, $^3J_{56}$ = 8.4 Hz, $^4J_{57}$ = unsolved, 1H, H5), 10.60 (bs, 1H, NH), and 12.28 (bs, 1H, NH). Anal. ($C_{22}H_{18}N_4O_2$·0.2H_2O) C, H, N.

***N*-(2-Methoxyphenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9f):** yield 32%; mp 227−229 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 3.62 (s, 3H, OCH_3), 7.00−7.13 (m, 4H, ArH), 7.51−7.69 (m, 3H, H3′, ArH, and H5′), 7.97−7.99 (m, 2H, H6 and H7), 8.19 (dd, $^3J_{87}$ = 8.1 Hz, $^4J_{86}$ = unsolved, 1H, H8), 8.45−8.49 (m, 2H, H4′ and H6′), 8.77 (dd, $^3J_{56}$ = 8.5 Hz, $^4J_{57}$ = unsolved, 1H, H5), 10.58 (bs, 1H, NH), and 11.89 (bs, 1H, NH). Anal. ($C_{22}H_{18}N_4O_2$·0.2H_2O) C, H, N.

***N*-(2-Chlorophenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9g):** yield 69%; mp 233−234 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 7.17 (t, $^3J_{4''3''}$ = 6.9 Hz, 1H, H4″), 7.35−7.58 (m, 5H, H3′, H2″ & H3″), 7.62−7.74 (m, 1H, H5′), 7.92−8.01 (m, 2H, H4′ & H8), 8.13 (d, $^3J_{6'5'}$ = 7.1 Hz, 1H, H6′), 8.28−8.46 (m, 2H, H6 & H7), 8.77 (d, $^3J_{56}$ = 7.3 Hz, 1H, H5), 10.78 (bs, 1H, NH), and 11.99 (bs, 1H, NH). Anal. ($C_{21}H_{15}ClN_4O$·0.2MeOH) C, H, N.

***N*-(2-Methylphenyl)-*N*′-(2-phenylquinazolin-4-yl)-urea (9h):** yield 45%; mp 120−121 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 2.18 (s, 3H, CH3), 7.05−7.32 (m, 3H, ArH), 7.48−7.56 (m, 3H, ArH), 7.60−7.78 (m, 2H, H6 & H7), 7.89−8.03 (m, 2H, ArH), 8.23−8.30 (m, 2H, H8 & H6′), 8.78 (d, $^3J_{56}$ = 8.2 Hz 1H, H5), 10.62 (bs, 1H, NH), and 11.71 (bs, 1H, NH). Anal. ($C_{22}H_{18}N_4O$·0.2MeOH) C, H, N.

***N*-(2-Methoxyphenyl)-*N*′-[2-(3-pyridyl)quinazolin-4-yl]-urea (10a):** yield 77%; mp 257−258 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 3.62 (s, 3H, OCH_3), 6.98−7.15 (m, 3H, H3″, H4″ & H5″), 7.62−7.79 (m, 2H, H6 & H7), 8.03 (m, 2H, H5′ & H6″), 8.11 (d, $^3J_{87}$ = 8.4 Hz, 1H, H8), 8.71−8.83 (m, 3H, H4′, H6′ & H5), 9.61 (s, 1H, H2′), 10.65 (bs, 1H, NH), and 11.80 (bs, 1H, NH). Anal. ($C_{21}H_{17}N_5O_2$) C, H, N.

***N*-(2-Methylphenyl)-*N*′-[2-(3-pyridyl)quinazolin-4-yl]-urea (10b):** yield 50%; mp 227−228 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) δ 2.19 (s, 3H, CH_3), 7.14−7.27 (m, 3H, H5′ & ArH), 7.61−7.81 (m, 3H, H8, H6 & H7), 8.02−8.04 (m, 2H, ArH), 8.62 (d, $^3J_{56}$ = 8.0 Hz, 1H, H5), 8.74−8.82 (m, 2H, H4′ & H6′), 9.41 (s, 1H, H2′), 10.70 (bs, 1H, NH), and 11.50 (s, 1H, NH). Anal. ($C_{21}H_{17}N_5O$) C, H, N.

***N*-(2-Methoxyphenyl)-*N*′-[2-(2-pyridyl)quinazolin-4-yl]-urea (11):** yield 68%; mp 190 °C; ^{1}H NMR (DMSO-d_6, ref DMSO-d_5-*H* = 2.49 ppm) two isomeric forms A:B = 4:1, A δ 3.40 (s, 3H, OCH_3), 6.98−7.10 (m, 4H, ArH), 7.55−8.14 (m, 5H, H4′, H5′, H6, H7 & H8), 8.39−8.43 (m, 1H, H3′), 8.77−8.84 (m, 2H, H6′ & H5), 10.65 (bs, 1H, NH), and 12.29 (bs, 1H, NH), B δ 3.90 (s, 3H, OCH_3), 6.98−7.10 (m, 4H, ArH), 7.55−8.11 (m, 5H, H4′, H5′, H6, H7 & H8), 8.52−8.56 (m, 1H,

H3′), 8.77−8.84 (m, 2H, H6′ & H5), 10.65 (bs, 1H, NH), 14.90 (bs, 1H, N···H···O). Anal. ($C_{21}H_{17}N_5O_2$) C, H, N.

Molecular Modeling. Calculations were performed with SPARTAN version 5.0 (Wavefunction, Inc., Irvine, CA)[33] running on a Silicon Graphics O2 workstation. The Merck force field was used in molecular mechanics minimizations.

Correlation Analysis. Thirteen descriptors for aromatic substituents were used in the correlation analysis for compounds **5a**−**k** and **9**: namely σ_m, σ_p, σ^0, σ^-, σ^+, π, log *P*, (log *P*)^2, E_s, *L*, B_1, B_{max}, and MR.[33] No correlation was observed between the A_3 receptor binding affinity and the descriptors other than B_{max}. Data analysis was with Microsoft Excel version 5.0 (Microsoft Corp.) running on MacOS 8.5.

Pharmacology. Materials. [^{3}H]cAMP, [^{3}H]DPCPX, and [^{3}H]CGS 21680 were commercially available from DuPont Nemours ('s Hertogenbosch, The Netherlands) with specific activities of 31, 120, and 38.3 Ci/mmol, respectively. [^{125}I]AB-MECA was prepared as described by Olah et al.[31] Adenosine deaminase was from Boehringer Mannheim (Mannheim, Germany). Forskolin was purchased from Sigma (The Netherlands) and XAC from Research Biochemicals International (Natick, MA). CGS 15943 was a gift from Dr. M. Williams and Dr. J. Watthey (Ciba-Geigy, Summit) and L-249313 was a gift form Dr. M. Jacobson (Merck). CHO cells expressing the human adenosine A_3 receptor were obtained from Dr. S. Rees (Glaxo Wellcome, U.K.) and HEK 293 cells stably expressing the human adenosine A_3 receptor were a gift from Dr. K.-N. Klotz (University of Würzburg, Germany).

Methods for Receptor Binding and Adenylase Cyclase Measurement. Binding of [^{3}H]DPCPX to adenosine A_1 receptors on rat cerebral cortex membranes and of [^{3}H]CGS 21680 to adenosine A_{2A} receptors from rat striatal membranes was performed as described previously.[28,29] Binding of [^{125}I]AB-MECA to membranes of HEK 293 cells stably expressing the human adenosine A_3 receptor was determined as described.[30,31] cAMP production was assayed in CHO cells stably expressing the human adenosine A_3 receptor. Assays were performed in DMEM/HEPES buffer (adjusted to pH 7.4, at 25 °C). The method involved addition of the ligand to membranes in the presence of ADA (10 IU/mL), NECA as agonist, forskolin (10 μM) to stimulate adenylate cyclase, and rolipram and cilostamide (50 μM each) as phosphodiesterase inhibitors. The reaction was terminated by addition of ice-cold 0.1 N HCl. cAMP determination was done via [^{3}H]cAMP and incubation for 2.5−18 h at 0 °C with protein kinase A. After filtration over Whatman GF/C filters using a Brandell cell harvester (Brandell, Gaithersburg, MD) under reduced pressure (200 mbar), the radioactivity was determined in a LKB1219 counter. The IC_{50} values and pA_2 were calculated using Prism (GraphPad, SanDiego, CA).

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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