Thiazole and Thiadiazole Analogues as a Novel Class of Adenosine Receptor Antagonists

Jacqueline E. van Muijlwijk-Koezen,* Hendrik Timmerman, Roeland C. Vollinga,‡ Jacobien Frijtag von Drabbe Künzel,‡ Miriam de Groote,‡ Sven Visser, and Adriaan P. IJzerman‡

Department of Pharmacochemistry, Division of Medicinal Chemistry, Leiden/Amsterdam Center for Drug Research, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands, and Gorlaeus Laboratories, Division of Medicinal Chemistry, Leiden/Amsterdam Center for Drug Research, Universiteit Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Received September 7, 2000

Novel classes of heterocyclic compounds as adenosine antagonists were developed based on a template approach. Structure−affinity relationships revealed insights for extended knowledge of the receptor−ligand interaction. We replaced the bicyclic heterocyclic ring system of earlier described isoquinoline and quinazoline adenosine A_3 receptor ligands by several monocyclic rings and investigated the influence thereof on adenosine receptor affinity. The thiazole or thiadiazole derivatives seemed most promising, so we continued our investigations with these two classes of compounds. The large difference between a pyridine and isoquinoline ring in binding adenosine A_1 and A_3 receptors showed the importance of the second ring of the isoquinoline ligands. We prepared several *N*-[4-(2-pyridyl)thiazol-2-yl]benzamides, and these compounds showed adenosine affinities in the micromolar range. Most surprising in the series of the *N*-[4-(2-pyridyl)thiazol-2-yl]amides were the retained adenosine affinities by introduction of a cylopentanamide instead of the benzamide. A second series of compounds, the thiadiazolobenzamide series of compounds, revealed potent and selective adenosine receptor antagonists, especially *N*-(3-phenyl-1,2,4-thiadiazol-5-yl)-4-hydroxybenzamide (LUF5437, **8h**) showing a K_i value of 7 nM at the adenosine A_1 receptor and *N*-(3-phenyl-1,2,4-thiadiazol-5-yl)-4-methoxybenzamide (LUF5417, **8e**) with a K_i value of 82 nM at the adenosine A_3 receptor. 4-Hydroxybenzamide **8h** is the most potent adenosine A_1 receptor antagonist of this new class of compounds. Structure−affinity relationships showed the existence of a steric restriction at the *para*-position of the benzamide ring for binding adenosine A_1 and A_3 receptors. The electronic nature of the 4-substituents played an important role in binding the adenosine A_3 receptor. *Cis*- and *trans*-4-substituted cyclohexyl derivatives were made next to the 4-substituted benzamide analogues. We used them to study the proposed specific interaction between the adenosine A_1 receptor and the 4-hydroxy group of this class of thiadiazolo compounds, as well as a suggested special role for the 4-methoxy group in binding the A_3 receptor. Both the adenosine A_1 and A_3 receptor slightly preferred the *trans*-analogues over the *cis*-analogues, while all compounds showed low affinities at the adenosine A_{2A} receptor. Our investigations provided the potent and highly selective adenosine A_1 antagonist *N*-(3-phenyl-1,2,4-thiadiazol-5-yl)-*trans*-4-hydroxycyclohexanamide (VUF5472, **8m**) showing a K_i value of 20 nM. A third series of compounds was formed by urea analogues, *N*-substituted with thiazolo and thiadiazolo heterocycles. The SAR of this class of compounds was not commensurate with the SAR of the previously described quinazoline urea. On the basis of these findings we suggest the existence of a special interaction between adenosine receptors and a region of high electron density positioned between the thia(dia)zole ring and phenyl(pyridyl) ring. Molecular electrostatic potential contour plots showed that for this reason the ligands need either a thiadiazole ring instead of a thiazole or a 2-pyridyl group instead of a phenyl. The derived novel classes of antagonists will be useful for a better understanding of the molecular recognition at the adenosine receptors.

Introduction

Extracellular adenosine regulates several physiological functions by activation of specific cell membrane receptors. The combination of pharmacological studies and molecular cloning revealed the existence of four distinct adenosine receptor subtypes which are identified and classified as A_1, A_{2A}, A_{2B}, and A_3. Whereas the adenosine A_1 and A_3 receptor subtypes are coupled to the G_i protein, inhibiting adenylate cyclase, A_{2A} and A_{2B} subtypes stimulate this enzyme via G_s.

The xanthine core structure has served as the basis for numerous selective antagonists for adenosine A_1, A_{2A}, and A_{2B} receptors.[1,2] The development of selective antagonists for the A_3 receptor has relied on chemically rather diverse structural leads. Triazolonaphthpyridine,[3,4] thiazolopyrimidine,[3] 1,4-dihydropyridines,[5−7] pyridines,[8] pyridinium salts,[9] pyrans,[10] triazoloquinazolines,[11,12] flavonoids,[13] and triazolopyrimidines[14] have

* To whom correspondence should be addressed. Tel: (+31) 20 4447604. Fax: (+31) 20 4447610. E-mail: muylwyk@chem.vu.nl.
‡ Universiteit Leiden.

10.1021/jm0003945 CCC: $20.00 © 2001 American Chemical Society
Published on Web 01/18/2001

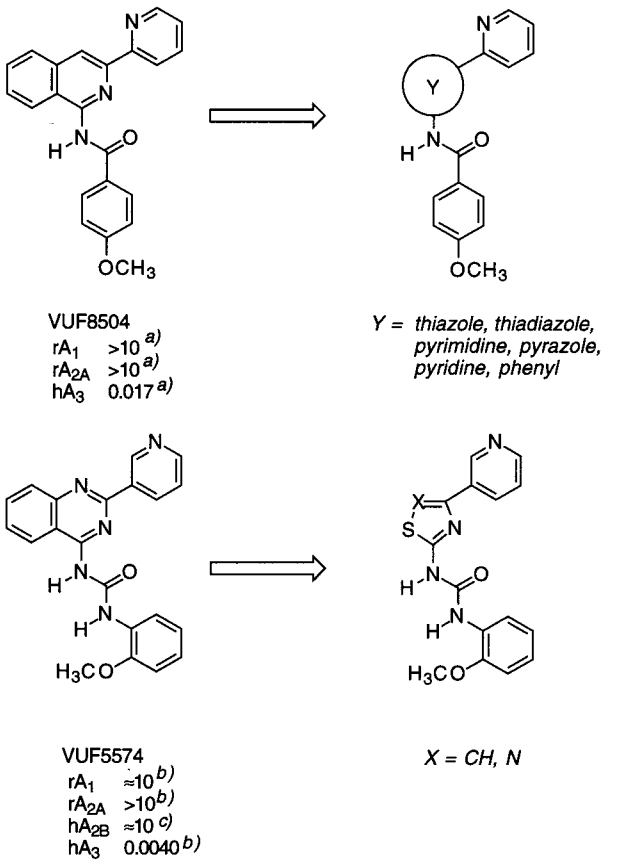


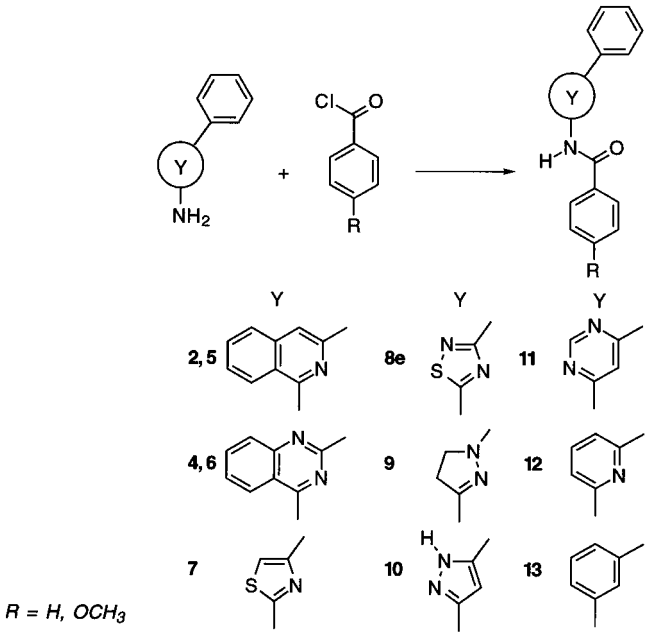
Figure 1. Template approach in the development of novel classes of adenosine analogues, i.e. replacement of the bicyclic ring system by a simple monocylic heterocycle. Affinities at adenosine receptors are expressed as K_i values in μM. [a]Data from ref 16. [b]Data from ref 17. [c]45% displacement of specific [^{3}H]DPCPX binding at human A_{2B} receptors, stably expressed in COS-7 cells ($n = 2$).

all been identified as adenosine A_3 receptor antagonists through the screening of chemical libraries.

Recently we reported on a series of isoquinoline and quinazoline analogues as adenosine A_3 receptor antagonists.[15−17] In these studies we showed that higher adenosine A_1 and A_3 receptor affinities resulted from spacer-coupled aromatic groups on the 1-position of the isoquinoline ring.[15] An amide[16] or urea[17] moiety as spacer between the heterocyclic ring and aromatic group on position 1 provided an increase in adenosine A_3 receptor affinity; in case the aromatic group is phenyl a substitution of this nucleus led to A_3-selective antagonists (Figure 1). We also found that an aromatic group at position 2 of the quinazoline ring increased adenosine A_3 receptor affinity.[17]

In the present study, we describe the development of a new class of heterocyclic compounds as adenosine receptor antagonists, based on a template approach (see also Figure 1). We replaced the bicyclic heterocyclic ring system by several monocyclic rings and investigated its influence on adenosine receptor affinity. Thiazole or thiadiazole derivatives appeared most promising; hence we focused our investigations on these two classes of compounds. We prepared several substituted *N*-thiadiazole- and *N*-thiazolebenzamide derivatives and investigated the influence of benzamide substituents on the adenosine receptor affinities.

Scheme 1



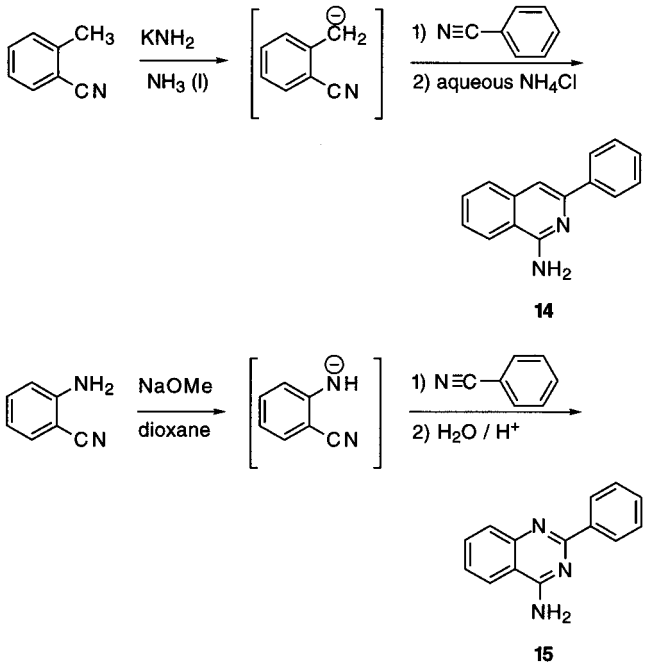
Chemistry

Compounds **2** and **5**−**13** were prepared from *p*-anisoyl chloride and phenyl-substituted heterocyclic amines, using different circumstances, depending on (1) the deactivating properties of the heterocyclic ring and (2) the reactivity of the resulting amide (Scheme 1). In case of strong deactivation of the amine, such as in the synthesis of isoquinoline derivative **5** or pyrimidine derivative **11**, high temperature and excess of acid chloride were needed, whereas the more reactive amines used in the synthesis of **6**, **7**, or **10** gave good results at low temperature. 2-Amino-4-phenyltriazine and 4-amino-2-phenylpyrimidine were not sufficiently reactive toward acid chlorides, even after deprotonation with a butyllithium reaction could not be achieved, probably due to delocalization of the negative charge. In case of **9** and **12** the benzamides were more reactive than the corresponding primary amines and the *N*,*N*-dibenzoyl analogues were easily obtained. Adjusting the reaction conditions of method C (Scheme 4) by using an excess of the amine and dilution of the reaction mixture led to the desired compound **12**. Hydrolysis of the dibenzoyl analogue with sodium methoxide in dioxane yielded the desired compound **9**. The amines **14** and **15** were prepared from the corresponding nitriles as described in previous studies[16,17] (Scheme 2).

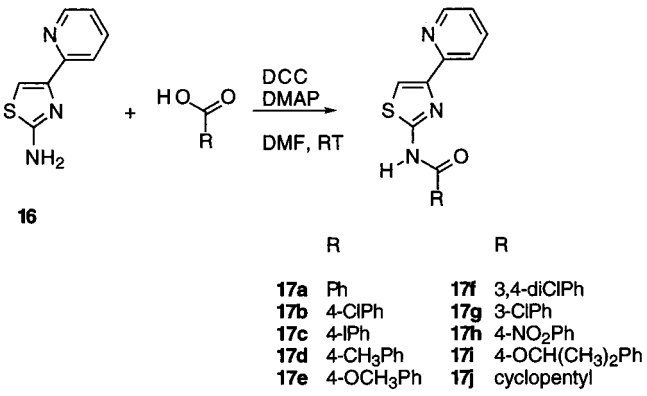
2-Amino-4-(2-pyridyl)thiazole **16** was prepared according to a literature procedure,[18] and the amides **17a**−**j** were synthesized by a reaction of amine **16** with the appropriate carboxylic acids in the presence of dicyclohexylcarbodiimide and DMAP (Scheme 3). Different methods were used for the synthesis of thiadiazole derivatives **8a**−**l** (Scheme 4). In methods A and B carboxylic acids were reacted with 5-amino-3-phenyl-1,2,4-thiadiazole, while in methods C and D the acid chlorides were used.

4-Hydroxybenzamide **8h** was prepared by cleavage of the methyl ether of **8e** under the influence of sodium cyanide (Scheme 5). The phenol **8h** in turn was alkylated by bromoethyl acetate and subsequently hydro-

Scheme 2



Scheme 3



	R		R
17a	Ph	**17f**	3,4-diClPh
17b	4-ClPh	**17g**	3-ClPh
17c	4-IPh	**17h**	4-NO_2Ph
17d	4-CH_3Ph	**17i**	4-$OCH(CH_3)_2$Ph
17e	4-OCH_3Ph	**17j**	cyclopentyl

lyzed, yielding the carboxylic acid **8i**. Methyl ethers **8k,l** were cleaved using alumina tribromide in the presence of ethanethiol,[19,20] yielding hydroxy derivatives **8m,n**. Other cleavage methods were explored, such as the use of BBr_3 at low temperature,[21] the application of this Lewis acid in the presence of 15-crown-5 and KI,[22] or the use of silylated compounds,[23,24] without success. Compound **18g** was prepared by oxidation of 4-methoxy-3-methylbenzaldehyde[25] to the corresponding carboxylic acid (**19**) which was easily converted into the acid chloride (Scheme 6).

Urea analogues **20**−**22** were prepared by coupling the aminothiazole or aminothiadiazole derivatives with substituted phenyl isocyanates in dry acetonitrile (Scheme 7).

Results and Discussion

Binding Studies. All synthesized compounds were tested in radioligand binding assays to determine their affinities for the adenosine A_1, A_{2A}, and A_3 receptors. The affinities at adenosine A_1 receptors were determined using membranes from rat brain cortex, with [^{3}H]DPCPX as radioligand.[26] The affinities at adenosine A_{2A} receptors were obtained on membranes from rat striatum with [^{3}H]CGS 21680[27] (Tables 1 and 2) or [^{3}H]ZM 241385 (Tables 3−5) as radioligands. 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 as radioligand. [28,29] The results are shown in Tables 1−5.

Template Approach. We have demonstrated earlier that A_3 receptor potency and selectivity could be achieved in isoquinoline and quinazoline derivatives through a combination of an aromatic group at the 2-position and an aromatic group coupled by a spacer at the 4-position of the quinazoline ring or at the appropriate 3- and 1-positions of an isoquinoline ring.[15−17] In the present study we applied a template approach in the development of novel classes of adenosine receptor ligands and replaced the bicyclic ring system by simpler monocyclic heterocycles (Figure 1).

For synthetic reasons we preferred the phenyl instead of the pyridinyl derivatives; earlier investigations had revealed that 2-(2-pyridinyl)-, 2-(4-pyridinyl)-, and 2-phenyl-substituted quinazoline derivatives showed comparable adenosine receptor affinities.[17] To check whether this also held in the benzamide series, we prepared the phenyl-substituted analogues **2** and **4** of VUF8507 (**1**)[16] and *N*-[2-(2-pyridinyl)quinazolin-4-yl]benzamide (**3**),[16] respectively, and tested their adenosine receptor binding (Table 1).

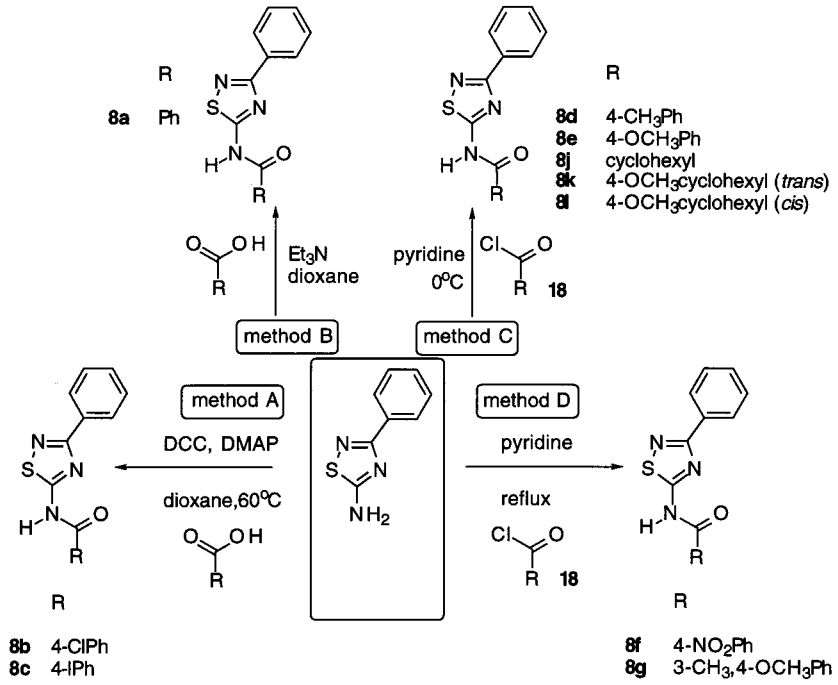
Isoquinoline as well as quinazoline analogues showed comparable adenosine A_1 and A_{2A} receptor binding for 2-pyridinyl and phenyl substituents. However, the affinity at the human adenosine A_3 receptor was the same for quinazolines **3** and **4**, but *N*-(3-phenylisoquinolin-1-yl)benzamide **2** showed a 6-fold decrease in adenosine A_3 receptor affinity compared to pyridinyl analogue **1**. In the molecular modeling section we will deal with this difference.

We altered the central heterocyclic ring to gain more insight into the structural requirements for binding adenosine receptors (compounds **5**−**13**). We chose several monocyclic ring systems and kept a phenyl ring and an *N*-4-methoxybenzamide (as in earlier studies) as substituents. For comparison, we prepared phenyl-substituted bicyclic heterocycles **5** and **6**. The various compounds and their adenosine receptor affinities are listed in Table 2.

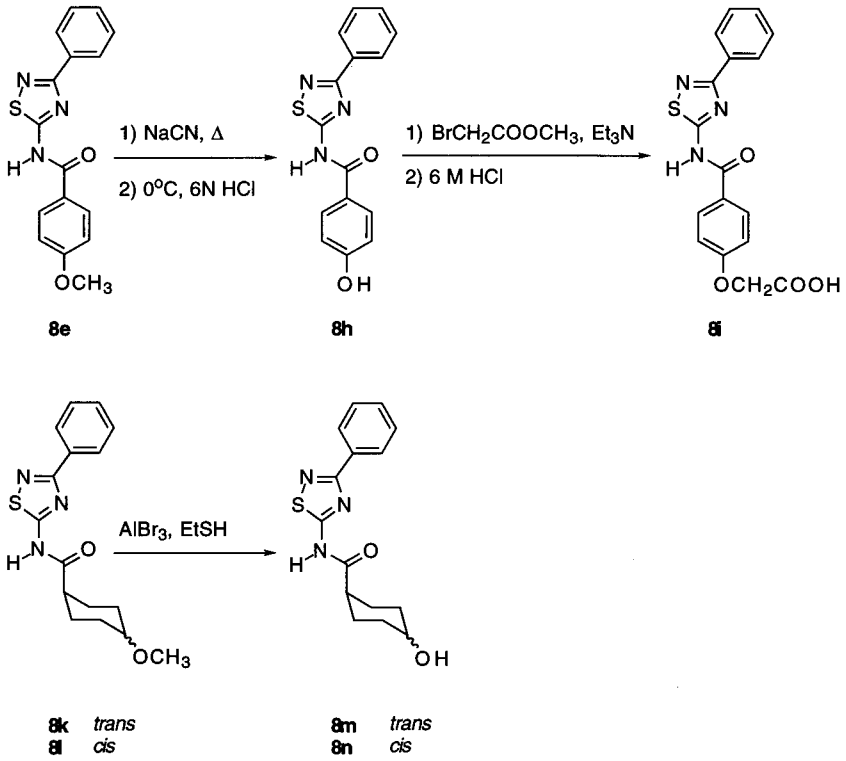
Isoquinoline **5** showed negligible affinity at the adenosine A_{2a} receptor but has a K_i value of 3 μM at the adenosine A_1 receptor and a K_i value of 79 nM at the adenosine A_3 receptor. The 4-fold lower adenosine A_3 receptor affinity of **5** compared to VUF8504[16] should be determined by the lack of a nitrogen atom in the 3-substituent. Quinazoline **6** is also an adenosine A_3 receptor-selective compound with a K_i value of 27 nM, i.e. 66-fold selectivity over A_1 and more than 350-fold over A_{2A}.

Replacement of the bicyclic heterocycle of **5** by a monocyclic thiazole moiety yielded LUF5433 (**7**), which showed remarkable adenosine receptor affinities. This thiazole derivative possessed reasonable affinities at all three receptor subtypes, with K_i values of 76 nM at the adenosine A_1 receptor, 2 μM at the adenosine A_{2A} receptor, and 0.7 μM at the adenosine A_3 receptor. With this compound as a lead we developed a new class of adenosine receptor antagonists (next section).

Scheme 4



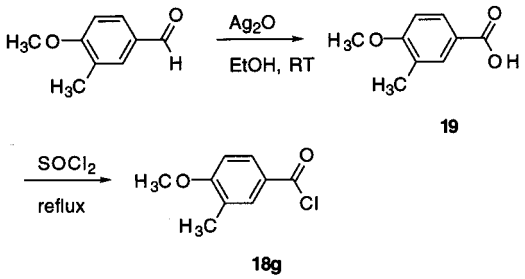
Scheme 5



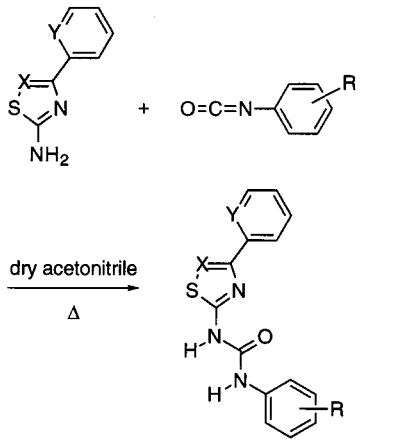
In analogy to compounds **5** and **7**, we replaced the quinazoline of **6** by a monocyclic thiadiazole moiety, resulting in LUF5417 (**8e**). Like thiazole **7**, thiadiazole **8e** showed affinities at adenosine receptors from the micromolar to the nanomolar range. The 8 times increased adenosine A_3 receptor affinity of **8e**, compared to thiazole **7**, is remarkable. This can be ascribed to the same effect as was seen in compounds **5** and **6**. Again, we will return to this in the molecular modeling section.

We continued our investigations on monocyclic heterocycles with two other five-membered rings and three six-membered ring systems. The choice of ring systems depended on synthetic accessibility. Dihydropyrazole **9** and pyrazole analogue **10** showed negligible affinity at all three adenosine subtypes. Different six-membered rings were used in compounds **11**−**13**. Compound **11** contains a pyrimidine ring, **12** a pyridyl ring, and **13** a phenyl ring. The pyrimidine analogue, although lacking a nitrogen between the two substituents, possessed substantial affinities at the adenosine A_1 and A_3 receptors with K_i values of 0.2 and 0.8 μM, respectively. The pyridine analogue **12** was slightly A_3-selective and

Scheme 6



Scheme 7



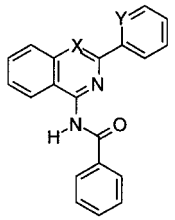
	X	Y	R
20a	N	CH	H
20b	N	CH	4-OCH_3Ph
20c	N	CH	2-OCH_3Ph
21a	CH	N	H
21b	CH	N	4-OCH_3Ph
22	CH	CH	H

showed a K_i value of 8.7 μM. This analogue can hardly be compared with the known class of 3,5-diacyl-2,4-dialkylpyridine analogues as adenosine A_3 receptor antagonists,[8] because of the large difference in substitution pattern of the compounds. Analogue **13**, lacking heteroatoms in the central ring, had a comparably low A_3 receptor affinity as **12**, with a K_i value of 10 μM. Comparison of **5** and **12** showed that the additional ring of **5** largely increased adenosine A_3 receptor affinity. It might be that substituents at positions 3 and 4 of the pyridine ring can fulfill the same role in binding.

Thiazolylbenzamide Analogues. Based on **1** and **7**, several substituted *N*-[4-(2-pyridyl)thiazol-2-yl]-benzamides were synthesized to explore the structure−activity relationships (SARs) at the benzamide ring. In Table 3 the affinities of compounds **17a**−**j** at the adenosine receptors are summarized.

The unsubstituted *N*-[4-(2-pyridyl)thiazol-2-yl]benzamide **17a** showed affinities in the micromolar range at the three adenosine receptors (A_1 > A_3 > A_{2A}). Introduction of a halogen atom at the *para*-position (**17b**,**c**) decreased the A_{2A} affinity and did not influence the adenosine A_3 receptor affinity. However, the 4-chloro-substituted benzamide **17b** possessed an 8-fold increased affinity at the adenosine A_1 receptor compared to **17a**, with a K_i value of 200 nM. Introduction of a methyl group (**17d**) does not influence adenosine receptor affinities, whereas the stronger electron-donating methoxy analogue (**17e**) increased adenosine A_3 receptor affinity and decreased adenosine A_1 receptor affinity. The methoxy group has a prominent role in different classes of adenosine A_3 receptor antagonists,[3,4,14−17] indicating that it could play an important role in the molecular recognition of the adenosine A_3 receptor.

Table 1. Affinities of Isoquinoline and Quinazoline Analogues in Radioligand Binding Assays at Adenosine Receptors



Compound	X	Y	rA_1 [a]	rA_{2A} [b]	hA_3 [c]
1 (VUF8507)	CH	N	3.2 ± 0.3	0 %	0.20 ± 0.04 [a]
2	CH	CH	1.2 ± 0.2	0 %	1.2 ± 0.3
3	N	N	16 %	9 %	0.23 ± 0.02 [d]
4	N	CH	34 %	17 %	0.24 ± 0.06

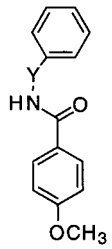
[a] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as percentage displacement of specific binding at a concentration of 10 μM (n = 2−3). [c] 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 μM (n = 3). [d] As published in ref 16.

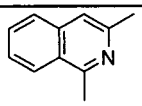
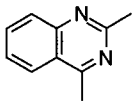
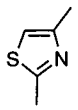
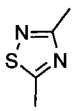
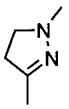
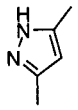
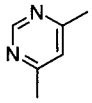
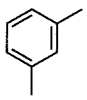
The 3,4-dichlorobenzamide analogue **17f**, 3-chlorobenzamide **17g**, and 4-nitrobenzamide **17h** showed affinities comparable to that of the unsubstituted benzamide **17a** toward adenosine A_1 and A_3 receptors. *m*-Chloro-substituted compound **17g** showed also A_{2A} affinity with a K_i value of 1.3 μM. Increasing the size of the ether group of **17e** resulting in isopropyl ether **17i** diminished both the adenosine A_1 and A_3 receptor affinities by 2-fold.

Furthermore we used a cyclopentyl group instead of the phenyl ring. This moiety showed A_1 selectivity in other classes of adenosine receptor ligands (DPCPX, N-0840, CPA, and CCPA).[29−33] Compound **17j** was also an adenosine A_1 receptor-selective ligand but showed also increased affinities at the adenosine A_{2A} and A_3 receptors compared to benzamide **17a**. This is surprising, since *N*-[3-(2-pyridyl)isoquinolin-1-yl]alkyl amides were hardly active at adenosine receptors with K_i values in the range of 10−50 μM,[16] whereas a benzamide instead of an alkylamide as in VUF8507 led to 55- and 10-fold increased adenosine A_3 and A_1 affinity, respectively. Thus the SAR of this class of *N*-[4-(2-pyridyl)-thiazol-2-yl]amides is not the same as the SAR of the *N*-[3-(2-pyridyl)isoquinolin-1-yl]amides. This observation has a precedent in the SAR of pyran derivatives and dihydropyridines not being parallel.[10] On the other hand, the *p*-methoxy-substituted compound **17e** was as expected the most active compound at the adenosine A_3 receptor within the series of benzamides.

Thiadiazolylbenzamide Analogues. We continued our investigations on the monocyclic thiadiazole adenosine ligands. In addition to the 4-methoxybenzamide

Table 2. Affinities of 4-Methoxybenzamide Analogues in Radioligand Binding Assays at Adenosine Receptors



Compound	Y	rA_1 [a]	rA_{2A} [b]	hA_3 [c]
5		3.0 ± 0.7	35 %	0.079 ± 0.02
6		1.8 ± 0.8	44 %	0.027 ± 0.02
7 (LUF5433)		0.076 ± 0.008	1.9 ± 0.5	0.67 ± 0.10
8e (LUF5417)		0.032 ± 0.005	2.34 ± 1.1	0.082 ± 0.004
9		26 %	19 %	41 %
10		20 %	16 %	35 %
11		0.20 ± 0.1	31 %	0.80 ± 0.05
12		46 %	16 %	8.7 ± 0.3
13		30 %	41 %	10 ± 1.6

[a] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] 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) or K_i ± SEM in μM (n = 3). [c] 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 μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2).

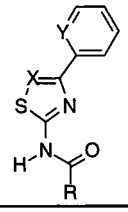
8e we prepared other benzamides **8a**−**i** to explore the structure−affinity relationships (Table 3).

Unsubstituted benzamide **8a** showed adenosine A_1 and A_{2A} receptor affinities comparable with **8e** but a 20-fold decreased adenosine A_3 receptor affinity. This is compatible with our earlier observation on the importance of the methoxyphenyl moiety.

At the adenosine A_1 receptor small substituents on the *para*-position were allowed, as in compounds **8a,b,d,e**. Introduction of more or less lipophilic substituents or changing their electronic nature did not influence the relatively high adenosine A_1 receptor affinity (K_i values 30−40 nM). However, larger substituents such as iodo in **8c** and nitro in **8f** decreased the adenosine A_1 receptor affinity tremendously.

Compounds **8a**−**f** showed little affinity for the adenosine A_{2A} receptor with the exception of the 4-methoxy-substituted thiadiazolobenzamide **8e** (K_i = 2 μM).

Table 3. Affinities of Thiazole and Thiadiazole Derivatives in Radioligand Binding Assays at Adenosine Receptors



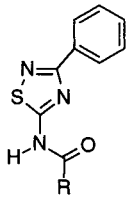
Compound	X	Y	R	rA_1 [a]	rA_{2A} [b]	hA_3 [c]
17a	CH	N	C_6H_5	1.7 ± 0.5	8.7 ± 0.6	3.4 ± 1
17b	CH	N	4-ClC_6H_4	0.20 ± 0.05	30 %	3.2 ± 2
17c	CH	N	4-IC_6H_4	2.4 ± 1	50 %	2.8 ± 1
17d	CH	N	4-$CH_3C_6H_4$	1.6 ± 0.4	44 %	3.2 ± 0.3
17e	CH	N	4-$OCH_3C_6H_4$	3.2 ± 0.7	40 %	1.8 ± 0.6
17f	CH	N	3,4-diClC_6H_4	1.6 ± 0.6	20 %	2.5 ± 1
17g	CH	N	3-ClC_6H_4	1.7 ± 0.7	1.3 ± 0.7	4.6 ± 1
17h	CH	N	4-$NO_2C_6H_4$	1.5 ± 0.4	29 %	3.5 ± 0.9
17i	CH	N	4-$OCH(CH_3)_2C_6H_4$	5.8 ± 1	44 %	3.7 ± 0.7
17j	CH	N	cyclopentyl	0.92 ± 0.08	1.3 ± 0.7	2.1 ± 0.8
8a	N	CH	C_6H_4	0.031 ± 0.007	4.4 ± 1.2	0.41 ± 0.1
8b	N	CH	4-ClC_6H_4	0.041 ± 0.02	36 %	0.52 ± 0.3
8c	N	CH	4-IC_6H_4	49 %	19 %	1.1 ± 0.9
8d	N	CH	4-$CH_3C_6H_4$	0.030 ± 0.002	38.2 %	0.14 ± 0.03
8e (LUF5417)	N	CH	4-$OCH_3C_6H_4$	0.032 ± 0.005	2.3 ± 1.1	0.082 ± 0.004
8f	N	CH	4-$NO_2C_6H_4$	34 %	5 %	28 %
8g	N	CH	3-CH_3-4-$OCH_3C_6H_4$	48%	8 %	0.58 ± 0.2
8h (LUF5437)	N	CH	4-OHC_6H_4	0.0073 ± 0.001	0.57 ± 0.07	0.13 ± 0.01
8i	N	CH	4-$OCH_2CO_2HC_6H_4$	0.10 ± 0.01	15 ± 3.0	1.2 ± 0.81
VUF8504[d]				37 %	19 %	0.017 ± 0.002
XAC				0.0012	0.063	0.11 ± 0.003[e]
L-249313						0.17 ± 0.009[e]
CGS 15943				0.021 ± 0.003[f]	0.0033 ± 0.002[f]	0.14 ± 0.02[e]

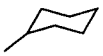
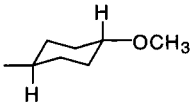
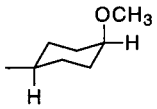
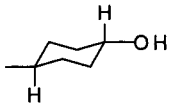
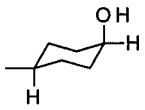
[a] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] Displacement of specific [^{3}H]ZM241385 binding in rat striatal membranes, expressed as percentage 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 [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [d] As published in ref 15. [e] As published in ref 16. [f] As published in ref 41.

At the adenosine A_3 receptor a quite different SAR was established. In addition to the steric restriction (an iodo atom or nitro moiety at the *para*-position appeared to be too large) electronic effects play an important role in the molecular recognition. The A_3 receptor potency order of 4-substituents is Cl < H < Me < OMe. The strong electron-donating methoxy substituent afforded a high K_i value of 82 nM. This SAR is similar as seen in adenosine A_3 receptor antagonizing *N*-isoquinolinebenzamides[16] and N^1-isoquinolinebenzamidines[15] and also to the SAR present in the adenosine A_3 receptor agonist class of N^6-(phenylcarbamoyl)adenosine-5′-*N*-ethyluronamides.[34]

N-(3-Phenyl-1,2,4-thiadiazol-5-yl)-3-methyl-4-methoxybenzamide (**8g**) was proof that the introduction of an extra 3-methyl next to the 4-methoxybenzamide **8e**

Table 4. Affinities of *N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)cyclohexanamide Derivatives in Radioligand Binding Assays at Adenosine Receptors



Compound	R	rA_1 [a]	rA_{2A} [b]	hA_3 [c]
8j		1.4 ± 0.5	16 %	16 ± 8
8k *(trans)*		0.032 ± 0.002	1.9 ± 0.8	1.7 ± 0.6
8l *(cis)*		0.11 ± 0.02	2.0 ± 0.5	5.3 ± 2
8m *(trans)* (VUF5472)		0.020 ± 0.007	37 %	1.9 ± 0.4
8n *(cis)*		0.042 ± 0.006	41 %	2.7 ± 0.6

[a] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] Displacement of specific [^{3}H]ZM241385 binding in rat striatal 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 [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2).

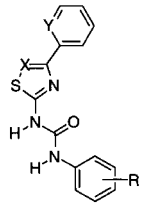
is adverse for binding to all three receptors. We also prepared compound **8i**, bearing the same oxyacetate substituent as in I-ABOPX, the only xanthine derivative with appreciable affintity for human adenosine A_3 receptors.[35] However, at all three receptors **8i** had decreased receptor affinities compared to the unsubstituted thiadiazole **8a**.

We also tested the 4-hydroxyl-substituted benzamide **8h**. This compound showed an increase in affinities at all three receptor subtypes when compared to **8a** and appeared to be a highly potent and selective adenosine A_1 receptor antagonist. *N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-hydroxybenzamide (LUF5437, **8h**) had a K_i value of 7.3 nM at the A_1 receptor and possessed 18- and 78-fold selectivity over the A_3 and A_{2A} receptors, respectively. A specific role for the *p*-hydroxyl group in binding to the A_1 receptor seems therefore likely, whereas the *p*-methoxy group plays an important role in binding to the A_3 receptor. We therefore further investigated the influence of these substituents on binding adenosine receptors by preparing *N*-(3-phenyl-1,2,4-thiadiazol-5-yl)cyclohexanamide **8j** and its 4-substituted derivatives **8k**−**n**. In the thiazolo series the cyclopentanamide analogue **17j** showed relatively high adenosine receptor affinities (better than benzamide **17a**), and therefore we chose also a cycloalkyl group to direct the hydroxy or methoxy substituent, as *cis*- and *trans*-isomers, respectively. The results are summarized in Table 4.

The cyclohexyl group of **8j** proved less favorable than the phenyl group of **8a** in binding adenosine receptors, which disagrees with the SAR of **17a**,**j**. This suggests that either a cyclopentyl group fits the adenosine receptor pocket better or the thiadiazole ligands behave differently, compared to the thiazole analogues. Introduction of a 4-*trans*-methoxy substituent increased adenosine A_3 receptor affinity. It also increased the adenosine A_1 and A_{2A} receptor affinities as in the thiadiazolobenzamides (**8e** vs **8a**), unlike the methoxy group in the benzamide analogues (**17e** vs **17a**). Introduction of a 4-*cis*-methoxy substituent (**8l**) led to similar changes, but to a lesser extent than observed for the *trans*-analogue. Apparently, adenosine A_1 and A_3 receptors slightly prefer the 4-*trans*-methoxycyclohexanamide over the *cis*-analogue.

The 4-*trans*-hydroxy analogue **8m** had an adenosine A_3 receptor affinity comparable to that of the 4-*trans*-methoxy analogue **8k**, a decreased A_{2A} receptor affinity, and a slightly increased A_1 receptor affinity. The *cis*-analogue **8n** showed lower affinity again at all three adenosine receptors. From Table 4 we concluded that all three receptor subtypes prefer 4-*trans*-substituted cyclohexanamides and, second, that a hydroxyl group is (slightly) favored over a methoxy group for binding the adenosine A_1 receptor. Furthermore, these investigations provided another potent adenosine A_1 receptor antagonist (VUF5472, **8m**). This 4-*trans*-hydroxycyclo-

Table 5. Affinities of Thiazole and Thiadiazole Urea Derivatives in Radioligand Binding Assays at Adenosine Receptors



Compound	X	Y	R	rA_1 [a]	rA_{24} [b]	hA_3 [c]
20a	N	CH	H	1.0 ± 0.9	48 %	4.0 ± 1
20b	N	CH	4-OCH_3	44 %	16 %	3.3 ± 2
20c	N	CH	2-OCH_3	27 %	38 %	20 %
21a	CH	N	H	0.93 ± 0.2	0.92 ± 0.06	5.0 ± 0.7
21b	CH	N	4-OCH_3	4 %	11 %	147 ± 159
22	CH	CH	H	37 %	19 %	9.9 ± 2

[a] Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2). [b] Displacement of specific [^{3}H]ZM241385 binding in rat striatal 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 [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as K_i ± SEM in μM (n = 3) or percentage displacement of specific binding at a concentration of 10 μM (n = 2).

hexanamide analogue showed a K_i value of around 20 nM and a 100-fold selectivity versus the adenosine A_3 receptor and even more than 500-fold versuss the A_{2A} receptor.

Thiazolyl- and Thiadiazolylurea Analogues. Earlier studies in a series of isoquinoline and quinazoline adenosine A_3 receptor antagonists have proven that a urea moiety as spacer increased adenosine A_3 receptor affinity compared to an amide moiety.[17] We therefore applied the template approach again and replaced the quinazoline ring of VUF5574 by a thiazole or thiadiazole moiety and investigated a urea series of compounds (Figure 1).

First, we prepared *N*-phenyl-*N*′-(3-phenyl-1,2,4-thiadiazol-5-yl)urea (**20a**) and determined its affinities at the three adenosine receptors (Table 5). Unsubstituted urea analogue **20a** had a K_i value of 1 and 4 μM at the adenosine A_1 and A_3 receptor, respectively, and possessed only 48% displacement at a concentration of 10 μM at the adenosine A_{2A} receptor. Second, we prepared the *o*- and *p*-methoxy-substituted phenylurea analogues according to the positive contributions of these substituents toward adenosine A_3 receptor affinity in the *N*-phenyl-*N*′-(2-phenylquinazolin-4-yl)urea[17] and various benzamide series, respectively. The SAR of this class of thiadiazole ureas differed from the SAR of the quinazoline urea analogues to a large extent. The 4-methoxyphenyl analogue **20b** showed a decrease in the adenosine A_1 and A_{2A} receptor affinities and did not affect the adenosine A_3 receptor affinity, compared to unsubstituted urea **20a**. Introduction of a 2-methoxy substituent as in **20c** decreased the binding at all three receptor subtypes substantially.

We also prepared *N*-phenyl-*N*′-[4-(2-pyridyl)thiazol-2-yl]urea analogues **21a,b**. Unsubstituted analogue **21a** had adenosine receptor affinities comparable to that of the amide analogue **17a** and the thiadiazole urea analogue **20a**. Introduction of a 4-methoxy substituent diminished adenosine receptor affinities. Finally, we studied the influence of the substituent on the 4-position of the thiazole ring and prepared compound **22**. A comparison of analogues **22** and **21a** revealed that a 2-pyridyl moiety at position 4 slightly improved binding to the adenosine A_3 receptor compared to a phenyl ring and is apparently essential for the adenosine A_1 receptor. Furthermore, compounds **22** and **20a** differ in the heterocyclic ring system only, and a comparison of the thiazole and thiadiazole analogues revealed that the lack of the nitrogen atom in **22** is detrimental toward the adenosine A_1 receptor, while also decreasing the adenosine A_3 receptor affinity.

Molecular Modeling. The binding data in Tables 1 and 5 show that for binding the adenosine A_1 and A_3 receptor ligands needed an additional nitrogen atom either in the heterocyclic central ring (as in thiadiazole rather than thiazole) or in the substituent at the 4-position of the thiazole ring (2-pyridyl instead of phenyl).

In earlier studies[17] it had been shown that in low-energy conformations of 3-(2-pyridyl)isoquinoline analogues, the nitrogen of the pyridyl group pointed "upward", i.e. the pyridyl group was turned away from the nitrogen of the isoquinoline ring. Therefore we built the structures of compounds **20a** and **21a** in Spartan,[36] and after conformational optimization we calculated their molecular electrostatic potentials (MEPs) semiempirically and ab initio. Indeed, also in this case, the low-energy conformation of **21a** has a pyridyl group in which the nitrogen atom is pointed "upward" (Figure 2). Both compounds showed comparable MEPs in the ab initio calculations as well as in the semiempiric study, indicating that the high electron density present in the plane of the thia(dia)zole or 2-pyridyl ring may function as proton acceptor for amino acid residues of the adenosine receptor (arrows in Figure 2). This could then also be the reason for the loss of adenosine receptor affinities of **22** compared to **20a** and **21a** and of **2** compared to **1** and **4**.

We have shown that our template approach yields thiazolo and thiadiazolo analogues as novel classes of adenosine receptor antagonists, and subsequently several potent *c.q.*-selective ligands were developed. These compounds contribute to a better understanding of the structural requirements necessary for the molecular recognition by adenosine A_1 and A_3 receptors.

Experimental Section

Abbreviations: APT, attached proton test; CGS 15943, 9-fluoro-2-(2-furyl-5,6-dihydro[1,2,4]triazolo[1,5-*c*]quinazin-5-imine; CHO cells, Chinese hamster ovary cells; CI, chemical ionization; COSY, correlated spectroscopy; DCC, dicyclohexylcarbodiimide; DEPT, distortionless enhancement by polarization transfer; DMAP, *N*,*N*-(dimethylamino)pyridine; DMSO, dimethyl sulfoxide; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; EA, ethyl acetate; [^{3}H]CGS 21680, [^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-(ethylcarbamoyl)adenosine; [^{3}H]-DPCPX, [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine; [^{3}H]ZM 241385; HEK cells, human embryonic kidney cells; [^{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]naphthpyridine; L-268605, 3-(4-meth-

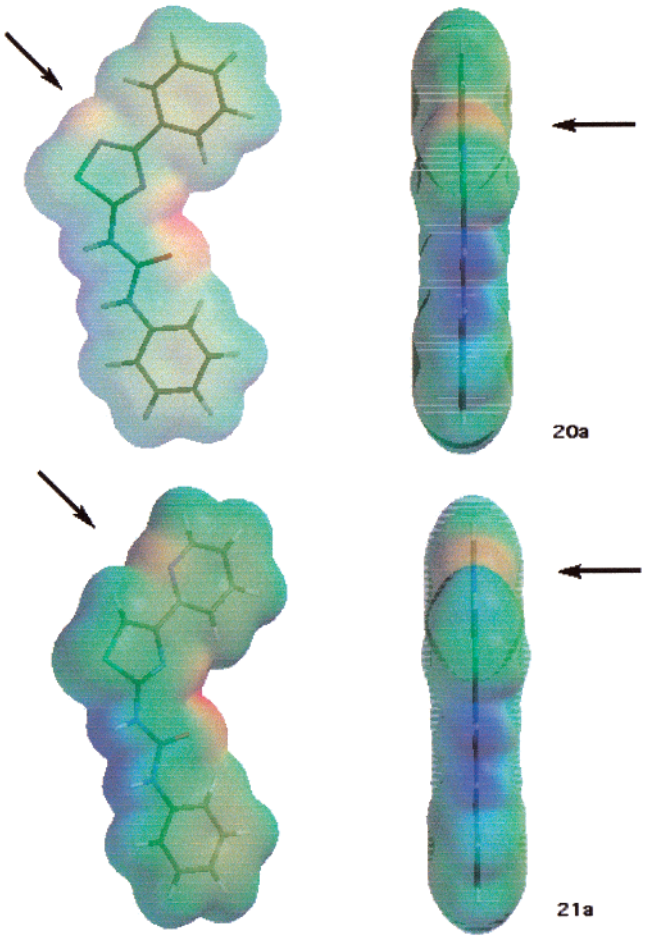


Figure 2. Molecular electrostatic potential energy isosurfaces of low-energy conformations of compounds **20a** and **21a**. Color coding is in the range from −60 (deepest red) to 60 kcal/mol (deepest blue).

oxyphenyl)-5-amino-7-oxothiazolo[3,2]pyrimidine; MEP, molecular electrostatic potential; MRS1191, 3-ethyl-5-benzyl-2-methyl-6-phenyl-4-(phenylethynyl)-1,4-(±)dihydropyridine-3,5-dicarboxylate; MRS1220, 9-chloro-2-(2-furyl)-5-phenylacetylamino[1,2,4]triazolo[1,5-*c*]quinazoline; N-0840, N^6-cyclopentyl-9-methyladenine; VUF8504, 4-methoxy-*N*-[3-(2-pyridinyl)isoquinolin-1-yl]benzamide; VUF5574, *N*-(2-methoxyphenyl)-*N*′-[2-(3-pyridyl)quinazolin-4-yl]urea; XAC, 8-[4-(((((2-aminoethyl)amino)carbonyl)methoxy)oxy)phenyl]-1,3-dipropylxanthine.

Materials. DMF was distilled and stored under linde type 4 Å molecular sieves. Dioxane, triethylamine, pyridine, and DMSO were distilled over CaH_2 and stored under linde type 4 Å molecular sieves as well. All other solvents used were analytical grade. 5-Amino-3-phenyl-1,2,4-thiadiazole, cyclohexanoic acid, *N*,*N*-dimethylaminopyridine (DMAP), 3-and 4-chlorobenzoic acid, *p*-anisoyl chloride, *p*-anisic acid, *p*-toluic acid, 3,4-dichlorobenzoic acid, 4-methoxyphenyl isocyanate and 4-nitrobenzoic acid were purchased from ACROS. 3-Amino-5-phenylpyrazole, 3-methyl-4-methoxybenzaldehyde, and 4-methoxycyclohexanoic acid were commercially available from Aldrich. Alumina tribromide, 4-iodobenzoic acid, 4-isopropoxybenzoic acid, and 2-amino-4-(4-chlorophenyl)thiazole were purchased from Merck, *n*-butyllithium and phenyl isocyanate from Fluka, and 3-amino-4,5-dihydro-1-phenylpyrazole, 2-amino-4-phenylthiazole and 1-(2-aminophenyl)pyrrole from Lancaster. Thionyl chloride was purchased from Riedel de Haen and 4-amino-6-phenylpyrimidine from SPECS. Compounds **2** and **4** were prepared as described previously[16] and 2-amino-4-(2-pyridyl)thiazole (**16**) was prepared by the method as described by Taurins and Blaga.[18]

Synthesis. Reaction scales were used as mentioned in the general methods, unless otherwise specified. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode, with tetramethylsilane as an internal standard or on a Bruker AC 200 (^{1}H NMR: 200 MHz; ^{13}C NMR: 50.29 MHz). 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. Mass spectra were measured on a Finnigan MAT TSQ-70 mass spectrometer, equipped with an electrospray interface (EI). Spectra were collected by constant infusion of the analyte dissolved in 80/20 methanol water (sometimes with 1% acetic acid). Experiments were done in positive ionization mode. Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected or measured on a Electrothermal IA9200 apparatus. Reactions were routinely monitored by thinlayer 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. Elemental analyses were performed by the analytical department of Organic and Molecular Inorganic Chemistry at the University of Groningen (The Netherlands) or at the Leiden University Gorlaeus Laboratories (The Netherlands) and are within ±0.4% of theoretical values unless otherwise specified.

Method A: General Procedure for the Synthesis of 8b,c. 0.5 g (2.8 mmol) of 5-amino-3-phenyl-1,2,4-thiadiazole, 0.52 g of DMAP (4.2 mmol), 4.2 mmol of the apparent benzoic acid and 0.87 g (4.2 mmol) of DCC were dissolved in 35 mL of dry dioxane. After stirring at 60 °C for 16 h, the yellow mixture was filtered and concentrated in vacuo. Recrystallization from a mixture of methanol and ethanol yielded white crystals.

Method B: 8a. 0.5 g (2.8 mmol) of 5-amino-3-phenyl-1,2,4-thiadiazole was dissolved in 20 mL of dry dioxane, containing 0.39 mL (2.8 mmol) of triethylamine. A solution of 0.5 mL (4.3 mmol) in 5.0 mL of dry dioxane was added and the solution stirred at 80 °C for 16 h. After filtration, the solution was concentrated in vacuo and recrystallized from methanol.

Method C: General Procedure for the Synthesis of 6, 8d,e,j−l, 9, and 10. 1.0 g (5.6 mmol) of 5-amino-3-phenyl-1,2,4-thiadiazole was dissolved in 2.5 mL of dry pyridine in a flame-dried 10-mL three-neck flask. This mixture was cooled to 0 °C and 8.2 mmol of acid chloride was added dropwise. The clear solution solidified. After 0.5 h, the solid was dissolved in a mixture of 50 mL of ethyl acetate and 50 mL of water. The H_2O layer was discarded and the ethyl acetate layer washed subsequently with 50 mL of 1 M HCl, 50 mL of 5% $NaHCO_3$ solution and 50 mL of brine. The organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. The remaining oil was stirred with 50 mL of *n*-pentane and a white solid resulted. Recrystallization from methanol resulted in white crystals.

Method D: General Procedure for the Synthesis of 5, 8f,g, and 11−13. 0.50 g (2.8 mmol) of 5-amino-3-phenyl-1,2,4-thiadiazole was dissolved in 1.0 mL of dry pyridine in a flame-dried 25-mL three-neck flask. This mixture was cooled to 0 °C and 3.2 mmol of acid chloride in 14 mL of dry pyridine was added dropwise. After stirring at room temperature for 1 h the mixture was refluxed for 5 h. After cooling to room temperature, a precipitate was formed which was filtrated and washed 3 times with methanol. Addition of methanol to the filtrate yielded a second batch of solidified product.

***N*-(3-Phenylisoquinolin-1-yl)benzamide (2):** yield 28% yellow-white crystals; mp 162−164 °C; R_f (MeOH) = 0.8; ^{1}H NMR ($CDCl_3$) δ 7.27−8.12 (m, 12H), 8.48 (d, 2H, $^3J_{BA}$ = 7.8 Hz, 2H, benzamide-2H), 8.98 (d, $^3J_{87}$ = 8.0.3 Hz, 1H, H8), and 14.76 (s, 1H, NH). Anal. ($C_{22}H_{16}N_2O$) C, H, N.

***N*-(2-Phenylquinazolin-4-yl)benzamide (4):** yield 32% white crystals; mp 166 °C (lit.[37] mp 168−169 °C); ^{1}H NMR (DMSO-d_6) δ 7.31−7.84 (m, 9H, H5 + H6 + H7 + H8 +

benzamide 2H + benzamide 3H + benzamide 4H), 7.93−8.22 (m, 5H, phenyl-2H + phenyl-3H + phenyl-4H), and 11.05 (s, 1H, NH). Anal. ($C_{21}H_{15}N_3O$) C, H, N.

***N*-(3-Phenylisoquinolin-1-yl)-4-methoxybenzamide (5):** method D; yield 47% white crystals; mp 144−146 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH_3), 6.98 (d, 2H, $^3J_{AB}$ = 8.8 Hz, 2H, benzamide-3H), 7.24−7.88 (m, 9H, H5 + H6 + H7 +phenyl-2H + phenyl-3H + phenyl-4H + NH), 8.42−8.52 (m, 3H, H8 + benzamide-2H), and 8.80−8.98 (m, 1H, H4). Anal. ($C_{23}H_{18}N_2O_2 \cdot 0.2H_2O$) C, H, N.

***N*-(2-Phenylquinazolin-4-yl)-4-methoxybenzamide (6):** method C used for 2.20 mmol of 4-amino-2-phenylquinazoline; yield 38% white powder; mp 165−168 °C; R_f (EA) = 0.6; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H, OCH_3), 7.12 (d, 2H, $^3J_{AB}$ = 8.8 Hz, 2H, benzamide-3H), 7.54−7.70 (m, 4H, H5 + H6 + H7 + H8), 7.95−8.18 (m, 5H, phenyl-2H + phenyl-3H + phenyl-4H), 8.45 (d, 2H, $^3J_{BA}$ = 8.6 Hz, 2H, benzamide-2H), and 11.21 (s, 1H, NH). Anal. ($C_{22}H_{17}N_3O_2$) C, H, N.

***N*-(4-Phenylthiazol-2-yl)-4-methoxybenzamide (7, LUF5433).** 47.0 mg (0.27 mmol) of 2-amino-4-phenylthiazole was dissolved in 5.0 mL of dry DMF and added to a solution of 155.0 mg (1.0 mmol) of *p*-anisic acid in 0.5 mL of dry DMF in a 2.2-mL Eppendorf cup. A solution of 210.0 mg (1.0 mmol) of DCC and 15.0 mg (0.12 mmol) of DMAP in 1.0 mL of DMF was added. After stirring at room for 4 days, the precipitate was removed and the solution concentrated under reduced pressure. Recrystallization of the residue from PE (40/60) and methanol resulted in 21% white crystals: mp 172 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, OCH_3), 7.08 (d, 2H, $^3J_{AB}$ = 8.9 Hz, benzamide-3H), 7.33 (d, 1H, $^3J_{43}$ = 7.2 Hz, phenyl-4H), 7.44 (dd, 1H, $^3J_{34}$ = 7.2 Hz, $^3J_{32}$ = 7.6 Hz, phenyl-3H), 7.7 (s, 1H, thiazolyl-5H), 7.9 (d, 2H, $^3J_{23}$ = 7.9 Hz, phenyl-2H), 8.13 (d, 2H, $^3J_{BA}$ = 8.9 Hz, benzamide-2H), and 12.6 (s, 1H, NH); MS (EI) *m*/*z* 311 (MH^+, 100%). Anal. ($C_{17}H_{14}N_2O_2S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)benzamide (8a):** method B; crystallization from CCl_4/MeOH yielded 35% white crystals; mp 145 °C; R_f value TLC (2.5% EA in CH_2Cl_2) 0.56; ^{1}H NMR (DMSO-d_6) δ 7.50−7.73 (m, 6H, phenyl-3H + phenyl-4H + benzamide-3H + benzamide-4H), 8.16−8.32 (m, 4H, phenyl-2H + benzamide-2H); MS (EI) *m*/*z* 282 (MH^+, 100%). Anal. ($C_{15}H_{11}N_3OS \cdot 0.03CCl_4$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-chlorobenzamide (8b):** method A; yield white crystals 38%; mp 258 °C; R_f value TLC (2.5% EA in CH_2Cl_2) 0.72; ^{1}H NMR (DMSO-d_6) δ 7.48−7.56 (m, 3H, phenyl-3H + phenyl-4H), 7.66 (ddd, 2H, *J* = 8.6; 2.8; 1.7 Hz, benzamide-3H), 8.15−8.22 (m, 4H, phenyl-2H + benzamide-2H); MS (EI) *m*/*z* 316 ($M(^{35}Cl)H^+$, 100%), 318 ($M(^{37}Cl)H^+$, 20%). Anal. ($C_{15}H_{10}ClN_3O_2S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-iodobenzamide (8c):** method A; yield white crystals 34%; mp 269 °C; R_f value TLC (2.5% EA in CH_2Cl_2) 0.63; ^{1}H NMR (DMSO-d_6) δ 7.51−7.54 (m, 3H, phenyl-3H + phenyl-4H), 7.90−8.02 (m, 4H, phenyl-2H + benzamide-2H), 8.18−8.22 (m, 2H, benzamide-3H); MS (EI) *m*/*z* 408 (MH^+, 100%). Anal. ($C_{15}H_{10}IN_3OS$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-methylbenzamide (8d):** method C; yield needlelike white crystals 17%; mp 177 °C; R_f value TLC (EA/tol) 0.69; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 7.22 (ddd, 2H, *J* = 8.25; 2.04;2.06 Hz, benzamide-3H), 7.24−7.40 (m, 3H, phenyl-3H + phenyl-4H), 7.71 (ddd, 2H, *J* = 8.25; 2.06; 2.05 Hz, benzamide-2H), 8.11−8.16 (m, 2H, phenyl-2H), 10.58 (bs,1H, NH). Anal. ($C_{12}H_{13}N_3OS$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-methoxybenzamide (LUF5417, 8e):** method C; yield needlelike white crystals 77%; mp 177 °C; R_f value TLC (CH_2Cl_2) 0.36; ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H, CH_3), 6.88 (ddd, 2H, *J* = 8.93; 2.06;2.06 Hz, benzamide-3H), 7.38−7.43 (m, 3H, phenyl-3H + phenyl-4H), 7.88 (ddd, 2H, *J* = 8.93; 2.06; 2.06 Hz, benzamide-2H), 8.12−8.16 (m, 2H, phenyl-2H). ^{13}C NMR ($CDCl_3$) δ 55.56 (CH_3), 114.44 (benzamide-3C), 122.53 (benzamide-1C), 127.78 + 128.63 + 129.88 + 130.26 (benzamide-2C + phenyl-3,4-C), 132.45 (phenyl-1C), 16402 (benzamide-4C), 165.08 (amide-C), 166.97 (thiadiazolyl-3C), 175.88 (thiadiazolyl-5C); MS (EI) *m*/*z* 312 (MH^+, 100%). Anal. ($C_{16}H_{13}N_3O_2S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-nitrobenzamide (8f):** method D; recrystallization of the combined solids from dichloromethane/methanol yielded 33% needlelike white crystals; mp >300 °C; R_f value TLC (CH_2Cl_2) 0.33; ^{1}H NMR (DMSO-d_6) δ 7.50−7.56 (m, 3H, phenyl-3H + phenyl-4H), 8.20−8.22 (m, 2H, benzamide-2H), 8.37−8.42 (m, 4H, phenyl-2H + benzamide-3H), 10.60 (bs,1H, NH). Anal. ($C_{15}H_{10}N_4O_3S \cdot 0.3CH_2Cl_2$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-methoxy-3-methylbenzamide (8g):** method D; purification by column chromatography (EA/tol as eluent) followed by recrystallization from methanol yielded 11% white needles; mp 160 °C; R_f value TLC (EA/tol) 0.90; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.73−6.78 (d, 1H, benzamide-5H), 7.34−7.37 (m, 3H, phenyl-3H + phenyl-4H), 7.67 (s, 1H, benzamide-2H), 7.71−7.75 (m, 1H, benzamide-6H), 8.10−8.15 (m, 2H, phenyl-2H), 10.64 (bs, 1H, NH). Anal. ($C_{17}H_{15}N_3O_2S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-hydroxybenzamide (LUF5437, 8h).** 0.5 g (1.6 mmol) of **8e** was dissolved in 5.0 mL of dry DMSO in a flame-dried pressure tube. 0.40 g (8.2 mmol) of NaCN was added and a nitrogen flow was let in the tube before closing. The solution was stirred at 165 °C for 90 h. The resulting black solution was poured on 30 mL of ice and acidified (carefully in a hood with good ventilation) with 6 N HCl. The black precipitate was collected by filtration and subsequently washed with water and *n*-pentane. A short Kieselgel column was used for purification with CH_2Cl_2 as eluent. 0.08 g of **8e** was recovered (pure). After this ethyl acetate was used as eluent and the product was isolated as a white/yellow solid, which was recrystallized from CCl_4/toluene: yield white crystals 70% (corrected for recovery of **8e**); mp 117 °C; ^{1}H NMR (CD_3OD) δ 6.93 (d, 2H, *J* = 8.6 Hz, benzamide-3H), 7.45 (m, 3H, phenyl-3H + phenyl-4H), 8.00 (d, 2H, *J* = 8.9 Hz, benzamide-2H), 8.24 (m, 2H, phenyl-2H); MS (EI) *m*/*z* 298 (MH^+, 100%). Anal. ($C_{15}H_{11}N_3O_2S \cdot 0.2CCl_4$) C, H, N.

2-(4-{[(3-Phenyl-1,2,4-thiadiazol-5-yl)amino]carbonyl}-phenoxy)acetic Acid (8i). 100.0 mg of **8h** was dissolved in 5.0 mL of dry dioxane in a flame-dried pressure tube under nitrogen gas. 200 μL of triethylamine and 200 μL of bromoethyl acetate were added. This light yellow solution was stirred overnight at ambient temperature. A precipitate was formed. A mixture of 20 mL of water and 20 mL of ethyl acetate was added. The ethyl acetate layer was collected, washed with a 1 M HCl solution (20 mL) and a brine solution (20 mL), dried on $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in 10 mL of ethanol (96%) and 10 mL of 1 M KOH (aq) was added. After 10 min 6 M HCl was added to acidify the solution. A precipitate formed and a mixture of 20 mL of ethyl acetate and 10 mL of water was added. The ethyl actetate layer was collected and washed with brine (20 mL). After drying and concentrating the ethyl acetate layer, a white "solid" oil remained (yield 100%), which was recrystallized twice from toluene with a little acetone: yield white needlelike crystals, 10% after recrystallization; mp >265 °C; ^{1}H NMR (DMSO-d_6) δ 4.82 (s, 2H, CH_2), 7.10 (d, 2H, *J* = 8.2 Hz, benzamide-3H), 7.52 (m, 3H, phenyl-3H + phenyl-4H), 8.17 (d, 2H, *J* = 8.2 Hz, benzamdie-2H), 8.21 (m, 2H, phenyl-2H); MS (EI) *m*/*z* 356 (MH^+, 100%). Anal. ($C_{17}H_{13}N_3O_4S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)cyclohexanamide (8j):** method C; crystallization from CCl_4/MeOH yielded 10% off-white crystals; mp 250 °C dec; R_f value TLC (EA/tol 4:1) 0.65; ^{1}H NMR ($CDCl_3$) δ 1.24−2.14 (m, 10H, chex), 2.70−2.82 (m, 1H, chex-H1), 7.44−7.61 (m, 3H, phenyl-3H + phenyl-4H), 8.52 (dd, 2H, 3*J* = 7.1 Hz, phenyl-2H), and 13.48 (bs, 1H, NH). Anal. ($C_{15}H_{17}N_3OS$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-*trans*-4-methoxycyclohexanamide (8k):** method C; purification by column chromatography (EA/CH_2Cl_2 1:7 as eluent) yielded the pure isomer; recrystallization from PE60−80/EA yielded 48% white crystals (corrected for *cis*/*trans*-mixture of starting material); mp 177 °C; R_f value TLC (2.5% EA/CH_2Cl_2) 0.31; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12−1.21 (m, 2H, H3-ax), 1.46−1.56 (m, 2H, H2-ax), 1.93−1.96 (m, 2H, H2-eq), 2.05−2.09 (m, 2H, H3-

eq), 2.52−2.56 (m, 1H, H1-ax), 3.06−3.13 (m, $^3J_{axax}$ = 15 Hz, 1H, H4-ax), 3.26 (s, 3H, OCH_3), 7.35−7.42 (m, 3H, phenyl-3H + phenyl-4H), 7.87−7.93 (m, 1H, phenyl-2H), 8.10−8.14 (m, 1H-phenyl-2H), 12.77 (bs, 1H, NH). Anal. ($C_{16}H_{19}N_3O_2S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-*cis*-4-methoxycyclohexanamide (8l):** method C; purification by column chromatography (EA/CH_2Cl_2 1:7 as eluent) yielded the pure isomer; recrystallization from PE60−80/EA yielded 32% (corrected for *cis*/*trans*-mixture of starting material); mp 221 °C; R_f value TLC (2.5% EA in CH_2Cl_2) 0.50; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42−1.48 (m, 2H, H3-ax), 1.60−1.64 (m, 2H, H2-eq), 1.74−1.83 (m, 2H, H2-ax), 1.86−1.91 (m, 2H, H3-eq), 2.58−2.65 (m, 1H, H1), 3.39 (dd, $^3J_{eqeq}$ = 2.1 Hz, 1H, H4-eq), 3.22 (s, 3H, OCH_3), 7.38−7.45 (m, 3H, phenyl-3H + phenyl-4H), 8.09−8.17 (m, 2H, phenyl-2H), 12.83 (bs, 1H, NH). Anal. ($C_{16}H_{19}N_3O_2S$) C, H, N.

General Procedure for the Synthesis of 8m,n. 100 mg of methoxy derivative **8k** or **8l** was added to a mixture of 0.80 g of $AlBr_3$ and 5.0 mL of EtSH and stirred for 3 h at room temperature. After quenching with 10 mL of water the solution was acidified with 3 drops of HCl (concentrated) and three times extracted with 15 mL of ethyl acetate. The combined organic layers were dried on Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography (EA/CH_2Cl_2 1:1 as eluent).

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-*trans*-4-hydroxycyclohexanamide (8m):** yield 78%; mp 236 °C; ^{1}H NMR (DMSO-d_6) δ 1.05−1.30 (m, 2H, H3-ax), 1.40−60 (m, 2H, H2-ax), 1.75−1.95 (m, 4H, H2-eq + H3-eq), 2.12−2.48 (m, 1H, H1-ax), 3.30−3.50 (m, $^3J_{axax}$ = 15 Hz, 1H, H4-ax), 4.64 (d ^3J = 6 Hz, 1H, OH), 7.50−7.60 (m, 3H, phenyl-3H + phenyl-4H), 8.60−8.70 (m, 2H, phenyl-2H), 13.08 (bs, 1H, NH). Anal. ($C_{15}H_{17}N_3O_2S$) C, H, N.

***N*-(3-Phenyl-1,2,4-thiadiazol-5-yl)-*cis*-4-hydroxycyclohexanamide (8n):** yield 47%; mp 175 °C; R_f value TLC (EA/CH_2Cl_2) 0.43; ^{1}H NMR (DMSO-d_6) δ 1.43−1.70 (m, 6H, H3-ax + H2-ax + H2-eq), 1.81−1.96 (m, 2H, H3-eq), 2.50−2.64 (m, 1H, H1), 3.46−3.50 (m, 1H, H4-eq), 3.74 (d, ^3J = 7 Hz, 1H, OH), 7.50−7.55 (m, 3H, phenyl-3H + phenyl-4H), 8.14−8.19 (m, 2H, phenyl-2H), 13.04 (bs, 1H, NH). Anal. ($C_{15}H_{17}N_3O_2S$) C, H, N.

***N*-(4,5-Dihydro-1-phenylpyrazol-3-yl)-4-methoxybenzamide (9).** Method C yielded the dibenzoyl analogue, which was dissolved in 5 mL of dioxane. Subsequently 1.5 mL of 1 M NaOMe was added and the mixture stirred at room temperature for 48 h. Purification by column chromatography (EA/*n*-hexane 3:1) yielded 3% yellow crystals: ^{1}H NMR ($CDCl_3$) δ 3.22 (t, ^3J = 10 Hz, 2H, CH_2), 3.81−3.93 (s + t, ^3J = 7.3 Hz, 5H, CH_2 + OCH_3), 6.73−6.88 (m, 5H, phenyl-2H, phenyl-4H, benzamide 3H), 7.19 (m, 2H, phenyl-3H), and 7.74 (d, 2H, $^3J_{BA}$ = 8.6 Hz, benzamide-2H). Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

***N*-(5-Phenylpyrazol-3-yl)-4-methoxybenzamide (10).** Method C was used for 2.08 mmol of 3-amino-5-phenylpyrazole and 2.28 mmol of anisoyl chloride. The white solid contained the desired product as well as the diamide and was purified by column chromatography (EA/tol 1:1). Recrystallization from methanol of the second fraction yielded 13% of **31** as white needles: mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H, OCH_3), 7.04−7.06 (m, 3H, benzamide-3H + pyrazol-4H), 7.37−7.46 (m, 3H, phenyl-3H + phenyl-4H), 7.72−7.76 (m, 2H, phenyl-2H), 8.0 (d, 2H, $^3J_{BA}$ = 8.7 Hz, benzamide-2H), 10.70 (bs, 1H, NH), and 12.92 (bs, 1H, pyrazol-NH). Anal. ($C_{17}H_{15}N_3O_2 \cdot 0.2CH_3OH$) C, H, N.

***N*-(6-Phenylpyrimidin-4-yl)-4-methoxybenzamide (11):** method D used for 0.292 mmol of 4-amino-6-phenylpyrimidine and 0.704 mmol of *p*-anisic acid; reflux period 12 h; preparative TLC (EA/CH_2Cl_2/TEA1:1 0.01) yielded 26% white solid; mp 160 °C; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, OCH_3), 6.98 (d, 2H, $^3J_{AB}$ = 9 Hz, benzamide-3H), 7.40−7.50 (m, 3H, phenyl-3H + phenyl-4H), 7.85 (d, 2H, $^3J_{BA}$ = 9 Hz, benzamide-2H), 8.02−8.12 (m, 2H, phenyl-2H), 8.61 (bs, 1H, NH), 8.75 (s, 1H, pyrimidine-H), and 8.88 (s, 1H, pyrimidine-H). Anal. ($C_{18}H_{15}N_3O_2$) C, H, N.

***N*-(6-Phenylpyridin-2-yl)-4-methoxybenzamide (12):** method D used for 3.00 mmol of 2-amino-6-phenylpyridine; yield 22% white crystals; mp 130 °C; ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3H, OCH_3), 6.93 (d, 2H, $^3J_{AB}$ = 7 Hz, benzamide-3H), 7.36−7.47 (m, 4H, H5 + phenyl-3H + phenyl-4H), 7.76 (dd, 1H, $^3J_{45}$ = 8.0 Hz, $^3J_{43}$ = 8.2 Hz, H4), 7.85−7.93 (m, 4H, benzamide-2H, phenyl-2H), 8.26 (d, $^3J_{34}$ = 8.2 Hz, 1 H, H3), and 8.55 (bs, 1H, NH). Anal. ($C_{19}H_{16}N_2O_2$) C, H, N.

***N*-(Biphen-3-yl)-4-methoxybenzamide (13):** method D used for 1.20 mmol of 3-aminobiphenyl; reflux period 8 h; yield 70% white powder; mp 137−138 °C; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, OCH_3), 6.97 (d, 2H, $^3J_{AB}$ = 8 Hz, benzamide-3H) and 7.38−7.87 (m, 11H, H2 + H4 + H5 + H6 + phenyl-2H + phenyl-3H + phenyl-4H + benzamide-2H). Anal. ($C_{20}H_{17}NO_2$) C, H, N.

General Procedure for 17a−j. *N*-[4-(2-Pyridyl)thiazol-2-yl]benzamide Hydrochloride (17a). 1.0 mmol of the appropriate benzoic acid was dissolved in 0.5 mL of dry DMF in a 2.2-mL Eppendorf vial. 45.0 mg (0.25 mmol) of 2-amino-4-phenylthiazole in 0.5 mL of dry DMF was added, together with a solution of 0.21 g (1.0 mmol) of DCC in 0.5 mL of dry DMF and 14.0 mg (0.1 mmol) of DMAP. This was stirred for 4 days with a small stirring bar. The precipitate was removed by centrifugation, washed with 1.0 mL of DMF, and the combined DMF solutions were concentrated in vacuo. The residue was dissolved in 10 mL of ethyl acetate (clear solution) and a stream of HCl gas was bubbled through the solution. A white precipitate formed, which was collected by centrifugation. The solid was recrystalized twice from EA/CCl_4/MeOH: yield off-white powder 49%; mp 176 °C; ^{1}H NMR (CD_3OD) δ 7.53−7.70 (m, 3H, benzamide-3H + benzamide-4H), 7.96 (ddd, 1H, J = 6.0; 6.0; 3.1 Hz, pyridyl-5H), 8.06 (dd, 2H, J = 7.2; 1.4 Hz, benzamide-2H), 8.42 (s, 1H, thiazolyl-5H), 8.57−8.64 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (d, 1H, J = 5.8 Hz, pyridyl-6H); MS (EI) *m*/*z* 282 (MH^+ (−HCl), 100%). Anal. ($C_{15}H_{11}N_3OS \cdot HCl$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-4-chlorobenzamide Hydrochloride (17b):** yield white crystals 59%; mp 245 °C; ^{1}H NMR (CD_3OD) δ 7.60 (ddd, 2H, J = 8.6; 2.4; 2.1 Hz, benzamide-3H), 7.97 (ddd, 1H, J = 6.2; 6.2; 2.7 Hz, pyridyl-5H), 8.06 (ddd, 2H, J = 8.9; 2.4; 2.1 Hz, benzamide-2H), 8.42 (s, 1H, thiazolyl-5H), 8.57−8.64 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (ddd, 1H, J = 5.5; 1.0; 1.0 Hz, pyridyl-6H); MS (EI) *m*/*z* 316 ($M(^{35}Cl)H^+$ (−HCl), 100%), 318 ($M(^{37}Cl)H^+$ (−HCl), 28%). Anal. ($C_{15}H_{10}ClN_3OS \cdot HCl \cdot 0.2CCl_4$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-4-iodobenzamide Hydrochloride (17c):** yield off-white powder 63%; mp 212 °C; ^{1}H NMR (CD_3OD) δ 7.81 (ddd, 2H, J = 8.6; 2.8; 2.1 Hz, benzamide-2H), 7.89 (m, 1H, pyridyl-5H), 7.98 (ddd, 2H, J = 8.6; 2.8; 2.1 Hz, benzamide-3H), 8.41 (s, 1H, thioazolyl-5H), 8.56−8.64 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (ddd, 1H, J = 5.5; 1.0; 1.0 Hz, pyridyl-6H); MS (EI) *m*/*z* 408 (MH^+) (−HCl, 100%). Anal. ($C_{15}H_{10}IN_3OS \cdot HCl \cdot 0.2CCl_4$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-4-methylbenzamide Hydrochloride (17d):** yield white crystals 78%; mp 221 °C; ^{1}H NMR (CD_3OD) δ 2.44 (s, 3H, CH_3), 7.38 (d, 2H, J = 7.9 Hz, bnzamide-3H), 7.90−8.00 (m, 1H, pyridyl-5H), 7.94 (d, 2H, J = 8.2 Hz, benzamide-2H), 8.39 (s, 1H, thiazolyl-5H), 8.52−8.64 (m, 2H, pyridyl-3H + pyridyl-4H), 8.76 (d, 1H J = 5.4 Hz, pyridyl-6H); MS (EI) *m*/*z* 296 (MH^+(−HCl), 100%). Anal. ($C_{16}H_{13}ClN_3OS \cdot HCl \cdot 0.2CCl_4$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-4-methoxybenzamide Hydrochloride (17e):** yield slightly yellow crystals 67%; mp 197 °C; ^{1}H NMR (CD_3OD) δ 3.90 (s, 3H, CH_3), 7.09 (ddd, 2H, J = 8.9; 3.1; 2.1 Hz, benzamide-3H), 7.97 (m, 1H, pyridyl-5H), 8.04 (ddd, 2H, J = 8.9; 3.1; 2.1 Hz, benzamide-2H), 8.40 (s, 1H, thiazolyl-5H), 8.62 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (dm, 1H, J = 5.8 Hz, pyridyl-6H); MS (EI) *m*/*z* 312 (MH^+ (−HCl) 100%). Anal. ($C_{16}H_{13}N_3O_2S$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-3,4-dichlorobenzamide Hydrochloride (17f):** yield white crystals 72%; mp 257 °C; ^{1}H NMR (CD_3OD) δ 7.75 (d, 1H, J = 8.6 Hz, benzamide-5H), 7.97 (ddd, 1H, J = 6.2; 5.8; 2.7 Hz, pyridyl-5-H), 8.00 (dd, 1H, J = 8.6, 2.1 Hz, benzamide-6H), 8.22 (d, 1H, J = 2.1 Hz, pyridyl-

2H), 8.42 (s, 1H, thiazolyl-5H), 8.56−8.63 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (ddd, 1H, J = 5.8; 1.0; 1.0 Hz, pyridyl-6H); MS (EI) m/z 350 (M(^{35}Cl, ^{35}Cl)H$^+$ (−HCl), 100%), 352 (M(^{35}Cl, ^{37}Cl)H$^+$ (−HCl), 70%), 354 (M(^{37}Cl, ^{37}Cl)H$^+$ (−HCl), 18%). Anal. ($C_{15}H_9Cl_2N_3OS\cdot HCl\cdot 0.1CCl_4$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-3-chlorobenzamide Hydrochloride (17g):** yield white crystals 62%; mp 188 °C; ^{1}H NMR (CD_3OD) δ 7.58 (dd, 1H, J = 8.2; 7.6 Hz, benzamide-5H), 7.78 (ddd, 1H, J = 7.9; 1.9; 1.9 Hz, benzamide-4H), 7.98 (ddd, 1J, J = 6.4; 5.7; 2.7 Hz, pyridyl-5H), 8.00 (m, 1H, benzamide-6H), 8.06 (t, 1H, J = 1.7 Hz, pyrdiyl-2H), 8.43 (s, 1H, thiazolyl-5H), 8.57−8.68 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (ddd, 1H, J = 5.5; 1.4; 1.4 Hz, pyridyl-6H); MS (EI) m/z 316 (M(^{35}Cl)H$^+$ (−HCl), 100%), 318 (M(^{37}Cl)H$^+$ −HCl), 60%). Anal. ($C_{15}H_{10}ClN_3OS\cdot HCl$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-4-nitrobenzamide Hydrochloride (17h):** recrystallization of the HCl salt from methanol yielded 53% white crystals; mp 261 °C; ^{1}H NMR (CD_3OD + 20% DMSO-d_6) δ 7.94 (m, 1H, pyridyl-5H), 8.30 (ddd, 2H, J = 9.3; 2.4; 2.1 Hz, benzamide-2H), 8.44 (ddd, 2H, J = 8.9; 2.4; 2.1 Hz, benzamide-3H), 8.45 (s, 1H, thiazolyl-5-H), 8.56−8.62 (m, 2H, pyrdiyl-3H + pyridyl-4H), 8.80 (dm, 1H, J = 6.1 Hz, pyrdiyl-6H); MS (EI) m/z 327 (MH$^+$ (−HCl), 100%. Anal. ($C_{15}H_{10}N_4O_3S\cdot HCl$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]-4-isopropoxybenzamide Hydrochloride (17i):** crystallization from methanol yielded 48% white needlelike crystals; mp 181 °C; ^{1}H NMR (CD_3OD) δ 1.37 (d, 6H, J = 5.8 Hz, CH_3), 4.75 (quin, 1H, J = 5.8 Hz, CH), 7.06 (ddd, 2H, J = 8.9; 2.1; 2.1 Hz, benzamide-3H), 7.95 (ddd, 1H, J = 6.2; 6.2; 3.1 Hz, pyridyl-5H), 8.02 (ddd, 2H, J = 8.9; 2.1; 2.1 Hz, benzamide-2H), 8.38 (s, 1H, thiazoly-5H), 8.53−8.63 (m, 2H, pyridyl-3H + pyridyl-4H), 8.78 (dm, 1H, J = 5.8 Hz, pyridyl-6H); MS (EI) m/z 340 (MH$^+$ (−HCl), 100%). Anal. ($C_{18}H_{17}N_3O_2S\cdot HCl$) C, H, N.

***N*-[4-(2-Pyridyl)thiazol-2-yl]cyclopentanamide Hydrochloride (17j):** yield white crystals 66%; mp 233 °C; ^{1}H NMR (DMSO-d_6 + 20% CD_3OD) δ 1.50−1.96 (m, 8H, cyclopentyl-2H + cyclopentyl-3H), 3.00 (m, 1H, cyclopentyl-1H), 7.73 (m, 1H, pyridyl-5H), 8.23−8.37 (m, 2H, pyridyl-3H + pyridyl-4H), 8.42 (s, 1H, thiazolyl-5H), 8.72 (d, 1H, J = 5.5 Hz, pyridyl-6H); MS (EI) m/z 274 (MH$^+$ (−HCl), 100%. Anal. ($C_{14}H_{15}N_3OS$) C, H, N.

General Procedure for the Synthesis of 20a,b, 21a,b, and 22. 90.0 mg (0.5 mmol) of 5-amino-3-phenyl-1,2,4-thiadiazole or 2-amino-4-phenylthiazole was dissolved in 2.0 mL of acetonitrile. This slightly turbid mixture was filtered and the clear solution was transferred in a 2.2-mL Eppendorf vial. 100 μL (0.9 mmol) of isocyanate was added and the solution was warmed at 56 °C for 2 h. Overnight a white solid precipitated from the reaction mixture, which was collected by centrifugation. The dry solid was recrystallized from PE (60/80) and methanol or absolute ethanol.

***N*-Phenyl-*N*′-(3-phenyl-1,2,4-thiadiazol-5-yl)urea (20a):** yield white crystals 38%; mp 218 °C; ^{1}H NMR (CD_3OD) δ 7.10 (t, 1H, J = 7.6 Hz, N-phenyl-4-H), 7.34 (t, 2H, J = 7.9 Hz, N-phenyl-3H), 7.41−7.53 (m, 5H, N-phenyl-2H + thiazolphenyl-3H + thiazolphenyl-4H), 8.16−8.21 (m, 2H, thiazolphenyl-2H); MS (EI) m/z 297 (MH$^+$, 100%). Anal. ($C_{15}H_{12}N_4OS$) C, H, N.

***N*-(4-Methoxyphenyl)-*N*′-(3-phenyl-1,2,4-thiadiazol-5-yl)urea (20b):** yield white crystals 43%; mp 210 °C; ^{1}H NMR ($CDCl_3$ + 10%CD_3OD) δ 3.81 (s, 3H, OCH_3), 6.90 (d, 2H, J = 8.6 Hz, N-phenyl-3H), 7.40 (d, 2H, J = 8.6 Hz, N-phenyl-2H), 7.43−7.49 (m, 3H, thiazolphenyl-3H + thiazolphenyl-4H), 8.13−8.20 (m, 2H, thiazolphenyl-2H); MS (EI) m/z 327 (MH$^+$, 100%). Anal. ($C_{16}H_{14}N_4O_2S$) C, H, N.

***N*-(2-Methoxyphenyl)-*N*′-(3-phenyl-1,2,4-thiadiazol-5-yl)urea (20c):** yield white crystals 37%; mp 178−179 °C; ^{1}H NMR ($CDCl_3$ + 10%CD_3OD) δ 3.71 (s, 3H, OCH_3), 6.95−7.15 (m, 4H, N-phenylH), 7.43−7.49 (m, 3H, thiazolphenyl-3H + thiazolphenyl-4H), 8.13−8.20 (m, 2H, thiazolphenyl-2H). Anal. ($C_{16}H_{14}N_4O_2S\cdot 0.2CH_3OH$) C, H, N.

***N*-Phenyl-*N*′-[4-(2-pyridyl)thiazol-2-yl]urea (21a):** yield white crystals 69%; mp 218 °C; ^{1}H NMR (DMSO-d_6) δ 7.10 (t, 1H, J = 7.2 Hz, N-penyl-4H), 7.03−7.38 (m, 3H, N-phenyl-3H + pyridyl-5H), 7.50 (dm, 2H, J = 8.6 Hz, N-phenyl-2H), 7.64 (s, 1H, thiazolyl-5H), 7.88 (ddd, 1H, J = 7.55; 7.55; 1.8 Hz, pyridyl-4H), 7.99 (dm, 1H, J = 7.9 Hz, pyridyl-3H), 8.57 (dm, 1H, J = 5.2 Hz, pyridyl-6H); MS (EI) m/z 297 (MH$^+$, 100%). Anal. ($C_{15}H_{12}N_4OS$) C, H, N.

***N*-(4-Methoxyphenyl)-*N*′-[4-(2-pyridyl)thiazol-2-yl]-urea (21b):** recystallization from methanol yielded 66% white crystals; mp 258 °C; ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H, CH_3), 6.90 (d, 2H, J = 8.6 Hz, phenyl-3H), 7.30 (m, 1H, pyridyl-5H), 7.39 (d, 2H, J = 0.86 Hz, phenyl-2H), 7.71 (s, 1H, thiazolyl-5H), 7.82−7.96 (m, 2H, pyridyl-3H + pyridyl-4H), 8.58 (m, 1H, pyridyl-6H), 8.74 (s, 1H, NH), 10.66 (bs, 1H, NH); MS (EI) m/z 327 (MH$^+$, 100%), 296 (MH$^+$ − OCH_3, 95%). Anal. ($C_{16}H_{14}N_4O_2S$) C, H, N.

***N*-Phenyl-*N*′-(4-phenylthiazol-2-yl)urea (22):** yield white crystals 80%; mp 214 °C; ^{1}H NMR (DMSO-d_6) δ 7.03 (t, 1H, J = 7.2 Hz, N-phenyl-4H), 7.28−7.54 (m, 8H, N-phenyl-2H + N-phenyl-3H + thiazolphenyl-3H + thiazolphenyl-4H + thiazolyl-5H), 7.88 (d, 2H, J = 7.6 Hz, thiazolphenyl-2H), 8.91 (s, 1H, NH_2), 10.68 (bs, 1H, NH); MS (EI) m/z 296 (MH$^+$, 100%). Anal. ($C_{16}H_{13}N_3OS$) C, H, N.

Molecular Modeling. Calculations were performed with SPARTAN version 5.0 (Wavefunction, Inc., Irvine)[36] running on a Silicon Graphics O2 workstation. Default values in the Merck force field[38] were used in molecular mechanics minimizations. Conjugate gradient energy minimizations were continued until the rms energy derivative was less than 0.01 kcal/mol·Å. The Monte Carlo method was used for conformational analysis (step size 15°). The semiempirical molecular orbital program AM1[39] with the AM1 Hamiltonian was used to optimize the conformation of the compounds. The keyword MMOK was used because the structures contain an amide moiety. The 3-21G* Gaussian basis set[40] (Hartree−Fock, closed shell) was used for ab initio calculations of the energy and the molecular electrostatic potential. The electrostatic potentials were sampled over the entire accessible surface of the molecules (corresponding roughly to a van der Waals contact surface). The most negative electrostatic potential is red, and the most positive electrostatic potential is blue.

Pharmacology. Materials: [^{3}H]CGS 21680 and [^{3}H]-DPCPX were commercially available from DuPont Nemours, ('s Hertogenbosch, The Netherlands). [^{3}H]ZM 241385 was purchased from TOCRIS (Bristol, U.K.). [^{125}I]AB-MECA was prepared as described by Olah et al.[28] Adenosine deaminase was from Boehringer Mannheim (Mannheim, Germany). 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: 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.[26,27] Binding of [^{3}H]ZM 241385 to adenosine A_{2A} receptors was performed in test tubes containing an aliquot of rat striatal membranes (100−200 μg of protein/mL) in incubation buffer (50 mM Tris-HCl, adjusted to pH 7.4 at 25 °C) with approximately 2 nM [^{3}H]ZM 241385 in a final volume of 1 mL After incubation at 25 °C for 120 min the binding reaction was terminated by filtration through Whatman GF/B filters under reduced pressure (200 mbar) using a Brandell cell harvester (Brandell Gaithersburg, MD). Filters were washed twice with ice-cold buffer (5 mL) and placed in scintillation vials. Bound radioactivity was determined using conventional liquid scintillation spectroscopy techniques in a LKB1219 counter.

Binding of [^{125}I]AB-MECA to membranes of HEK 293 cells stably expressing the human adenosine A_3 receptor was determined as described.[28,29]

Acknowledgment. This research was financially supported by Byk Gulden (Zwanenburg, The Netherlands).

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

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JM0003945