A Novel Class of Adenosine A3 Receptor Ligands. 1. 3-(2-Pyridinyl)isoquinoline Derivatives

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A series of 3-(2-pyridinyl)isoquinoline derivatives was synthesized as potential antagonists for the human adenosine A3 receptor by substitution of the 1-position. The compounds were obtained by various synthetic routes from 1-amino-3-(2-pyridinyl)isoquinoline. The affinity was determined in radioligand binding assays for rat brain A1 and A2A receptors and for the cloned human A3 receptor. A structure−activity relationship analysis indicated that a phenyl group when coupled by a spacer allowing conjugation on position 1 of the isoquinoline ring increased the adenosine A3 receptor affinity. In contrast, such a phenyl group directly bound to position 1 of the isoquinoline ring decreased affinity. Since the combination of a phenyl group together with a spacer raised adenosine A3 receptor affinity, various spacers were investigated. VUF8501 (*N*-[3-(2-pyridinyl)isoquinolin-1-yl]benzamidine (**15**) showed an affinity at the human adenosine A3 receptor of 740 nM. Substituent effects on the phenyl group were investigated by in vitro evaluation of a series of substituted benzamidines. Electron-donating groups at the para position of the benzamidine ring increased adenosine A3 receptor affinity. These investigations led to VUF8505 (4-methoxy-*N*-[3-(2-pyridinyl)isoquinolin-1-yl]benzamidine **(22)**), which is a moderately potent and selective ligand for the human adenosine A3 receptor with an affinity of 310 nM in our test system having negligible affinity for rat A1 and A2A receptors.

Introduction

All actions known of extracellular adenosine are mediated by G protein-coupled receptors. These receptors have been initially classified into A1 and A2 subtypes based on pharmacological criteria and their coupling to adenylate cyclase. The A2 receptor has been subdivided into A2A and A2B subtypes, based on cloning and functional characterization.[1] In 1991 a new a-denosine receptor-like sequence has been identified from a rat testis cDNA library.[2] This sequence was shown to correspond to a new subtype of the adenosine receptor family, the A3 receptor.[3]

The adenosine A3 receptor has now also been cloned from and characterized pharmacologically in several other species: human,[4,5] sheep,[6] mouse,[7] and recently, rabbit.[8] Considerable species differences have been found for the A3 receptors.[9,10] The rat receptor in particular behaves anomalously in ligand binding. Moreover, a low degree of homology in its DNA sequence with those of other species has been found. For these reasons the question has been raised as to whether A3 subtypes exist. This question has not yet been answered.

Stimulation of the adenosine A3 receptor promotes the release of inflammatory mediators from mast cells, like histamine,[10,11] mediating processes of inflammation[9] and hypotension.[10] Stimulation of the adenosine A3 receptor has also been suggested to play a role in immunosuppression[12] and brain ischemia.[13,14] In in vivo studies it was shown that an adenosine A3 receptor agonist induces bronchospasm.[15] Selective A3 receptor antagonists are thought to be antiasthmatic and/or antiinflammatory agents.[9,16] For these reasons the adenosine A3 receptor became a target in drug research.[17]

The classical antagonists known for the adenosine A1 and A2A receptors are xanthine derivatives, but at adenosine A3 receptors, these compounds have shown rather low affinities and optimization has not led to truly selective ligands.[18] Therefore, we started a search for a new lead structure for the development of potent and selective adenosine A3 receptor antagonists. Recently, non-xanthine structures such as 1,4-dihydropyridines,[19] triazoloquinazolines,[20] and the triazolonaphtpyridine L-249313[21] have been shown to possess moderate to high affinity at the adenosine A3 receptor with appreciable selectivity.

In a screening program of compounds from our laboratory stock, we found a series of 3-(2-pyridinyl)-isoquinoline derivatives showing adenosine A3 receptor affinities. In the past, we have determined the copper-dependent antimycoplasmal activity of such isoquinoline derivatives.[22−26] In the present study we investigated the structure−activity relationship of a series of 3-(2-
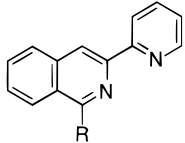
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Table 1. Affinities at the Human Adenosine A_3 Receptor of Reference Compounds

compound	present study hA_3 K_i (nM)[a]	literature hA_3 K_i (nM)	ref
XAC	108 ± 3.0	71^b	5
DPCPX	1700 ± 160	759^b	5
		200 ± 126^c	36
L-249313	172 ± 9.1	13	21
CGS15943	143 ± 19	13.8 ± 2.4^d	20
		7.9 ± 4.6^c	36

[a] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as $K_i \pm$ SEM in nM ($n = 3–5$). [b] Displacement of specific [^{125}I]ABA binding at human adenosine A_3 receptors expressed in CHO cells, expressed as pK_i. [c] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in CHO cells, expressed as $pK_i \pm$ SEM ($n = 3$). [d] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as $K_i \pm$ SEM in nM ($n = 3$).

Table 2. Affinities at the Adenosine Receptors of 3-(2-Pyridinyl)Isoquinoline Derivatives

| compound	R	$A_3{}^a$	$A_1{}^b$	$A_{2A}{}^c$
(1)	$-NH_2$	17%	19%	28
(2)	$-OH^d$	27 ± 10	21%	35
(3)	$-Cl$	2.6 ± 0.76	43%	29
(4)	$-CH_2CH_3$	5.1 ± 0.83	30%	25
(5)	$-OCH_3$	5.6 ± 1.1	3.3 ± 1.2	19
(6)	$-SCH_3$	3.9 ± 0.70	12 ± 3	7
(7)	$-SO_2CH_3$	15 ± 2.3	1.8 ± 0.4	54
(8)	$-Ph$	12 ± 8.6	1.9 ± 0.44	31
(9)	$-N=CHNH_2$	20 ± 6.6	47%	42
(10)	$-N(COCH_3)_2$	35 ± 21	34%	18
(11)	$-N(COPh)_2$	0.23 ± 0.091	4.3 ± 2.8	27
(12)	$-N((CO)2,6\text{-}diCH_3Ph)_2$	3.2 ± 1.3	0%	0
(13)	$-CH_2COPh$	0.66 ± 0.23	0.24 ± 0.1	22
(14)	$-CH(CH_3)COPh$	0.37 ± 0.19	0.70 ± 0.2	5
VUF8501 (15)	$-N=C(NH_2)Ph$	0.74 ± 0.15	34%	4

[a] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A_3 receptors expressed in HEK 293 cells, expressed as $K_i \pm$ SEM in mM ($n = 3–5$) or percentage displacement of specific binding at a concentration of 10 mM ($n = 2–3$). [b] Displacement of specific [^{3}H]DPCPX binding in rat cortical membranes, expressed as $K_i \pm$ SEM in mM ($n = 3$) or percentage displacement of specific binding at a concentration of 10 mM. [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] Isocarbostyryl exists predominantly in the keto tautomeric form.

pyridinyl)isoquinoline derivatives on the human adenosine A_3 receptor.

Chemistry

The 1-substituted 3-(2-pyridinyl)isoquinoline derivatives (Table 2) were all prepared from 1-amino-3-(2-pyridinyl)isoquinoline (**1**) via replacement of, or coupling to, the amino group. Amine **1** was obtained according to the procedure as described by De Zwart et al.[27] by condensation of 2-methylbenzenecarbonitrile with picolinonitrile at −78 °C in Et$_2$O/THF under strongly alkaline conditions, i.e., potassium amide prepared in situ (Scheme 1).

For the synthesis of derivatives **6** and **7**, amine **1** was converted into 1-chloro-3-(2-pyridinyl)isoquinoline (**3**) by

Scheme 1
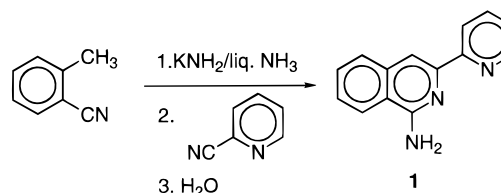
treatment with sodium nitrite in acetic acid, resulting in 3-(2-pyridinyl)-2H-1-isoquinolone (**2**), followed by treatment with POCl$_3$ as described by Pijper et al.[28] The chloroisoquinoline **3** was then treated with methanethiol, yielding the methylthioether **6**. Oxidation of the sulfide by permanganate yielded the methylsulfonyl derivative **7**. Both preparations are represented in Scheme 2.

Formamidine **9** was synthesized according to the method described by Linschoten et al. for N-[2-(2-pyridinyl)quinazoline-4-yl]formamidine.[29] In the first step, amine **1** was converted into a formimidate by refluxing in triethyl orthoformate in the presence of an acid.[30] This acidic environment is required, because otherwise the corresponding N,N'-disubstituted formamidine is formed. When treated with ammonia/chloroform the imidate yielded the desired formamidine **9** (Scheme 2).

Various N-acyl derivatives **10**−**12** were synthesized from **1** using mixed anhydrides prepared in situ (Scheme 2). For the preparation of the 1-phenacylisoquinolines **13** and **14**, a good leaving group at position 1 of the isoquinoline system was needed, and therefore, the amino group of **1** was replaced by chlorine (vide supra). The resulting chloroisoquinoline **3** was added to a solution of the anion of the corresponding acetophenone prepared in situ in an aprotic solvent such as DMSO or HMPT. This type of reaction has been described by Coudert et al.[31] for the formation of 1-(2-oxoalkyl)-quinolines from 2-chloroquinoline. These authors showed that yields could be improved by using a polar aprotic solvent. This route leading to the 1-phenacylisoquinolines **13** and **14**, is depicted in Scheme 2.

Results and Discussion

We used 1-substituted 3-(2-pyridinyl)isoquinolines as a lead structure in order to find antagonists for the human adenosine A_3 receptor (Figure 1). 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 and [^{3}H]CGS 21680 as radioligands, respectively.[32,33] 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 (Tables 1, 2, and 3).[34,35]

We first checked our binding assay by testing the reference ligands XAC, DPCPX, L-249313, and CGS 15943 (Table 1). We compared the results with the data from literature[5,20,21,36] and found substantial differences, in particular for L-249313 and CGS 15943. A trivial explanation is that experimental conditions in the various studies are quite different, for example, cell type or radioligand used and levels of receptor expression. Also the limited solubility of the compounds may be a

Scheme 2[a]
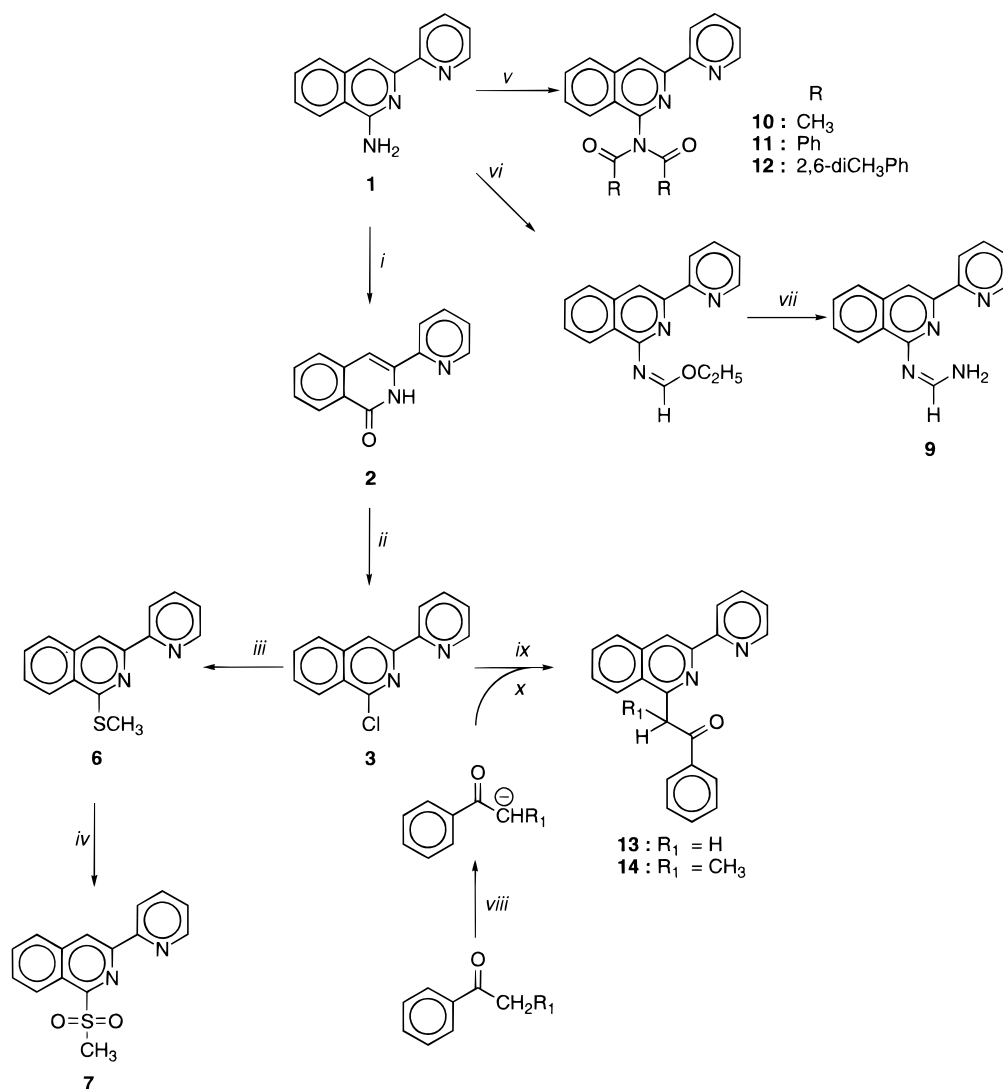
a (i) NaNO₂/HOAc; (ii) POCl₃; (iii) NaSCH₃; (iv) KMnO₄/HOAc; (v) RCOOH/Ac₂O; (vi) CH(OC₂H₅)₃/H⁺; (vii) NH₃; (viii) NaNH₂; (ix) HMPT or DMSO; (x) H₂O/H⁺.
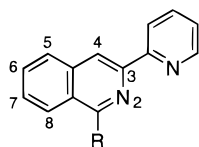

Figure 1. Lead structure: 1-substituted 3-(2-pyridinyl)isoquinoline.

complicating factor. The results however, may serve as a warning not to compare binding data from different laboratories in an absolute way.

Various structural modifications were made on position 1 of the isoquinoline moiety to investigate the effects on binding at the A₃ receptor (Table 2). The affinities of the 1-substituted 3-(2-pyridinyl)isoquinolines of Table 2 range from negligible, i.e., 17% displacement at 10 mM (**1**), to K_i values in the submicromolar range.

The 1-amino derivative (**1**), which was the starting point in the synthesis of derivatives, had a very low affinity at all three adenosine receptors.

3-(2-Pyridinyl)-2*H*-1-isoquinolone (**2**) showed low adenosine A₃ receptor affinity. This could be due to the

existence of an isocarbostyryl tautomeric form (see also Scheme 2). This form of **2** was preferred according to the resonance signal at 10.32 ppm of the NH proton in the ¹H NMR (in CDCl₃) spectrum and to the UV spectrum, published by Ewing and Steck.[37]

Compound **1** and 1-chloro-3-(2-pyridinyl)isoquinoline (**3**) showed a large difference in adenosine A₃ receptor affinity. Whereas the 1-substituent of **1** had a strong electron-donating resonance effect through p orbitals and a small electron-withdrawing inductive effect through s orbitals, **3** contained an electron-withdrawing chloro substituent on the 1-position. The 10-fold increase in adenosine A₃ receptor affinity of compound **3** compared to that of compound **1** suggested that the effect of the 1-substituent of the isoquinoline ring on the adenosine A₃ receptor affinity is either lipophilic or electronic in nature. The electron-withdrawing chloro and the electron-donating ethyl substituent, as in **3** and **4** respectively, yielded an adenosine A₃ receptor affinity in the micromolar range. This increased adenosine A₃ affinity compared to **1** could therefore not be ascribed to an electronic effect of the 1-substituent, but probably to its lipophilic property. Both compounds (**3** and **4**)

Table 3. Affinities at the Adenosine Receptors of Substituted Benzamidines Derivatives
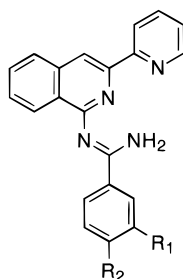
compound		R₁	R₂	A₃[a]	A₁[b]	A₂ₐ[c]
VUF8501	(**15**)	−H	−H	0.74 ± 0.15	34	4
	(**16**)	−Cl	−H	7.8 ± 5.0	49	14
	(**17**)	−CH₃	−H	1.3 ± 0.38	43	0
	(**18**)	−OCH₃	−H	0.98 ± 0.32	43	0
	(**19**)	−Cl	−Cl	2.3 ± 0.54	21	2
	(**20**)	−H	−Cl	2.3 ± 0.18	20	7
	(**21**)	−H	−CH₃	0.58 ± 0.082	12	34
VUF8505	(**22**)	−H	−OCH₃	0.31 ± 0.099	18	5

[a] Displacement of specific [^{125}I]AB-MECA binding at human adenosine A₃ receptors expressed in HEK 293 cells, expressed as $K_i \pm$ 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 ($n = 2-3$).

showed selectivity for the adenosine A₃ receptor, suggesting that these 1-substituted isoquinolines formed a good starting point for the development of potent and selective A₃ ligands. For this reason, more modifications were made, yielding 1-methoxy-, 1-methylthio-, and 1-methylsulfonyl-3-(2-pyridinyl)isoquinolines (**5**−**7**). These isoquinolines also showed adenosine A₃ receptor affinity in the micromolar range, but were not A₃ selective. They were equally or more potent on the rat A₁ receptor than on the human A₃ receptor.

Replacing the alkyl substituent of **4** with a phenyl ring, as in **8**, did not influence the affinity at the human adenosine A₃ receptor significantly but did show a loss in A₃/A₁ selectivity. Unsubstituted amidine **9** with delocalized electrons showed an affinity at the adenosine A₃ receptor of 20 mM which was also in the same range as **8**. The bulky tertiary amide **10** had a K_i value of 35 mM at the adenosine A₃ receptor and no affinity at adenosine A₁ and A₂ₐ receptors. It still showed minor affinity, indicating enough space in the receptor pocket to hold a tertiary amide.

Compound **11** showed a 100-fold increased affinity at the adenosine A₃ receptor, compared to compound **10**. The large difference in affinity, afforded by replacement of the methyl by a phenyl group, could possibly be ascribed to two effects. First, one benzamide group was nearly coplanar with the isoquinoline ring. This large conjugating moiety could be necessary for human adenosine A₃ receptor affinity. This will also be discussed later. Second, p−p stacking could play a role in this part of receptor−ligand interaction.

The role of a spacer between a phenyl group and position 1 of the isoquinoline ring was investigated in compounds **11**−**14**. Compound **11** showed an affinity at the adenosine A₁ receptor of 4.3 mM, but was still more than 10-fold A₃ selective. Compound **12** contained

two bulky disubstituted benzoyl groups. The adenosine A₃ receptor affinity was decreased 10-fold compared to that of **11** by the methyl substituents on the phenyl rings. There was no displacement of the radioligands at the adenosine A₁ and A₂ₐ receptors at a concentration of 10 mM ligand. Preliminary results pointed out that N1-(4-chlorobenzoyl)-N1-[3-(2-pyridyl)-1-isoquinolyl]4-chlorobenzamide showed affinities at the adenosine receptors comparable to **11**. From these three dibenzoyl derivatives it was concluded that the receptor pocket is large enough to contain these bulky tertiary amides and that aromatic substitution affects adenosine receptor affinities. This latter effect was investigated with the aid of compounds **15**−**22**. Coplanarity and conjugation play an important role in compound **12**. The two methyl groups on the ortho positions of the benzoyl might force the phenyl ring out of the amide−isoquinoline plane.

Compounds **13** and **14** both showed high affinities at the adenosine A₃ receptor, indicating that a ketone function was also a good spacer and that monosubstitution of the amine was allowed. In a subsequent study we focus on a monosubstituted carboxamide group as spacer. Compounds **13** and **14** met the above suggested condition for human adenosine A₃ receptor affinity of conjugation and coplanarity because of the existence of their tautomeric iminol forms.

From the results shown in Table 2, we decided to continue with 1-substituted 2-pyridinylisoquinolines containing a second aromatic ring via a spacer allowing conjugation between the isoquinoline ring and the second aromatic ring. To investigate substituent effects on the phenyl ring unambiguously, we preferred secondary amines. We chose an amidine bridge rather than a ketone spacer for synthetic reasons only.[38] These choices resulted in an amidine substituent at position 1 of the isoquinoline ring as a spacer to the phenyl ring, i.e., N-[3-(2-pyridinyl)isoquinolin-1-yl)benzamidines.

The in vitro results of the substituted N-[3-(2-pyridinyl)isoquinolin-1-yl)benzamidines are shown in Table 3. The meta substituted analogues **16**, **17**, and **18** showed equal or slightly diminished adenosine A₃ receptor affinity in comparison to the unsubstituted benzamidine **15**. Substitution with an electron-withdrawing substituent as in **16** resulted in a 10-fold decrease in potency. Electron-donating substituents had less influence. The 3,4-dichloro derivative **19** had higher affinity at the adenosine A₃ receptor compared to the m-chloro derivative **16**. Chloro substitution on the para position only (compound **20**) showed an affinity equal to **19**.

Electron-donating groups on the para position (compounds **21** and **22**) were more conducive to affinity at the adenosine A₃ receptor than those of the unsubstituted analogue **15**. The 4-methyl analogue **21** showed slightly higher affinity than the unsubstituted benzamidine **15**, whereas 4-methoxy substitution (compound **22**) afforded the adenosine A₃ receptor ligand with the highest affinity of this series. 4-Methoxy-N-(3-(2-pyridinyl)isoquinolin-1-yl)benzamidine (**22**, VUF8505) displayed a K_i value of 310 nM and showed marked selectivity versus the rat A₁ and A₂ₐ receptors, since radioligands for these two receptors were hardly displaced at a concentration up to 10 mM of **22**.

Conclusions

This study showed that 1-substituted 3-(2-pyridinyl)-isoquinoline derivatives provide new lead structures for selective and potent adenosine A₃ ligands. Although the adenosine A₃ receptor binding assay cannot be used to discriminate between agonists and antagonists, the absence of a ribose group in the compounds in the present study suggests these isoquinolines to behave as antagonists.

A phenyl group, coupled by a spacer to position 1 of the isoquinoline seemed pivotal for substantial adenosine A₃ receptor affinity. An enolizable ketone, diacyl group, and amidine, all three with conjugating properties (diacyl group by its resonance forms), can be used as spacers.

The investigations in the present study led to VUF8505 (**22**), a 4-methoxybenzamidine derivative. This compound is a moderately potent human adenosine A₃ receptor antagonist with a K_i value of 310 nM in our test system. It showed marked selectivity versus the rat A₁ and A₂A receptors.

This selective adenosine A₃ receptor antagonist could be a useful tool in characterizing the human adenosine A₃ receptor and its physiological role.

Experimental Section

Materials. Acetophenone, 4-chlorobenzoic acid, benzoic acid, 2,6-dimethylbenzoic acid, hexamethyl phosphorus triamide, propiophenone, and triethylorthoformate were purchased from ACROS (Belgium). Methanethiol, sodium amide, and sulfur dioxide (lecture bottle) were commercially available from Aldrich (The Netherlands). THF was distilled from LiAlH₄. DMSO was distilled over CaH₂ and stored under linde type 4 Å molecular sieves. All other solvents used were of analytical grade. 1-Amino-3-(2-pyridinyl)isoquinoline (**1**) was prepared as described by de Zwart et al.[27] 3-(2-Pyridinyl)-1-isocarbostyril (**2**), 1-chloro-3-(2-pyridinyl)isoquinoline (**3**), and 1-phenyl-3-(2-pyridinyl)isoquinoline (**8**) were prepared from **1** according to Pijper et al.[28] Compounds **4**,[28] **5**,[28] and **15−22**[38] were from laboratory stock (VU Amsterdam).

Synthesis. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker AC 200 (¹H NMR, 200 MHz; ¹³C NMR, 50.29 MHz) spectrometer with tetramethylsilane or sodium 3-(trimethyl-silyl)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. Melting points were measured on a Electrothermal IA9200 apparatus and are uncorrected. Column chromatography was performed with silica gel 60H, <230 mesh, purchased at Merck. Thin layer chromatography was used a few times to follow the reaction (Merck silicagel 60; F_{254}, 0.25 mm).

1-(Methylsulfonyl)-3-(2-pyridinyl)isoquinoline (6). Sodium (2.3 g, 100 mmol) was dissolved in methanol (75 mL), and condensed methanethiol (15 g, 300 mmol) was added at 0 °C and stirred for 1 h at this temperature. The mixture was heated to 70 °C, and 11.93 g (50 mmol) of **3** in 100 mL of THF was added and heated to reflux for 14 h. The reaction mixture was poured into water, and the yellow-white precipitate was filtered off and crystallized from Et₂O yielding 11.9 g white needles (94%): mp 128−129 °C; ¹H NMR (CDCl₃, ref CHCl₃ = 7.27 ppm) δ 2.79 (s, 3H, SCH_3), 7.26 (ddd, $^3J_{4'3'}$ = 4.8 Hz, $^3J_{4'5'}$ = 7.5 Hz, $^4J_{4'6'}$ = 1.2 Hz, 1H, pyr-H4′), 7.49−7.68 (m, 2H, H7 and H6), 7.79 (dd, $^3J_{56}$ = 8.0 Hz, $^4J_{54}$ = 1.8 Hz, 1H, H5),

7.40−7.90 (ddd, $^3J_{5'6'}$ unresolved, $^3J_{5'4'}$ unresolved, $^4J_{5'3'}$ unresolved, 1H, pyr-H5′), 8.17 (d, $^3J_{87}$ = 8.2 Hz, H8), 8.48 (s, 1H, H4), 8.56 (ddd, $^3J_{6'5'}$ = 7.99 Hz, $^4J_{6'4'}$ unresolved, $^5J_{6'3'}$ = 0.9 Hz, 1H, pyr-H6′), and 8.69 (ddd, $^3J_{3'4'}$ = 4.8 Hz, $^4J_{3'5''}$ = 1.7 Hz, $^5J_{3'6'}$ = 0.8 Hz, 1H, pyr-H3′); ¹³C NMR (CDCl₃, ref CDCl₃ = 77.0 ppm) δ 13.0 (s, 1C, SCH₃), 113.9 (s, 1C, C4), 121.0 (s, 1C, pyr-C6′), 123.1 (s, 1C, pyr-C4′), 124.2 (s, 1C, C8), 126.91 (s, 1C, C9), 127.2 (s, 1C, C7), 128.2 (s, 1C, C5), 130.3 (s, 1C, C6), 135.9 (s, 1C, C10), 136.8 (s, 1C, pyr-C5′), 148.0 (s, 1C, C1), 149.0 (s, 1C, pyr-C3′), 156.2 (s, 1C, C3), and 159.3 (s, 1C, pyr-C1′). Anal. (C₁₅H₁₂N₂S) C, H, N.

1-(Methylsulfonyl)-3-(2-pyridinyl)isoquinoline (7). To a solution of 7.56 g (30 mmol) of **6** in 175 mL of 6 N acetic acid was added dropwise 9.48 g (60 mmol) of KMnO₄ in 250 mL of H₂O. After cooling to room temperature, the reaction mixture was bubbled with SO₂ to colorlessness. After filtration, the filtrate was neutralized with NaHCO₃, extracted with chloroform, and evaporated to dryness yielding a pink solid which was crystallized from toluene: yield 3.55 g (42%) white needles: mp 133 °C; ¹H NMR (CDCl₃, ref CHCl₃ = 7.27 ppm) δ 3.64 (s, 3H, SO₂CH_3), 7.33 (ddd, $^3J_{4'3'}$ = 4.81 Hz, $^3J_{4'5'}$ = 7.5 Hz, $^4J_{4'6'}$ = 1.1 Hz, 1H, pyr-H4′), 7.68−7.89 (m, 3H, H7, H5 and H6), 8.05 (ddd, $^3J_{5'6'}$ = 7.1 Hz, $^3J_{5'4'}$ unresolved, $^4J_{5'3'}$ = 1.7 Hz, 1H, pyr-H5′), 8.35 (d, $^3J_{87}$ = 8.0 Hz, H8), 8.71 (ddd, $^3J_{3'4'}$ = 4.0 Hz, $^4J_{3'5''}$ unresolved, $^5J_{3'6'}$ = 0.8 Hz, 1H, pyr-H3′), 8.92 (ddd, $^3J_{6'5'}$ = 7.2 Hz, $^4J_{6'4'}$ unresolved, $^5J_{6'3'}$ = 0.9 Hz, 1H, pyr-H6′), and 8.98 (s, 1H, H4); ¹³C NMR (CDCl₃, ref CDCl₃ = 77.0 ppm) δ 40.3 (s, 1C, SO₂CH₃), 120.9 (s, 1C, C4), 120.9 (s, 1C, pyr-C6′), 121.9 (s, 1C, pyr-C4′), 122.9 (s, 1C, C8), 128.5 (s, 1C, C7 or C9), 128.6 (s, 1C, C7 or C9), 129.5 (s, 1C, C5), 131.5 (s, 1C, C6), 137.2 (s, 1C, C10), 138.5 (s, 1C, pyr-C5′), 148.0 (s, 1C, C1), 149.3 (s, 1C, pyr-C3′), 154.3 (s, 1C, C3), and 157.2 (s, 1C, pyr-C1′); IR (KBr cm⁻¹) 1330, 1140 (RSO₂R′). Anal. (C₁₅H₁₂N₂SO₂) C, H, N.

N1-[3-(2-Pyridinyl)-1-isoquinolyl]iminoformamide (9). To a stirred solution of 50 mL of triethyl orthoformate and 0.25 mL of sulfuric acid was added 200 mmol of **1**. This suspension was heated to reflux for 3 h, cooled to room temperature, and subsequently neutralized with potassium carbonate.

The mixture was concentrated under reduced pressure, 250 mL of chloroform saturated with ammonia was added, and the inorganic material was filtered off. Anhydrous ammonia was bubbled through the solution for 30 min, the mixture was left standing overnight, and the excess ammonia could evaporate. The precipitate was filtered off, boiled in chloroform for 3 h and filtered off hot, yielding 60% of **9** as white needles: mp 37 °C; HRMS (CI, NH₃) m/z 248.2990 (M⁺), 248.2992 (C₁₅H₁₂N₄); ¹H NMR ([D₆]DMSO, ref DMSO[D₅H] = 2.49 ppm) δ 7.36−7.42 (ddd, $^3J_{4'3'}$ unresolved, $^3J_{4'5'}$ unresolved, $^4J_{4'6'}$ unresolved, 1H, pyr-H4′), 7.43−7.72 (m, 3H, form-H, H6 and H7), 7.80 (bs, 1H, NH), 7.89−7.97 (m, 3H, H5, H8 and pyr-H5′), 8.39 (s, 1H, H4), 8.47 (d, $^3J_{6'5'}$ = 8.2 Hz, 1H, pyr-H6′), 8.67 (d, $^3J_{3'4'}$ = 4.4 Hz, 1H, pyr-H3′), and 8.75 (bs, 1H, NH). Anal. (C₁₅H₁₂N₄) C, H, N.

General Procedure for Preparation of Derivatives 10−12. A solution of **1** (8.84 g, 0.04 mol), 50 mL of RCOOH, and 40 mL of acetic anhydride was heated to reflux for 1 h. After cooling to room temperature, the mixture was poured onto ice, and the precipitate was filtered by suction, dissolved in chloroform, washed twice with 5% NaHCO₃ and water, dried over potassium carbonate, and evaporated to dryness under reduced pressure. The residue was purified by crystallization from ethyl acetate.

N1-Acetyl-N1-[3-(2-pyridinyl)-1-isoquinolyl]acetamide (10): yield 66%; mp 152 °C; HRMS (CI, NH₃) m/z 305.3508 (M⁺), 305.3510 (C₁₈H₁₅N₃O₂); ¹H NMR (CDCl₃, ref CHCl₃ = 7.24 ppm) δ 2.32 (s, 6H, COCH_3), 7.28 (ddd, $^3J_{4'3'}$ = 4.8 Hz, $^3J_{4'5'}$ = 7.5 Hz, $^4J_{4'6'}$ = 1.2 Hz, 1H, pyr-H4′), 7.75−7.90 (m, 4H, H7, H6, H5 and pyr-H5′), 7.99 (d, $^3J_{87}$ = 8.1 Hz, 1H, H8), 8.40−8.44 (ddd, $^3J_{6'5'}$ unresolved, $^4J_{6'4'}$ unresolved, $^5J_{6'3'}$ unresolved, 1H, pyr-H6′) 8.69 (ddd, $^3J_{3'4'}$ = 4.8 Hz, $^4J_{3'5''}$ = 1.8 Hz, $^5J_{3'6'}$ = 0.9 Hz, 1H, pyr-H3′), and 8.86 (s, 1H, H4); ¹³C NMR (CDCl₃, ref CDCl₃ = 77.0 ppm) δ 26.5 (s, 2C, COCH₃), 119.2

(s, 1C, C4), 121.4 (s, 1C, pyr-C6′), 123.5 (s, 1C, pyr-C4′), 123.6 (s, 1C, C8), 125.6 (s, 1C, C9), 128.4 (s, 1C, C7), 129.1 (s, 1C, C5), 130.9 (s, 1C, C6), 137.0 (s, 1C, pyr-C5′), 138.8 (s, 1C, C10), 148.9 (s, 1C, C1), 149.2 (s, 1C, pyr-C3′), 151.1 (s, 1C, C3), 155.1 (s, 1C, pyr-C1′), and 172.7 (s, 2C, $COCH_3$). Anal. ($C_{18}H_{15}N_3O_2$·$0.3H_2O$) C, H, N.

N1-(Benzoyl)-N1-[3-(2-pyridinyl)-1-isoquinolyl]benzamide (11): yield 38%; mp 207−208 °C; HRMS (CI, NH_3) m/z 429.4974 (M^+), 429.4978 ($C_{28}H_{19}N_3O_2$); ^{1}H NMR ([D_6]DMSO, ref DMSO[D_5H] = 2.49 ppm) δ 7.57−8.11 (m, 10H, Ar−H), 8.13−8.22 (m, 3H, H7, H6, and pyr-H4′), 8.25−8.33 (m, 2H, H5 and pyr-H5′), 8.56 (d, $^3J_{87}$ = 7.9 Hz, 1H, H8), 8.70−8.75 (m, 1H, pyr-H3′), 8.77 (d, $^3J_{6'5'}$ = 8.7 Hz, 1H, pyr-H6′), and 8.91 (s, 1H, H4). Anal. ($C_{28}H_{19}N_3O_2$·$0.07CH_3CO_2C_2H_5$) C, H, N.

N1-(2,6-Dimethylbenzoyl)-2,6-dimethyl-N1-[3-(2-pyridinyl)-1-isoquinolyl]benzamide (12): yield 68%; mp 258 °C; ^{1}H NMR ($CDCl_3$, ref $CHCl_3$ = 7.24 ppm) δ 2.56 (bs, 12H, CH_3), 6.68−7.02 (m, 2H, phenyl-H3″ and phenyl-H4″), 7.29−7.35 (ddd, $^3J_{4'3'}$ unresolved, $^3J_{4'5'}$ unresolved, $^4J_{4'6'}$ unresolved, 1H, pyr-H4′) 7.64−7.72 (m, 2H, H7 and H6), 7.83−7.91 (m, 2H, H5 and pyr-H5′), 8.20−8.25 (ddd, $^3J_{3'4'}$ unresolved, $^4J_{3'5''}$ unresolved, $^5J_{3'6'}$unresolved, 1H, pyr-H3′), 8.33 (d, $^3J_{87}$ = 8.0 Hz, H8), and 8.67−8.69 (m, 2H, pyr-H6′ and H4); ^{13}C NMR ($CDCl_3$, ref $CDCl_3$ = 77.0 ppm) δ 20.2 (s, 4C, phenyl-CH_3), 119.3 (s, 1C, C4), 120.9 (s, 1C, pyr-C6′), 123.5 (s, 1C, pyr-C4′), 125.0 (s, 1C, C8), 126.9 (s, 1C, C9), 127.0 (s, 4C, phenyl-C3″), 128.1 (s, 1C, C7), 128.2 (s, 1C, C5), 128.5 (s, 2H, phenyl-C4″), 130.6 (s, 1C, C6), 132.4 (s, 1C, C10), 137.3 (s, 1C, pyr-C5′), 138.3 (s, 4C, phenyl-C2″), 148.7 (s, 1C, pyr-C3′), 149.1 (s, 1C, C1), 154.3 (s, 1C, C3), 154.6 (s, 2C, phenyl-C1″), 159.4 (s, 1C, pyr-C1′), and 180.1 (s, 2C, NCO-phenyl). Anal. ($C_{32}H_{27}N_3O_2$) C, H, N.

1-Phenyl-2-[3-(2-pyridinyl)-1-isoquinolyl]-1-ethanone (13). A suspension of 50% sodium amide in toluene (3.0 g) was stirred with 20 mL of anhydrous THF, and acetophenone (38 mmol) in 20 mL of anhydrous THF was added dropwise. The mixture was kept at 45 °C for 2 h and 30 mL of HMPT was added, after which 1-chloro-3-(2-pyridinyl)-isoquinoline (3) (15 mmol) in 20 mL of anhydrous THF was added dropwise. The mixture was stirred for 1 h, and subsequently the reaction mixture was poured into ice/water and acidified with concentrated HCl. After the mixture was washed with diethyl ether three times, the remaining water layer was neutralized with sodium bicarbonate to pH = 8 and subsequently extracted with chloroform. The combined organic layers were dried over potassium carbonate, and after evaporation to dryness, the residue was purified by colum chromatography (diethyl ether/hexane 6:4 (v/v)). The product was crystallized from absolute ethanol: yield 1.5 g (31%); mp 148 °C; HRMS (CI, NH_3) m/z 324.1250 (M^+), 324.1263 ($C_{22}H_{16}N_2O$); ^{1}H NMR ($CDCl_3$, ref $CHCl_3$ = 7.27 ppm) δ 6.76 (s, 1H, CH), 7.26−8.34 (m, 13 H, Ar−H), 8.90 (d, $^3J_{5'6'}$ = 5.0 Hz, pyr-H-6′), and 16.70 (s, 1H, OH); IR (KBr cm^{-1}) 3440 (OH), 3050, 3010 (CH), 1595, 1580, 1545, 1535, 1500, 1485 (C=C, C=N). Anal. ($C_{22}H_{16}N_2O$) C, H, N.

1-Phenyl-2-[3-(2-pyridinyl)-1-isoquinolyl]-1-propanone (14). In 100 mL of ammonia, sodium amide (0.05 mol) was freshly prepared, and after evaporation of the remaining ammonia, 30 mL of anhydrous DMSO was added slowly. Propiophenone (0.05 mol) in 10 mL of anhydrous DMSO was added dropwise at 45 °C, and at the same temperature the mixture was stirred for 1.5 h. Subsequently, 1-chloro-3-(2-pyridinyl)-isoquinoline (3) (0.02 mol) in 40 mL of anhydrous DMSO was added dropwise, and after the reaction mixture was stirred for 30 min it was poured into ice/water and acidified with concentrated HCl. The water layer was washed with diethyl ether and the pH adjusted to 8 by adding sodium carbonate. After extraction with chloroform, the combined organic layers were dried over potassium carbonate, and the solvent was evaporated. The residue was purified by preparative TLC (silica, diethyl ether/hexane 6:4) to give 1.28 g of **14** (19%): mp 148 °C; HRMS (CI, NH_3) m/z 338.4210 (M^+), 338.4270 ($C_{23}H_{18}N_2O$); ^{1}H NMR ($CDCl_3$, ref $CHCl_3$ = 7.27 ppm)

δ 1.77 (d, 3H, ^3J = 6.9 Hz, CHCH_3), 5.54 (q, 1H, ^3J = 6.9 Hz, CHCH_3), 7.18−7.38 (m, 4H, pyr-H4′, phenyl-H3″, and phenyl-H4″), 7.60−7.73 (m, 3H, H5, H6, and H7), 7.76−7.99 (m, 3H, pyr-H5′ and phenyl-H2″), 8.25−8.32 (m, 2H, H8 and pyr-H3′), and 8.63 (s, 1H, H4); ^{13}C NMR ($CDCl_3$, ref $CDCl_3$ = 77.0 ppm) δ 16.8 (s, 1C,.CHCH_3), 47.1 (s, 1C, CHCH_3), 116.4 (s, 1C, C4), 121.4 (s, 1C, pyr-C6′), 123.2 (s, 1C, pyr-C4′), 124.2 (s, 1C, C8), 125.9 (s, 1C, C9), 127.9 (s, 1C, C7), 128.2 (s, 2C, phenyl-C2″), 128.3 (s, 2C, phenyl-C3″), 129.0 (s, 1C, C5), 130.1 (s, 1C, phenyl-C4″), 132.2 (s, 1C, C6), 136.7 (s, 1C, C10 or phenyl-C1″), 136.9 (s, 1C, pyr-C5′), 137.5 (s, 1C, C10 or phenyl-C1″), 148.1 (s, 1C, C1), 148.7 (s, 1C, pyr-C3′), 155.9 (s, 1C, C3), and 160.6 (s, 1C, pyr-C1′); IR (KBr cm^{-1}) 3090, 3065, 3050 (CH), 2980, 2940 (CH_3), 1690 (C=O), 1620, 1580, 1560, 1494 (C=C, C=N). Anal. ($C_{23}H_{18}N_2O$) C, H, N.

Pharmacology. Binding of [^{3}H]DPCPX to A_1 receptors from rat cerebral cortex membranes and of [^{3}H]CGS 21680 to adenosine A_{2A} receptors from rat striatal membranes was performed as described previously.[32,33]

Binding of [^{125}I]AB-MECA to human A_3 receptors stably expressed in HEK 293 cells was determined as described.[35,36]

Abbreviations

AcOH	acetic acid
Ac_2O	acetic anhydride
APT	attached proton test
CaH_2	calcium hydride
CGS15943	9-fluoro-2-(2-furyl-5,6-dihydro[1,2,4]-triazolo{1,5-c}-quinazin-5-imine
CI	chemical ionization
COSY	correlated spectroscopy
DMSO	dimethyl sulfoxide
Et_2O	diethyl ether
DEPT	distortionless enhancement by polarization transfer
[^{3}H]-DPCPX	[^{3}H]-1,3-dipropyl-8-cyclopentylxanthine
[^{3}H]CGS 21680	[^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-N-(ethylcarbamoyl)adenosine
HEK cells	human embryonic kidney cells
HMPT	hexamethylphosphoric triamide
HRMS	high-resolution mass spectrometer
[^{125}I]ABA	[^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine
[^{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]-naphtpyridine
$LiAlH_4$	lithium aluminum hydride
THF	tetrahydrofuran
TLC	thin layer chromatography
XAC	8-[4-(((((2-aminoethyl)-amino)carbonyl)-methoxy)oxy)phenyl]-1,3-dipropylxanthine

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