A Novel Class of Adenosine A_3 Receptor Ligands. 2. Structure Affinity Profile of a Series of Isoquinoline and Quinazoline Compounds

Jacqueline E. van Muijlwijk-Koezen,* Henk Timmerman, Regina Link,† Henk van der Goot, and Adriaan P. IJzerman†

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

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1-Substituted 3-(2-pyridinyl)isoquinolines have been shown to form a novel class of adenosine A_3 receptor ligands. In the present study further investigations of this new lead and the structure affinity relationships of this class of compounds are described. First, the influence of an amide group at position 1 of the isoquinoline ring on the adenosine A_3 receptor affinity was determined. A carboxamide proved to be a useful spacer between the isoquinoline and a phenyl ring. *N*-[2-(2-pyridinyl)isoquinolin-4-yl]benzamide (VUF8507, compound **6**) had an affinity of 200 nM at the adenosine A_3 receptor. Second, we investigated the effects of substitution of the benzamide ring of **6** with a series of mono- and disubstituted *N*-[3-(2-pyridinyl)isoquinoline]benzamides. The ratio of the tautomers of the benzamides was determined in the solid state and in solution by spectroscopic techniques (IR and NMR). Affinities were determined in radioligand binding assays at rat brain A_1 and A_{2A} receptors and at cloned human A_3 receptor. The benzamides showed higher adenosine A_3 receptor affinity than aliphatic amides. We propose that the adenosine A_3 receptor affinity of the different benzamides is related to their presence in either the iminol or amide form. Ligands present in the iminol form showed relatively high adenosine A_3 receptor affinity. Finally, we explored the influence of replacement of C_4 of the isoquinoline ring by a nitrogen atom. Comparison of isoquinolines with the corresponding quinazolines revealed that both compounds showed similar adenosine A_3 receptor affinity. These investigations led to potent and selective human adenosine A_3 receptor ligands with affinities in the nanomolar range. The subtype-selective compound 4-methoxy-*N*-[2-(2-pyridinyl)quinazolin-4-yl]benzamide (VUF8504, **13**) with an affinity of 17.0 nM at the human adenosine A_3 receptor might become a useful tool in the pharmacological characterization or the investigation of the physiological function of this receptor.

Introduction

Extracellular adenosine modulates a wide range of physiological functions by activation of G protein-coupled adenosine receptors. Four subclasses of adenosine receptors have been identified: A_1, A_{2A}, A_{2B}, and A_3. The latter subtype has only been cloned recently[2,3] and is the subject of intensive pharmacological characterization.[4−7]

Activation of the adenosine A_3 receptor causes hypotension and promotes the release of inflammatory mediators from mast cells.[8,9] Selective A_3 antagonists could act as antiasthmatic,[10] cerebroprotective,[11,12] and antiinflammatory agents.[7]

For the A_1 and A_{2A} adenosine receptors, potent and selective antagonists have been found by optimization of the xanthine skeleton,[13] but these classical adenosine antagonists usually bind only weakly at adenosine A_3 receptors.[14] Recently, nonxanthine structures with high affinity at the adenosine A_3 receptor have been reported.[15−17] We have started a search for nonxanthine A_3 antagonists and found 3-(2-pyridinyl)isoquinoline derivatives as a novel class of A_3 ligands.[1]

The previous study from our group[1] has suggested that an additional phenyl group on 2-pyridinylisoquinoline increased adenosine A_3 receptor affinity, provided that this aromatic ring is coupled by a spacer allowing conjugation. We also have reported on the influence of different spacers between the isoquinoline ring and this second aromatic ring. In Figure 1 different spacers, e.g., diacylamine **(1)**, methyl ketone **(2)**, and amidine **(3)**, are shown.

In the present study we describe another spacer, the amide moiety. The influence on adenosine receptor affinity of substituents on the aromatic ring coupled to this amide moiety was investigated using a series of substituted benzamides. Moreover, we explored the effects of the replacement of C_4 in the isoquinoline ring by a nitrogen atom, yielding quinazoline derivatives. The structure−activity relationship of these derivatives is discussed as well.

Chemistry

Intermediate 1-amino-3-(2-pyridinyl)isoquinoline was prepared under strong alkaline conditions from 2-meth-

* Author for correspondence. Address: LACDR, Division of Medicinal Chemsitry, De Boelelaan 1083, 1081 HV Amsterdam. Tel: (+31) 20 4447604. Fax: (+31) 20 4447610. E-mail: muylwyk@chem.vu.nl.
† Rijks Universiteit Leiden.

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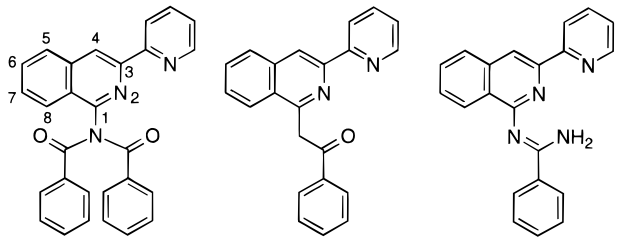


Figure 1. 1-Substituted 3-(2-pyridinyl)isoquinoline derivatives with different spacers between the isoquinoline ring and the second aromatic ring.

ylbenzenecarbonitrile and picolinonitrile according to a method described by De Zwart et al.[18] with some modifications. At a temperature of −40 °C potassium was added to condensed ammonia with ferric nitrate as catalyst. Subsequently, the nitriles were added to this freshly prepared KNH_2 and stirred overnight at room temperature to evaporate the ammonia. Hydrolysis took place by addition of aqueous ammonium chloride solution to obtain a NH_3/NH_4^+ buffer in situ. After acid/base extraction the compound was obtained by sublimation.

3,4-Dimethoxy-*N*-[3-(2-pyridinyl)isoquinolin-1-yl]benzamide **(19)** was prepared according to a modified method described by De Zwart et al.[18] In this procedure 1-amino-3-(2-pyridinyl)isoquinoline was condensed with the appropriate acyl chlorides, yielding the corresponding amide. These reactions are depicted in Scheme 1. The acylation took place in two steps: (i) abstraction of an amine proton and (ii) nucleophilic attack of the acid chloride. After proton abstraction, an intermediate was formed in which the negative charge is delocalized. This could be the reason that the yields of the benzamides were only moderate (10−60%), although the isomeric amide was not detected or isolated during the purification steps.

Compound **28**, 4-methoxy-*N*-[2-(2-pyridinyl)quinazolin-4-yl]benzamide, was synthesized similarly to the corresponding isoquinoline benzamide, viz. by acylation of 4-amino-2-(2-pyridinyl)quinazoline. Preparation of this precursor was carried out according to Linschoten et al.[19] The synthetic route yielding quinazolines **27** and **28** is depicted in Scheme 2.

Results and Discussion

Binding Studies. All 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 or [^{3}H]CGS 21680 as the radioligands, respectively.[20,21] The affinity at adenosine A_3 receptors was determined on membranes from HEK 293 cells, stably expressing the human A_3 receptor with [^{125}I]AB-MECA as radioligand.[22,23] These radioligand binding data are summarized in Tables 1, 2, and 4.

As mentioned in our previous report,[1] we found K_i values of some reference compounds different from literature data. Therefore, the absolute K_i values of the ligands cannot be compared unambiguously with affinities of other ligands reported in the literature.

Amide Group as Spacer. We used 1-substituted 3-(2-pyridinyl)isoquinolines, mentioned in our previous report,[1] as lead structure in an effort to find antagonists for the human adenosine A_3 receptor. We described the optimization of this class and found that addition of a phenyl ring via a spacer allowing conjugation to the 3-(2-pyridinyl)isoquinoline skeleton largely increased the affinity at the A_3 receptor.

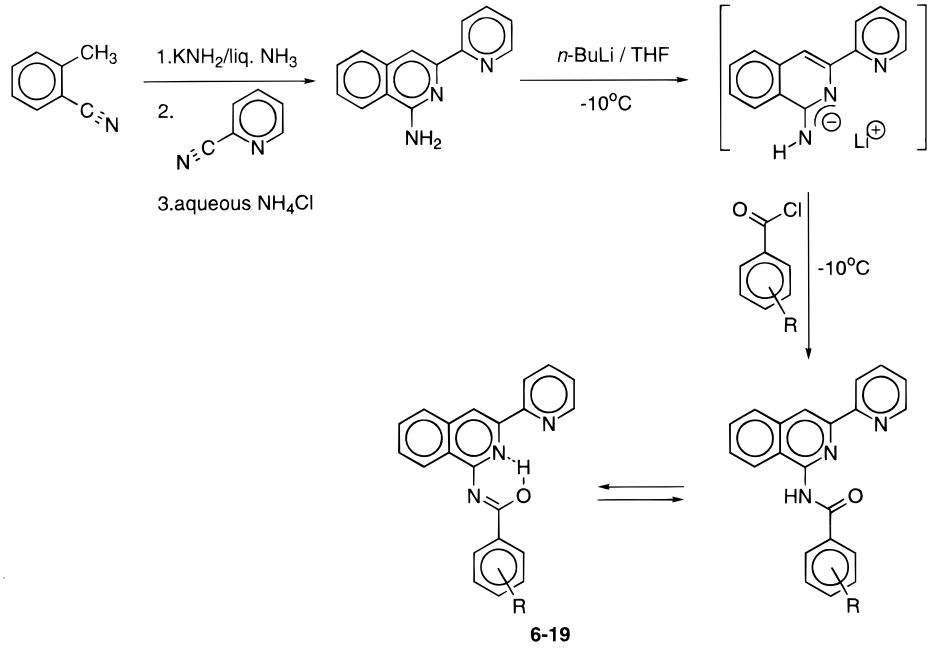
We investigated several spacers between the phenyl ring and the isoquinoline moiety. Among these were diacylamine (in **1**), methyl ketone (in **2**), and amidine (in **3**) moieties (Figure 1). The rather bulky compound **1**, containing an extra phenylacyl group compared to compounds **2** and **3**, showed significant A_3/A_1 selectivity. Compounds **2** and **3** showed equal affinities at the human adenosine A_3 receptor, but the A_3/A_1 selectivity was at least 4-fold more favorable for the amidine. Compounds **2** and **3** can be present in two tautomeric forms. In the enol or iminol form, the hydroxyl group may form a hydrogen bond with N(2) of the isoquinoline according to IR data.[1,24] In compound **1** this hydrogen bond could also exist according to its resonance structures. We decided to explore the influence of an amide bond as spacer on the adenosine A_3 receptor affinity with a monosubstituted amide. The importance of the aromatic ring at position 1 of the isoquinoline ring was checked by comparing the adenosine receptor affinities of compounds **4** and **5** with **6**.

Table 1 showed that replacement of the methyl group of **4** by a phenyl substituent **(6)** resulted in a 55-fold increase of adenosine A_3 receptor affinity. The isopropyl derivative **5** showed an affinity of approximately 10 mM, not very different from the methyl derivative **4**. From these data we concluded that an aromatic ring has to be used. The affinity for the human adenosine A_3 receptor of **6** increased more than 3-fold compared to compounds **2** and **3**, and compound **6** showed high A_3/A_1 selectivity, too. The difference in selectivity between compounds **3**, **6**, and **2** was remarkable. The amidine derivative **3** and the amide derivative **6** showed substantial A_3 selectivity, whereas methyl ketone derivative **2** showed a small preference for the adenosine A_1 receptor. Comparison of compounds **6** and **1** showed about equal affinities as well as A_3/A_1 selectivities. To investigate the influence of substituents at the phenyl ring on the adenosine receptor affinities unambiguously, we decided to use the monosubstituted amide bond as spacer.

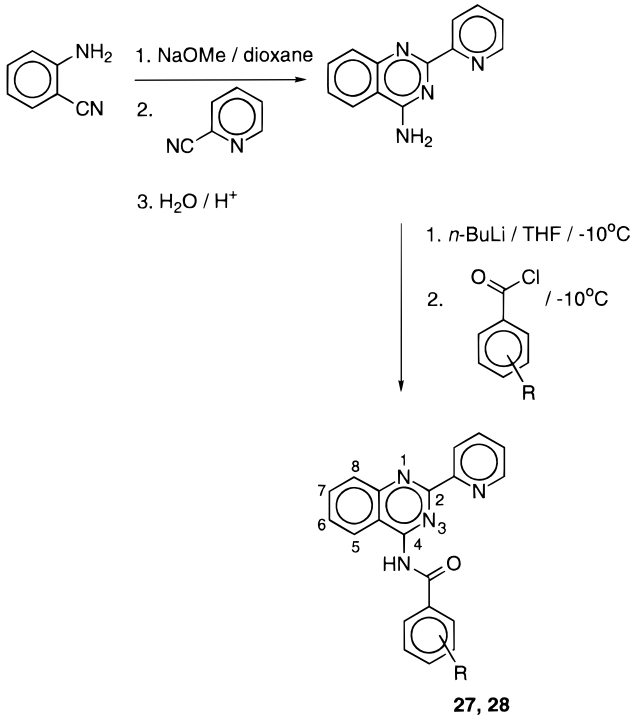
Aromatic Substitution of Benzamide. Next, the influence of substituents at the benzamide ring was investigated. The affinities of substituted benzamides **7**−**19** are shown in Table 2. A chloro substituent at the meta position resulted in lower adenosine A_3 receptor affinity (compound **7**), whereas a slightly electron-donating group (methyl compound **8**) showed equal affinity in comparison to the unsubstituted benzamide **6**. The electron-donating methoxy substituent on the meta position (compound **9**) raised the adenosine A_3 receptor affinity slightly.

For the para substituted benzamides a trend similar to that observed for the substituted *N*-[3-(2-pyridinyl)-isoquinolin-1-yl]benzamidines[1] was apparent. Para elec-

Scheme 1



Scheme 2



tron-donating substituents present in **12** and **13** rendered these analogues more potent than the unsubstituted phenyl analogue **6**, and an electron-withdrawing group as in **11** resulted in an equally potent compound. In the case of benzamides, para substitution contributed more convincingly to adenosine A_3 affinity. An electron-withdrawing chloro substituent, which in the series of benzamidines resulted in lower A_3 affinity,[1] showed no effect here (adenosine A_3 receptor affinity of 200 nM for both **11** and **6**). The influence of an electron-donating group, however, seemed to be larger since a 2-fold increase in adenosine A_3 receptor affinity was observed for 4-methylbenzamide **12**.

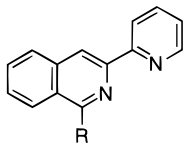
The methoxy substituent at the para position contributed most to adenosine A_3 receptor affinity. VUF8504 **(13)** showed a K_i value as low as 17 nM at the A_3 receptor. This potent A_3 antagonist was also a highly selective ligand. Only very low affinity was found for the A_1 and A_{2A} receptor with 14 and 0% displacement of the radioligand at a concentration of 10^{-5} M, respectively.

We also tested disubstituted benzamides to investigate the influence of the position of various substituents (Table 2). The 3,4-dichlorobenzamide **10** showed an affinity for the adenosine A_3 receptor equal to that of benzamide **6**. Larger differences were seen in the dimethyl derivatives **14**−**18**. The 3,4-dimethylbenzamide derivative **17** showed the highest adenosine A_3 receptor affinity from this series of disubstituted compounds. The adenosine A_3 receptor affinities for the 2,5-, 2,4-, and 3,5-dimethylbenzamides (compounds **15**, **16**, and **18**) were almost equal. The 2,6-dimethylbenzamide **14**, however, showed a 10-fold lower affinity. An explanation for this large difference in adenosine A_3 receptor affinity could be steric hindrance of the ortho substituents which could influence the tautomeric equilibrium.

In both pairs of compounds **8**−**9** and **12**−**13**, the methoxy derivatives showed higher adenosine A_3 receptor affinities than did the methyl derivatives. Because of the relatively high adenosine A_3 receptor affinity of **17**, higher than the unsubstituted or the 3-methyl derivative and comparable to the 4-methyl derivative, we decided to synthesize and measure adenosine A_3 receptor binding of the 3,4-dimethoxy derivative **19**. However, the adenosine A_3 receptor affinity of **19** decreased compared to **17**.

From compounds **8**, **12**, **17**, and **18** we concluded that a para electron-donating group is necessary for high adenosine A_3 receptor affinity and that methyl groups on the meta position were allowed (**17**) but did not contribute to positive ligand−receptor interaction (**18**).

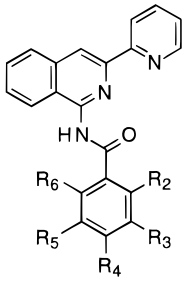
Table 1. Affinities at Adenosine Receptors of 1-Substituted 3-(2-Pyridinyl)isoquinoline Derivatives



compound	R	A_3[a]	A_1[b]	A_{2A}[d]	A_1/A_3[e]
(**1**)	$N(COPh)_2$	0.23 ± 0.091	4.3 ± 2.8[c]	27	19
(**2**)	CH_2COPh	0.66 ± 0.23	0.24 ± 0.1[c]	22	0.36
(**3**)	$N{=}C(NH_2)Ph$	0.74 ± 0.15	34	4	
(**4**)	$NH(CO)CH_3$	13 ± 1.9	24	18	
(**5**)	$NH(CO)CH(CH_3)_2$	48% (10^{-5} M)	38	48	
VUF8507 (**6**)	NH(CO)Ph	0.20 ± 0.04	3.2 ± 0.3[c]	0	16

[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 mM (n = 3−5) or percentage displacement of specific binding at a concentration of 10 mM (n = 3). [b] Displacement of specific [^{3}H]DPCPX binding in rat cortical membranes, expressed as percentage displacement of specific binding at a concentration of 10 mM (n = 2−3). [c]Displacement of specific [^{3}H]DPCPX binding in rat cortical membranes, expressed as K_i ± SEM in mM (n=3). [d] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as percentage displacement of specific binding at a concentration of 10 mM (n = 2−3). [e] $A_1/A_3 = K_iA_i/K_iA_3$.

Table 2. Affinities at the Adenosine Receptors of Aromatic *N*-[3-(2-Pyridinyl)isoquinolin-1-yl]amides



compound	R_2	R_3	R_4	R_5	R_6	A_3[a]	A_1[b]	A_{2A}[d]
VUF8507 (**6**)	H	H	H	H	H	0.20 ± 0.042	3.2 ± 0.3[c]	0
(**7**)	H	Cl	H	H	H	0.77 ± 0.50	42	0
(**8**)	H	CH_3	H	H	H	0.24 ± 0.084	35	0
(**9**)	H	OCH_3	H	H	H	0.15 ± 0.023	22	0
(**10**)	H	Cl	Cl	H	H	0.21 ± 0.049	0	0
(**11**)	H	H	Cl	H	H	0.20 ± 0.049	11	0
(**12**)	H	H	CH_3	H	H	0.096 ± 0.026	37	0
VUF8504 (**13**)	H	H	OCH_3	H	H	0.017 ± 0.0017	14	0
(**14**)	CH_3	H	H	H	CH_3	2.6 ± 0.74	2	0
(**15**)	CH_3	H	H	CH_3	H	0.43 ± 0.10	29	7
(**16**)	CH_3	H	CH_3	H	H	0.36 ± 0.13	14	0
(**17**)	H	CH_3	CH_3	H	H	0.069 ± 0.021	16	0
(**18**)	H	CH_3	H	CH_3	H	0.32 ± 0.13	8	0
(**19**)	H	OCH_3	OCH_3	H	H	0.31 ± 0.12	41	20

[a] *D*isplacement 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 cortical membranes, expressed as percentage displacement of specific binding at a concentration of 10 mM (n = 2−3). [c] Displacement of specific [^{3}H]DPCPX binding in rat cortical membranes, expressed as K_i ± SEM in mM (n = 3). [d] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as percentage displacement of specific binding at a concentration of 10 mM (n = 2−3).

Tautomerism and Conformation. In general, benzamides can exist in the amide or in the iminol form. It appeared that the unsubstituted compound **6** was present in solution in the iminol form as detected by NMR and IR spectroscopy. Because of the solubility properties of these benzamides, chloroform instead of water was used as solvent. In DMSO the NMR spectra were comparable to those in chloroform. The iminol group in compound **6** formed a hydrogen bond with the nitrogen atom of the isoquinoline ring which was shown by a signal at 16.3 ppm for the intramolecular hydrogen bond in the NMR spectrum. However, in the solid state, benzamide **6** existed in the amide form, according to the absorption of the C=O vibration at 1660 cm^{-1} in the IR spectrum of **6** in a KBr pellet. A solution IR spectrum of **6** in chloroform, however, showed that the carbonyl absorption had disappeared and an absorption at 3400 cm^{-1} according to the OH vibration had arisen. From these measurements we concluded that, in chloroform solution, compound **6** existed predominantly in the highly conjugated iminol form (Table 3). Similar findings have been reported by other authors.[25−28] They have investigated the equilibria of the two tautomers of different amides of 1-aminoisoquinolines by photochemistry and mass spectrometric studies.

The structures of the benzamides **6** and **12**−**18** were established in the solid phase (KBr platelets) with IR spectroscopy and in chloroform solution with IR and NMR spectrometry (Table 3).[18] Increased affinities of the disubstituted benzamides paralleled their presence

Table 3. Tautomeric Forms of *N*-[3-(2-Pyridinyl)isoquinolin-1-yl]benzamides

	solution		solid phase	
compound	^{1}H NMR $CDCl_3$ δ (ppm)	IR chloroform solution *A* (cm^{-1})	IR KBr platelets (cm^{-1})	affinity A_3 K_i (nM)
VUF8507 (**6**)	16.30 (N−HO)[18]	3400 (OH)	3240 (NH)/1660 (C═O)[18]	204 ± 42
(**12**)	16.33 (N−HO)[18]	3400 (OH)	3240 (NH)/1655 (C═O)[18]	96.3 ± 26
VUF8504 (**13**)	16.26 (N−HO)[18]	3400 (OH)	3400 (OH)[18]	17.0 ± 1.7
(**14**)	10.92 (NH)[18]	1660 (C═O)	3170 (NH)/1640 (C═O)[18]	2560 ± 740
(**15**)	16.20 (N−HO)	3400 (OH)/1660 (C═O)	1660 (C═O)	426 ± 101
(**16**)	16.18 (N−HO)	3400 (OH)/1660 (C═O)	1660 (C═O)	358 ± 129
(**17**)	16.24 (N−HO)[18]	3400 (OH)	3400 (OH)[18]	68.8 ± 21
(**18**)	16.22 (N−HO)[18]	3400 (OH)	3400 (OH)[18]	322 ± 133
(**19**)	15.15 (0.2 N−HO) 10.96 (0.8 NH)[a]	3400 (OH)	1650 (C═O)	306 ± 115

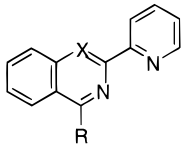
[a] Measured in DMSO solution because of its low solubility in chloroform.

in the iminol form. Compound **14**, existing in the amide form both in the solid state and in chloroform solution, had very low affinity at the adenosine A_3 receptor (2.56 mM). Compounds **15** and **16** were in the amide form in the solid state and for the larger part in the iminol form in solution. Indeed, C═O absorption in the IR spectrum was markedly reduced in the chloroform solution, whereas in the KBr pellets, high C═O absorption was observed. The two compounds had moderate affinity at the adenosine A_3 receptor (0.43 and 0.36 mM, respectively). Compound **17** existed in the iminol form in the solid as well as in the liquid phase and showed high adenosine A_3 receptor affinity (69 nM). From these observations we suggest that the influence of the positions of the substituents on affinity at the human adenosine A_3 receptor could be two-fold; first, direct steric and electronic effects of the substituents govern the conformations of the molecule, and second, the substituents determine the tautomeric form in which the ligand is present. For example, 2,6-dimethyl-*N*-[3-(2-pyridinyl)isoquinolin-1-yl]benzamide (**14**) contained two methyl groups on the ortho positions. This caused steric hindrance with the amide moiety and forced the phenyl ring out of the plane of the isoquinoline ring and amide moiety, resulting in the decoupling of the amide−aromatic system interaction. Because of this decrease in conjugation, the amide form would be preferred over the iminol form. The amide tautomer could fit the adenosine A_3 receptor pocket worse for yet unknown reasons. Second, if the adenosine A_3 receptor prefers a nearly coplanar conformation of the isoquinolinylbenzamides, as in the iminol form, the substituents have large influence. In that case, the ortho substituents of **14** prevented a good fit on the receptor.

The preference of the human adenosine A_3 receptor for a flat conformation of the ligand might also cause the low adenosine A_3 receptor affinity of the aliphatic amides **4** and **5**, besides their lower lipophilicity and bulkiness. The iminol form is favored by conjugation of an aromatic phenyl group with the isoquinoline moiety.[25−28] The adenosine A_3 receptor affinity decreased without this conjugation, as explained above. Also, in case of the substituted benzamidines, conjugation is highly conducive to adenosine A_3 receptor affinity.[1] All in all the tautomeric preference observed in KBr platelets may resemble the receptor-bound conformation of the ligands most.

Aza and Deaza Analogues. It has been shown that the influence of each nitrogen atom on the affinity at adenosine receptors can be established very well with the aid of deaza compounds such as the deazaxanthines[29] and the deazaadenines.[30,31] In analogy, we studied the adenosine receptor affinities of the closely related quinazolines (Table 4).

Table 4. Affinities at Adenosine Receptors of Quinazoline and Isoquinoline Compounds



compound	X	R	A_3[a]	A_1[b]	A_{2A}[c]
(**20**)	N	$NH(CO)CH_3$	11 ± 5.4	25	17
(**21**)	N	$NH(CO)CF_3$	86 ± 5.2	8	0
(**22**)	N	$N(COPh)_2$	0.47 ± 0.06	3	3
(**23**)	CH	OH	27 ± 10	21	35
(**24**)	N	OH	22 ± 9.2	16	10
(**25**)	CH	$N{=}CHNH_2$	20 ± 6.6	47	42
(**26**)	N	$N{=}CHNH_2$	4 (10^{-5} M)[d]	38	17
(**27**)	N	NH(CO)Ph	0.23 ± 0.02	0	2
(**28**)	N	$NH(CO)(4\text{-}OCH_3Ph)$	0.15 ± 0.12	41	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 mM (n = 3−5). [b] Displacement of specific [^{3}H]DPCPX binding in rat cortical membranes, expressed as percentage displacement of specific binding at a concentration of 10 mM (n = 2−3). [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 mM (n = 2−3). [d] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as percentage displacement of specific binding at a concentration of 10 mM (n = 3).

Aliphatic amides **4** and **20** showed equal affinities at the different adenosine receptors as did the quinolone **23** and quinazolone **24**. The adenosine A_3 receptor affinities of the tertiary amides **1** and **22** differed by a factor 2 in favor of the isoquinoline. Quinazoline **21** having a bulky, strong electron-withdrawing group showed a decrease in adenosine A_3 receptor affinity compared to quinazoline **20**. Because of the fact that the relatively potent compound **22**, having two phenyl groups, was bulky too, the decrease in affinity could be subscribed to the electronic effect.

Whereas the tertiary amides showed a small difference in adenosine A_3 receptor affinities, the secondary benzamides **6** and **27** showed equal affinities. The largest difference between quinazoline and the "1-deaza compound" isoquinoline was seen in the amidine series. The quinazoline **26** showed no adenosine A_3 receptor affinity (only 4% displacement of the radioligand at a concentration of 10 mM), whereas the corresponding isoquinoline **25** showed a K_i value of 20 mM at the

adenosine A_3 receptor. From these data it can be concluded that in these cases the nitrogen atom at the 1-position of the quinazoline ring or the carbon atom at the 4-position of the isoquinoline ring does not play an important role in the ligand−adenosine A_3 receptor interaction.

In two cases, an interesting difference between isoquinoline and quinazoline in their adenosine A_1 receptor affinities was seen. Compound **1** showed an adenosine A_1 receptor affinity of 4.3 mM, whereas the corresponding quinazoline derivative **22** showed no adenosine A_1 receptor affinity at all. The aromatic benzamides **6** and **27** differed likewise. Isoquinoline derivative **6** showed an adenosine A_1 receptor affinity of 3.2 mM, whereas the corresponding quinazoline derivative **27** showed no adenosine A_1 receptor affinity at all.

This latter finding prompted us to prepare compound **28** which is the quinazoline derivative of the potent adenosine A_3 antagonist **13** in order to increase the A_3/A_1 selectivity even more. However, the adenosine A_3 receptor affinity decreased almost 10-fold by this substitution, whereas the adenosine A_1 receptor affinity slightly increased.

Conclusions

In this study we optimized the affinity of isoquinoline compounds selectively for the adenosine A_3 receptor versus A_1 and A_{2A} receptors by use of an amide bond on position 1 of the isoquinoline ring and by aromatic substitution of the benzamide ring.

These investigations resulted in the potent and selective antagonist VUF 8504 **(13)** for the human adenosine A_3 receptor with an affinity of 17 nM. This compound showed negligible affinity at the rat A_1 or A_{2A} receptor. This potent and selective compound may become a useful tool in a further pharmacological characterization of the human adenosine A_3 receptor and in the investigation of its physiological role. We are currently exploring analogues of VUF8504 as potential adenosine A_3 receptor antagonists.

Experimental Section

Materials. *n*-Butyllithium was purchased from ACROS (Belgium) as a 2.5 M solution in hexane. *p*-Anisic acid, picolinonitrile, 3,4-dimethoxybenzoic acid, ferric nitrate, and 2-methylbenzonitrile were purchased from ACROS (Belgium). THF was predried over CaH_2 and distilled from $LiAlH_4$. All other solvents used were of analytical grade. 1-Amino-3-(2-pyridinyl)isoquinoline was prepared by a modified method of de Zwart et al.,[18] and 4-amino-2-(2-pyridinyl)quinazoline was prepared as described by Linschoten et al.[19] The synthesis and identification of compounds **1**−**3**, **23**, and **25** were described in the previous paper.[1] Compounds **4**−**18**,[18] **20**−**22**,[19] **24**,[19] and **26**−**27**[19] are from our laboratory stock (VU Amsterdam).

Synthesis. ^{1}H NMR 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 combined with a CH correlation. The symbols used are (p) for primary, (s) for secondary, (t) for tertiary, and (q) for quaternary carbon signals. HRMS measurements were performed on a Finnigan MAT 90 mass spectrometer (direct inlet) at an ionization potential of 70 eV. FT-IR spectra were recorded on a Mattson Instruments 6030 Galaxy Series spectrophotometer. Melting points were measured on a Electrothermal IA9200 apparatus and are uncorrected.

1-Amino-3-(2-pyridinyl)isoquinoline. In a flame-dried three-necked flask, equipped with a mechanical stirrer, containing 50 mL condensed ammonia under nitrogen atmosphere, 30 mmol of potassium was added in portions at −40 °C together with some crystals of ferric nitrate as catalyst. The gray suspension was then cooled to −78 °C, and 30 mmol of 2-methylbenzonitrile in 20 mL of anhydrous THF was added slowly with appearance of a dark red color, directly followed by the addition of 30 mmol of 2-cyanopyridine in 20 mL of anhydrous THF. The reaction mixture was stirred overnight at room temperature to evaporate the ammonia and hydrolyzed with 40 mL of 2 M aqueous ammonium chloride solution, creating a buffer of pH ≈ 9. THF was removed under reduced pressure, and 30 mL of diethyl ether was added. The organic layer was separated and dried with Na_2SO_4, and after filtration, ether/HCl was added dropwise under stirring until precipitation ceased. The dark precipitate was isolated by filtration, washed with ether, and solved in ether/0.5 M NaOH. The organic layer was separated, dried with Na_2SO_4, filtrated, and evaporated until dryness under reduced pressure. Sublimation yielded 58% free base as white crystals.

General Procedure for Preparation of Derivatives 19 and 28. A solution of 20 mmol of 1-amino-3-(2-pyridinyl)-isoquinoline or 4-amino-2-(2-pyridinyl)quinazoline in 40 mL of anhydrous THF was cooled to −10 °C in a nitrogen atmosphere, and 12.5 mL of 1.6 M *n*-butyllithium in hexane was added dropwise while stirring. After the mixture was stirred for 10 min at this temperature, a solution of 20 mmol of freshly distilled acyl chloride in 5 mL of anhydrous THF was added, and stirring was continued for 1 h at −10 °C. After warming to room temperature, the mixture was hydrolyzed with water and extracted with chloroform (3 × 50 mL), and the combined organic layers were washed twice with a 5% $NaHCO_3$ solution, dried over sodium sulfate, and evaporated to dryness under reduced pressure. The residue was purified by crystallization from methanol/ethyl acetate.

3,4-Dimethoxy-*N*-[3-(2-pyridinyl)isoquinolin-1-yl]benzamide (19): yield 46%; mp 157 °C; ^{1}H NMR ([D$_6$]DMSO, ref DMSO[D$_5$H] = 2.49 ppm) δ 3.87 (s, 6H, OCH_3), 7.15 (d, $^3J_{5''6''}$ = 8.4 Hz, 1H, H5″), 7.47 (ddd, $^3J_{4'3'}$ = 4.9 Hz, $^3J_{4'5'}$ = 7.5 Hz, $^4J_{4'6'}$ = 1.2 Hz, 1H, pyr-H4′), 7.64−7.77 (m, 4H, H5, H6, H7, and H2″), 7.82−7.98 (m, 2H, pyr-H3′ and H6″), 8.20 (ddd, $^3J_{5'6'}$ = 8.0 Hz, $^3J_{5'4'}$ unsolved, $^4J_{5'3'}$ = 1.7 Hz, 1H, pyr-H5′), 8.44 (d, $^3J_{87}$ = 8.1 Hz, 1H, H8), 8.74 (ddd, $^3J_{6'5'}$ = 8.0 Hz, $^4J_{6'4'}$ = 1.2 Hz, $^5J_{6'3'}$ unsolved, 1H, pyr-H6′), 8.83 (s, 1H, H4), 10.96 (s, 0.8H, NH) and 15.15 (bs, 0.2H, OH). Anal. ($C_{23}H_{19}N_3O_3$) C, H, N.

4-Methoxy-*N*-[2-(2-pyridinyl)quinazolin-4-yl]benzamide (28): yield 52%; mp 147−148 °C; HRMS (EI) *m*/*z* 356.1270 (M$^+$), 356.1273 ($C_{21}H_{16}N_4O_2$); ^{1}H NMR ([D$_6$]DMSO, ref DMSO[D$_5$H] = 2.49 ppm) δ 3.87 (s, 3H, CH_3), 7.07 (d, $^3J_{AB}$ = 8.9 Hz, 2H, AA′BB′ ArH), 7.70−7.77 (m, $^3J_{4'5'}$ = 7.5 Hz, 2H, H8, and pyr-H4′), 7.90−8.18 (m, 3H, H6, H7, and pyr-H5′), 8.39 (d, $^3J_{BA}$ = 8.9 Hz, 2H, AA′BB′ ArH), 8.58 (dd, $^3J_{56}$ = 8.1 Hz, $^4J_{57}$ = unsolved, 1H, H5), 8.78 (dd, $^3J_{6'5'}$ = 8.2 Hz, $^4J_{6'4'}$ = 1.4 Hz, 1H, pyr-H6′), 8.89 (ddd, $^3J_{3'4'}$ = 4.2 Hz, $^4J_{3'5'}$ unresolved, $^5J_{3'6'}$ unresolved, 1H, pyr-H3′), and 15.53 (bs, 1H, OH). Anal. ($C_{21}H_{16}N_4O_2$) C, H, N.

Pharmacology. Radioligand Binding Studies. Adenosine A_1 receptor affinities were determined on rat cerebral cortex membranes with [^{3}H]DPCPX as radioligand as described previously.[20] Adenosine A_{2A} receptor affinities were determined on rat striatal membranes with [^{3}H]CGS 21680 as radioligand according to a protocol published previously.[21]

Binding of [^{125}I]AB-MECA to human A_3 receptors in stably transfected HEK 293 cell membranes was performed as described.[22,23]

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Abbreviations

AcOH	acetic acid
Ac_2O	acetic anhydride
APT	attached proton test
n-BuLi	*n*-butyllithium
CI	chemical ionization
COSY	correlated spectroscopy
DEPT	distortionless enhancement by polarization transfer
DMSO	dimethylsulfoxide
Et_2O	diethyl ether
FT-IR	Fourier transformation infrared
[3H]DPCPX	[3H]-1,3-dipropyl-8-cyclopentyl-xanthine
[^{3}H]CGS 21680	[^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-(ethylcarbamoyl)adenosine
HEK cells	human embryonic kidney cells
HMPT	hexamethylphosphoric triamide
[^{125}I]AB-MECA	[^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine
K_i	equilibrium inhibition constant
THF	tetrahydrofuran
TLC	thin layer chromatography

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