1-Substituted 4-[Chloropyrazolyl][1,2,4]triazolo[4,3-*a*]quinoxalines: Synthesis and Structure-Activity Relationships of a New Class of Benzodiazepine and Adenosine Receptor Ligands☆

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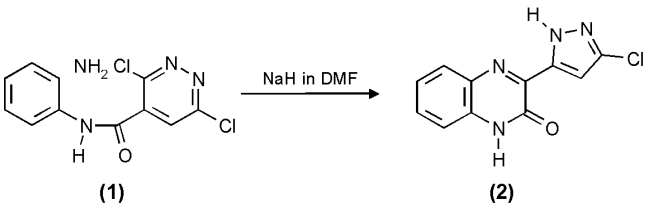
Key Words: *Adenosine receptors; $GABA_A$ receptor; benzodiazepine; 4-[chloropyrazolyl][1,2,4]triazolo[4,3-a]quinoxalines; structure-activity relationships*

Summary

Starting from 3-(3-chloro-1*H*-pyrazol-5-yl)-1*H*-quinoxalin-2-one (**2**) a series of substituted [1,2,4]triazolo[4,3-*a*]quinoxalines (**3a–f**) was prepared *via* a multistep reaction sequence. Affinities of the novel derivatives **3a–f** for benzodiazepine as well as for adenosine A_1- and A_{2A}-receptors of rat brain were determined by radioligand binding assays. 1-Methyl-4-(3-chloro-1*H*-pyrazol-5-yl) derivative **3a** exhibited submicromolar affinity for the benzodiazepine binding site of $GABA_A$ receptors (K_i = 340 nM) and was less potent at A_1- (K_i = 7.85 μM) and A_{2A}- (K_i = 1.43 μM) adenosine receptors (AR). Derivatives with larger substituents in the 1-position showed reduced binding to benzodiazepine and A_{2A}-AR, but increased A_1-AR affinity, the 2-thienylmethyl derivative **3f** being the most potent and selective A_1-AR ligand of the present series (K_i = 200 nM).

Introduction

Recently we have observed a novel type of a 1,2-diazine → 1,2-diazole ring transformation [1]. The product of this reaction – a pyrazolyl substituted quinoxalinone **(2)** – was found to represent an attractive educt for the development of potentially bio-active compounds [2a–b].



(1) (2)

Scheme 1. Formation of the pyrazolyl-substituted quinoxalinone (**2**) from (**1**).

In view of the interesting biological activities described for [1,2,4]triazolo-annelated heterocycles (*e.g.* benzodiazepine receptor binding [3a–d] and adenosine receptor antagonistic properties [4a–c]), the pyrazolyl substituted [1,2,4]triazoloquinoxalines of type **3** became an object of our interest as potential benzodiazepine and/or adenosine receptor ligands.

Benzodiazepine derivatives have attracted the attention of researchers owing to their interesting activities and their low toxicity. They are widely used as anxiolytics, antidepressants, hypnotics, anticonvulsants, and muscle relaxants [5a–c]. Due to the side effects such as psychological and physical dependence, there is continuing interest in the development of novel central benzodiazepine receptor ligands with improved properties. It has been found that many compounds, whose structures were apparently unrelated to benzodiazepines, are able to bind competitively to the benzodiazepine binding site of the $GABA_A$ receptor [5b–c], *e.g.* [1,2,4]triazolo[1,5-*c*]quinazolin-5(6*H*)-ones [3a], [1,2,4]triazolo[4,3-*b*]pyridazines [3d], and imidazo[1,5-*a*]quinoxaline derivatives [6].

Adenosine receptors (AR) – which belong to the superfamily of G-protein-coupled receptors – can be divided into two 'high affinity' subtypes (A_1 and A_{2A}) and two 'low affinity' subtypes (A_{2B} and A_3) [7]. Whereas the first types of receptors are stimulated by adenosine in nanomolar concentrations the latter require micromolar concentrations of adenosine for activation [7]. Antagonists of the A_1-adenosine receptors are of interest for the treatment of dementias (*e.g.* Alzheimer's disease), depression, or as diuretics, antihypertensives, antiarrhythmics, and for the prevention of acute renal failure [8a–b]. On the other hand, adenosine A_{2A} antagonists may be used as drugs for the treatment of Morbus Parkinson [8a–b]. During recent years a number of potent and selective AR antagonists have been discovered [8a–c], and some of them are currently under clinical development. Most of the adenosine receptor antagonists are aromatic nitrogen-containing heterocyclic compounds, *e.g.* xanthines, pyrazolo[1,5-*a*]pyridines, pyrrolo[2,3-*d*]pyrimidines, pyrazolo[3,4-*d*]pyrimidines, [1,2,4]triazolo[1,5-*a*]quinoxalines, and [1,2,4]triazolo- [4,3-*a*]quinoxalines [4b–c, 8a–c].

Herein we report on the synthesis of tricyclic compounds of type **3** structurally related to recently described adenosine receptor antagonists **4** [4b–c]. Moreover we present the results of biological evaluation as benzodiazepine and adenosine A_1- and A_{2A}-receptor ligands.

Results and Discussion

Chemistry

The [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives of type **3** were prepared as shown in Scheme **2**. Starting from the ring contraction product **2** [1], the chloro substituted quinoxaline

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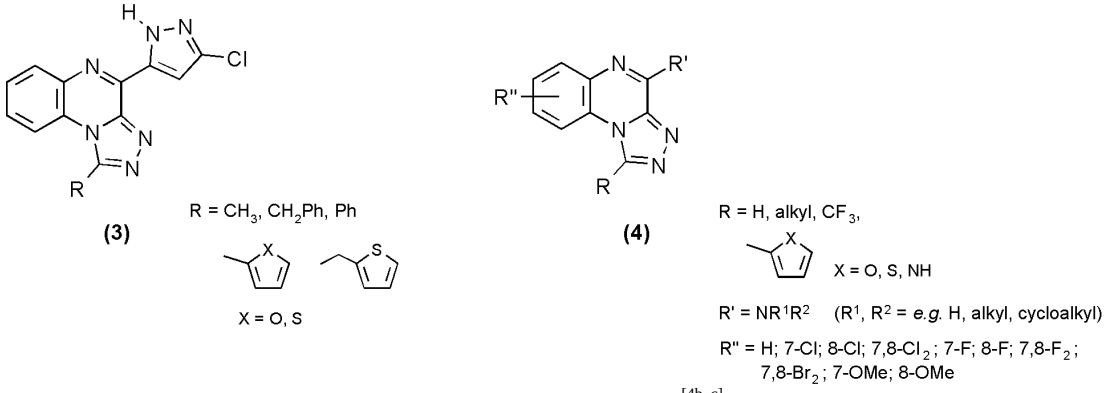


Figure 1. Structures of the target compounds of type **3** and adenosine receptor antagonists **4** [4b–c].

5 was prepared by refluxing **2** in phosphorus oxychloride in the presence of pyridine [2a]. The novel 2-hydrazino derivative **6** became accessible by heating of **5** in hydrazine hydrate at 150 °C.

Acylation of the hydrazino function was achieved by treatment of **6** in dry *N*,*N*-dimethyl formamide with carboxylic acid derivatives (acetyl chloride, benzoyl chloride, phenylacetyl chloride, furan-2-carbonyl chloride, thiophene-2-carbonyl chloride, and thiophen-2-yl-acetyl chloride, respectively) in the presence of triethylamine. The hydrazide derivatives **7a–f** were converted into the tricyclic compounds of type **3** by heating in a mixture of 1,2-dichloroethane and phosphorus oxychloride. Interestingly, cyclisation of compound **7d** was already observed while recrystallising the compound in a mixture of 1,4-dioxane and *N*,*N*-dimethyl formamide. Under these conditions a 68% yield of **3d** was obtained. However, it turned out that this facilitated cyclisation was restricted to compound **7d** and the approach could not be generalised.

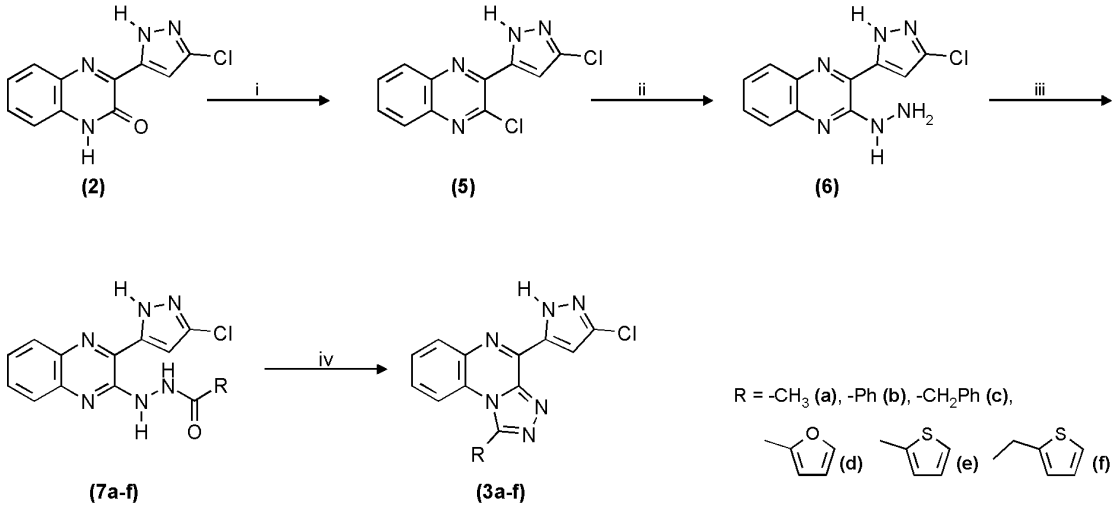
Analytically pure hydrazides as well as the cyclised products could be obtained by recrystallisation from appropriate solvents (see Experimental). Structural proof for all newly synthesised derivatives is based on correct elemental analyses as well as on IR, ^{1}H-NMR, and mass spectral data.

Biological Evaluation of Compounds **3a–f** *as Ligands for Benzodiazepine Binding Sites*

The compounds were investigated in radioligand binding assays at rat brain cortical membranes. Initially, all compounds were screened for their potency to displace [^{3}H]diazepam from its binding site at a single concentration (10 μM). For the most potent compound, *i.e.* **3a**, the K_i value was determined additionally.

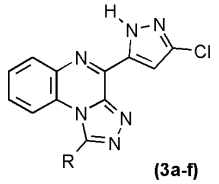
Structure-Activity Relationships

The results obtained (see Table 1) indicate that the methyl substituted tricycle is the most potent derivative in this series (K_i = 340 nM) which exhibits an approximately 40-fold lower activity than the reference compound diazepam. Formal replacement of the methyl substituent by a (hetero)aromatic (phenyl, 2-thienyl, 2-furyl) or (hetero)arylmethyl (benzyl, 2-thienylmethyl) moiety was found to result in a decrease in benzodiazepine receptor affinity.



Scheme 2. Synthesis of 4-[3(5)-chloro-1*H*-pyrazol-5(3)-yl][1,2,4]triazolo[4,3-*a*]quinoxalines (**3a-f**). Reagents used: (i) $POCl_3$, pyridine, reflux; (ii) hydrazine monohydrate, reflux; (iii) R-COCl, $N(C_2H_5)_3$ in *N*,*N*-dimethyl formamide, room temperature; (iv) $POCl_3$, 1,2-dichloroethane, reflux or heating in a mixture of *N*,*N*-dimethyl formamide and 1,4-dioxane (for R = 2-furyl).

Table 1. Inhibition of [^{3}H]diazepam binding to rat brain by test compounds.



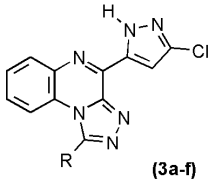
(3a-f)

Compd.	R	Percent specific inhibition ± SEM (*n* = 3) of [^{3}H]diazepam binding to rat brain cortical membranes by test compound (10 μM)	K_i ± SEM (n = 3) [μM]
diazepam		n.d.[a)]	0.009 ± 0.003 [9]
3a	methyl	85 ± 1	0.34 ± 0.08
3b	phenyl	57 ± 5	n.d.[a)]
3c	benzyl	42 ± 2[b)]	n.d.[a)]
3d	2-furyl	51 ± 9	n.d.[a)]
3e	2-thienyl	57 ± 2	n.d.[a)]
3f	2-thienylmethyl	39 ± 19	n.d.[a)]

[a)] n.d. = not determined

[b)] Inhibition of [^{3}H]diazepam binding may be underestimated due to low solubility of **3c** (estimated < 3 μM). Radioligand could be absorbed to the precipitate formed and thus result in high counts feigning low affinity.

Table 2. A_1-Adenosine receptor affinities:



(3a-f)

Compd.	R	A_1-affinity K_i ± SEM [μM] (n = 2) Rat brain cortical membranes [^{3}H]CCPA
caffeine		23.5 ± 3.0
3a	methyl	7.85 ± 0.95
3b	phenyl	1.62 ± 0.68
3c	benzyl	0.31 ± 0.09[a)]
3d	2-furyl	0.41 ± 0.16
3e	2-thienyl	0.71 ± 0.06
3f	2-thienylmethyl	0.20 ± 0.01

[a)] Estimated value through extrapolation of the curve. A complete curve could not be recorded due to low solubility of the compound.

Biological Evaluation of Compounds **3a–f** *as Adenosine Receptor Antagonist***s**

The pyrazolyl-substituted [1,2,4]triazolo[4,3-*a*]quinoxalines **3** were further tested in radioligand binding assays for affinity at A_1- and A_{2A}-adenosine receptors in rat cortical membrane and rat striatal membrane preparations, respectively. The A_1-selective agonist [^{3}H]2-chloro-N^6-cyclopentyladenosine (CCPA) [10] was used as A_1 ligand and [^{3}H]3-(3-hydroxypropyl)-8-(3-methoxystyryl)-7-methyl-1-propargylxanthine (MSX-2) [11] as A_{2A} ligand, caffeine was chosen as a standard reference compound. The results thus obtained are shown in Tables 2 and 3.

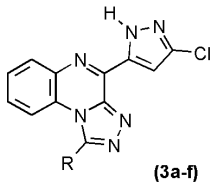
Structure-Activity Relationships

The results of these binding assays indicate that all new compounds prepared show affinity to adenosine receptors. A_1-Affinity of the novel derivatives is higher than that of caffeine, which was tested as a reference compound. Furthermore, the methyl substituted tricyclic compound was about 20-fold more potent at A_{2A}-receptors than caffeine. Since a virtually intact ribose moiety is required for agonistic activity at AR [8] it can be assumed that triazoloquinoxalines **3a–f**, which are lacking a ribose residue, are antagonists at AR, like caffeine.

A substituent in position 1 of the tricyclic system has a profound effect on affinity as well as selectivity.

For A_1-adenosine receptor affinity the following structure-activity relationships could be established: the methyl substituted derivative **3a** exhibits only low affinity, (formal) exchange of methyl by a (hetero)aryl or (hetero)arylmethyl moiety leads to increased potency. Comparing these compounds, the rank order of A_1-AR affinity according to the nature of the substituent is as follows: phenyl (**3b**) < 2-thienyl (**3e**) < 2-furyl (**3d**) < benzyl (**3c**) < 2-thienylmethyl (**3f**). In this series, the heteroaryl substituted derivatives (**3d**/**3e**) turned out to be about 2- to 4-fold more potent than the corresponding phenyl substituted compound **3b**. Formal replacement of the (hetero)aryl moiety by a (hetero)arylmethyl substituent, however, leads to a further increase in A_1-AR affinity. The most active compound in this series (**3f**) was approximately 120-fold more potent than caffeine.

Table 3. A_{2A}-Adenosine receptor affinities



(3a-f)

Compd.	R	A_{2A}-affinity Percent specific inhibition ± SEM (n = 2) of [^{3}H]MSX-2-binding to rat striatal membranes by test compound (25 μM)	K_i [μM]
caffeine		36 (at 30 μM)	32.5
3a	methyl	96 ± 1	1.43 [a]
3b	phenyl	0	n.d. [b]
3c	benzyl	6.1 ± 1.5 [c]	n.d. [b]
3d	2-furyl	31 ± 3	n.d. [b]
3e	2-thienyl	22 ± 13	n.d. [b]
3f	2-thienylmethyl	21 ± 10	n.d. [b]

[a] result from single experiment

[b] n.d. = not determined

[c] Inhibition of [^{3}H]MSX-2 binding may be underestimated due to low solubility of **3c** (estimated < 3 μM). Radioligand could be absorbed to the precipitate formed and thus result in high counts feigning low affinity.

These structure-activity relationships are in accordance with pharmacophore models described for A_1-adenosine receptor antagonists, as shown for the potent A_1-selective AR antagonist ADPEP [8b, 12], a pyrrolo[2,3-*d*]pyrimidine derivative (see Figure 2 [8b]). A_1-AR ligands typically contain a hydrogen bond donor at a certain distance from a hydrogen bond acceptor (see Figure 2). A lipophilic domain increases A_1 selectivity of AR antagonists versus A_{2A}-AR since the A_{2A}-AR shows restricted bulk tolerance in that receptor domain [8b, 12d]. A major drawback of the derivatives bearing a lipophilic moiety (especially benzyl), however, is the low solubility of these compounds.

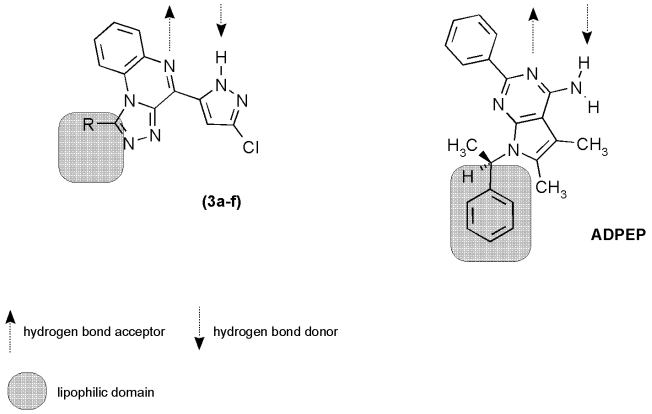


Figure 2. Pharmacophore model for A_1-adenosine receptor antagonists [8b, 12d].

Structure-activity relationships for compounds **3** at A_{2A}-AR can be described as follows: the 1-methyl substituted derivative **3a** represents the most active A_{2A}-AR ligand in this series, (formal) replacement of methyl by (hetero)aryl or (hetero)arylmethyl leads to a reduction of receptor affinity. Compound **3a** is 5-fold selective for the A_{2A}-AR versus A_1, while compounds with larger substituents are A_1-selective.

In conclusion, the pyrazolyl-substituted [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives show affinity to benzodiazepine and/or adenosine receptors. Whereas the 1-(hetero)aryl and 1-(hetero)arylmethyl substituted derivatives represent selective A_1-adenosine receptor ligands, compound **3a** (bearing a methyl group in position 1 of the tricycle) shows affinity to benzodiazepine and A_{2A}-adenosine receptors (A_{2A}:A_1 = 5), with four-fold higher affinity to benzodiazepine receptors as compared to A_{2A} adenosine receptors. Based on these structure-activity relationships further structural modifications are in progress in order to increase affinity and selectivity of this new class of AR antagonists and ligands for the benzodiazepine binding site of $GABA_A$ receptors.

Acknowledgement

The authors are very grateful to Dr. Dietmar Rakowitz (Institute of Pharmaceutical Chemistry, University of Innsbruck) for recording the mass spectra. E.P. was on leave from the Department of Chemical Technology of Drugs, laboratory of Dr. habil. Kiec-Kononowicz, Collegium Medicum of the Jagiellonian University, Kraków, Poland, with support from 'Deutscher Akademischer Austauschdienst' (DAAD), which is gratefully acknowledged.

Experimental

Melting points were determined on a Kofler hot-stage microscope (Reichert) and are uncorrected. IR spectra were taken on a Mattson Galaxy Series FT-IR 3000 spectrophotometer (KBr pellets). Mass spectra were obtained on a Finnigan MAT SSQ 7000. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer (^{1}H: 199.98 MHz). The centre of the solvent multiplet ([D_6]DMSO) was used as internal standard (chemical shifts in δ ppm), which was related to TMS with δ 2.49 ppm for ^{1}H. Reactions were monitored by TLC using Polygram® SIL G/UV$_{254}$ (Macherey-Nagel) plastic-backed plates (0.25 mm layer thickness). Microanalyses were performed at the Institute of Physical Chemistry (Mag. J. Theiner), University

of Vienna, Austria. All analyses were within ±0.4% of the calculated values. Light petroleum refers to the fraction of bp 40–60 °C. The yields are not optimised.

Syntheses

Starting materials: 2-Chloro-3-(3-chloro-1*H*-pyrazol-5-yl)-quinoxaline **(5)** was prepared by treatment of **(2)** [1] with phosphorus oxychloride and pyridine [2]. 3-(3-Chloro-1*H*-pyrazol-5-yl)-1*H*-quinoxalin-2-one **(2)** became accessible by reaction of *N*-(2-aminophenyl)-3,6-dichloropyridazine-4-carboxamide **(1)** with sodium hydride in dry *N*,*N*-dimethyl formamide [2].

*3-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-2-hydrazinoquinoxaline (**6**)*

A mixture of 2-chloro-3-(3-chloro-1*H*-pyrazol-5-yl)-quinoxaline **(5)** [2] (0.512 g, 2 mmol) in hydrazine monohydrate (15 mL) was heated at 150 °C until TLC indicated completion of the reaction (ca. 2 h). After cooling, the mixture was poured into ice-water (150 mL). The crystals thus obtained were collected by filtration, washed with water and light petroleum and were recrystallised from tetrahydrofuran to yield 460 mg (88%) of a yellow powder, mp 243–250 °C.– $C_{11}H_9ClN_6$ (260.69) Anal. C,H,N.– IR (KBr): 3308, 3147 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO + 1 drop of D_2O): δ = 7.71 (d, *J* = 7.8 Hz, 1H), 7.50–7.48 (m, 2H), 7.31–7.22 (m, 1H) (quinoxaline-H-5, H-6, H-7, H-8), 7.08 (s, 1H, pyrazole-H-4).– EI MS (70 eV): *m/z* = 260 [M^+].

General Procedure for the Synthesis of Compounds of Type **7***:*

To a stirred mixture of **(6)** (0.261–0.521 g, 1.0–2.0 mmol) and triethylamine (0.121–0.242 g, 1.2–2.4 mmol, 1.2 equiv.) in dry *N*,*N*-dimethyl formamide (5–10 mL) was added slowly a solution of the corresponding carboxylic acid chloride (1.1 equiv.) (acetyl chloride, benzoyl chloride, phenylacetyl chloride, furan-2-carbonyl chloride, thiophene-2-carbonyl chloride, and thiophen-2-yl-acetyl chloride, respectively) in 1–2 mL of dry *N*,*N*-dimethyl formamide at 0 °C under a nitrogen atmosphere. The mixture was allowed to warm to room temperature and stirred over night at this temperature. Then the mixture was poured into 150–300 mL of water. The crystals thus obtained were collected by filtration, washed with water and light petroleum and were recrystallised (see below).

*2-Acetylhydrazino-3-[3(5)-chloro-1H-pyrazol-5(3)-yl]-quinoxaline (**7a**)*

Recrystallisation from ethyl acetate yielded 83% of a dark yellow powder, mp 293–297 °C.– $C_{13}H_{11}ClN_6O \times 0.2\ CH_3COOC_2H_5$ (320.35) Anal. C,H,N.– IR (KBr): 3217, 1640 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO + 1 drop of D_2O): δ = 7.89 (d, *J* = 8.0 Hz, 1H), 7.66–7.60 (m, 2H), 7.55–7.42 (m, 1H) (quinoxaline-H-5, H-6, H-7, H-8), 7.16 (s, 1H, pyrazole-H-4), 2.00 (s, 3H, CH_3).– EI MS (70 eV): *m/z* = 302 [M^+].

*2-Benzoylhydrazino-3-[3(5)-chloro-1H-pyrazol-5(3)-yl]-quinoxaline (**7b**)*

Recrystallisation from tetrahydrofuran/ethyl acetate (ca. 1:1) yielded 80% of a yellow powder, mp 222–225 °C.– $C_{18}H_{13}ClN_6O$ (364.80) Anal. C,H,N.– IR (KBr): 3447, 3217, 1636 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): δ = 14.35 (s, br, 1H, D_2O-exchangeable, NH), 10.88 (s, 1H, D_2O-exchangeable, NH), 10.22 (br, 1H, D_2O-exchangeable, NH), 8.00–7.89 (m, 3H), 7.62–7.50 (m, 6H) (quinoxaline-H-5, -H-6, -H-7, -H-8, phenyl-H), 7.22 (s, 1H, pyrazole-H-4).– EI MS (70 eV): *m/z* = 364 [M^+].

*3-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-2-phenylacetylhydrazinoquinoxaline (**7c**)*

Recrystallisation from tetrahydrofuran yielded 49% of a yellow powder, mp 225–231 °C.– $C_{19}H_{15}ClN_6O$ (378.82) Anal. C,H,N.– IR (KBr): 3213, 3040, 1637 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO + 1 drop of D_2O): δ = 7.91 (d, *J* = 8.2 Hz, 1H), 7.70–7.63 (m, 2H), 7.55–7.19 (m, 7H), (quinoxaline-H-5, -H-6, H-7, -H-8, phenyl-H, pyrazole-H-4), 3.64 (s, 2H, CH_2).– EI MS (70 eV): *m/z* = 378 [M^+].

*3-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-2-(2-furoyl)-hydrazinoquinoxaline (**7d**)*

Recrystallisation from ethyl acetate/tetrahydrofuran (ca. 1:1) yielded 63% of a yellow powder, mp 212–215 °C.– $C_{16}H_{11}ClN_6O_2 \times 0.7\ H_2O$ (367.37) Anal. C,H,N.– IR (KBr): 3461, 3230, 1637 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO + 1 drop of D_2O): δ = 7.92–7.91 (m, 2H), 7.62 ('s' br, 1H), 7.51 ('s' br, 1H), 7.20 ('s' br, 1H) (quinoxaline-H-5, -H-6, -H-7, -H-8, pyrazole-H-4, furan-H-5), 7.29 (d, *J* = 3.5 Hz, 1Hz, furan-H-3), 6.70 (dd, *J* = 3.5 Hz, *J* = 1.8 Hz, 1H, furan-H-4).– EI MS (70 eV): *m/z* = 354 [M^+].

*3-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-2-(2-thenoyl)-hydrazinoquinoxaline (**7e**)*

Recrystallisation from a mixture of 1,4-dioxane and *N*,*N*-dimethyl formamide (ca. 2:1) yielded 65% of yellow needles, mp 195–200 °C.– $C_{16}H_{11}ClN_6OS$ (370.82) Anal. C,H,N.– IR (KBr): 3282, 2923, 1643 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO + 1 drop of D_2O): δ = 8.00–7.98 (m, 1H), 7.92–7.83 (m, 2H), 7.62–7.61 (m, 2H), 7.52–7.44 (m, 1H), 7.25–7.20 (m, 2H) (quinoxaline-H-5, -H-6, -H-7, -H-8, pyrazole-H-4, thiophene-H-3, -H-4, -H-5).– EI MS (70 eV): *m/z* = 370 [M^+].

*3-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-2-(2-thienylacetyl)-hydrazinoquinoxaline (**7f**)*

Recrystallisation from 1,4-dioxane yielded 61% of a yellow powder, mp 223–229 °C, crystallised at 230 °C, then mp 250–252 °C.– $C_{17}H_{13}ClN_6OS$ (384.85) Anal. C,H,N.– IR (KBr): 3210, 3041, 1640 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO + 1 drop of D_2O): δ = 8.23 (dd, *J* = 6.0 Hz, *J* = 3.5 Hz, 1H), 8.12 (dd, *J* = 6.0 Hz, *J* = 3.5 Hz, 1H), 7.73–7.63 (m, 2H) (quinoxaline-H-5, -H-6, -H-7, -H-8), 7.62 (s, 1H, pyrazole-H-4), 7.41 (dd, *J* = 4.9 Hz, *J* = 1.5 Hz, 1H, thiophene-H-5), 6.96–6.92 (m, 2H, thiophene-H-3, -H-4), 5.18 (s, 2H, CH_2).– EI MS (70 eV): *m/z* = 384 [M^+].

General Procedure for the Synthesis of Compounds of Type **3a–c** *and* **3e–f***:*

A mixture of the corresponding 2-acylhydrazino-3-[3(5)-chloro-1*H*-pyrazol-5(3)-yl]-quinoxaline **(7a–c, 7e–f)** (0.5–1.0 mmol) and phosphorus oxychloride (4–8 mL) in 1,2-dichloroethane (10–20 mL) was heated under reflux until TLC indicated completion of the reaction (ca. 3 h). After cooling, the mixture was poured into ice-water (150 mL) and was extracted exhaustively with ethyl acetate. The organic layer was washed with 2N NaOH, water and brine, dried over sodium sulphate and evaporated *in vacuo*. The products thus obtained were purified by recrystallisation (see below).

*4-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-1-methyl-[1,2,4]triazolo[4,3-a]-quinoxaline (**3a**)*

Recrystallisation from 1,4-dioxane/H_2O (ca. 2:1) yielded 71% of light beige needles, mp 283–289 °C (sublimation above 230 °C).– $C_{13}H_9ClN_6$ (284.71) Anal. C,H,N.– IR (KBr): 3421, 3100 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): δ = 14.22 (s, 1H, NH), 8.42–8.37 (m, 1H), 8.14–8.09 (m, 1H), 7.85–7.70 (m, 2H) (H-6, H-7, H-8, H-9), 7.60 (s, 1H, pyrazole-H-4), 3.14 (s, 3H, CH_3).– EI MS (70 eV): *m/z* = 284 [M^+].

*4-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-1-phenyl-[1,2,4]triazolo[4,3-a]-quinoxaline (**3b**)*

Recrystallisation from ethyl acetate yielded 69% of light pink needles, mp 267–270 °C.– $C_{18}H_{11}ClN_6$ (346.78) Anal. C,H,N.– IR (KBr): 3447, 3144, 3074 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): δ = 14.30 (s, 1H, NH), 8.13 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H), 7.83–7.64 (m, 7H), 7.57–7.49 (m, 1H), 7.41–7.36 (m, 1H) (H-6, H-7, H-8, H-9, phenyl-H, pyrazole-H-4).– EI MS (70 eV): *m/z* = 346 [M^+].

*1-Benzyl-4-[3(5)-chloro-1H-pyrazol-5(3)-yl]-[1,2,4]triazolo[4,3-a]-quinoxaline (**3c**)*

Recrystallisation from tetrahydrofuran/ethyl acetate (ca. 2:1) yielded 62% of light yellow needles, mp 268–270 °C.– $C_{19}H_{13}ClN_6 \times 0.8\ H_2O$ (375.22) Anal. C,H,N.– IR (KBr): 3108 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): = 14.25 (s, 1H, NH), 8.19–8.08 (m, 2H), 7.73–7.65 (m, 3H), 7.36–7.23 (m, 5H) (H-6, H-7, H-8, H-9, pyrazole-H-4, phenyl-H), 5.01 (s, 2H, CH_2).– EI MS (70 eV): *m/z* = 360 [M^+].

*4-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-1-(2-thienyl)-[1,2,4]triazolo[4,3-a]-quinoxaline (**3e**)*

Recrystallisation from tetrahydrofuran yielded 63% of light beige needles, mp 307–310 °C.– $C_{16}H_9ClN_6S$ (352.81) Anal. C,H,N.– IR (KBr): 3448, 3149 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): δ = 14.29 (s, 1H, NH), 8.09 (dd, *J* = 5.1 Hz, *J* = 1.2 Hz, 1H, thiophene-H-5), 8.16–8.12 (m, 1H), 7.76–7.65 (m, 2H), 7.61–7.58 (m, 2H) (H-6, H-7, H-8, H-9, pyrazole-H-4), 7.72 (dd, *J* = 3.6 Hz, *J* = 1.2 Hz, 1H, thiophene-H-3), 7.43 (dd, *J* = 5.1 Hz, *J* = 3.6 Hz, 1H, thiophene-H-4).– EI MS (70 eV): *m/z* = 352 [M^+].

*4-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-1-(2-thienylmethyl)-[1,2,4]triazolo-[4,3-a]quinoxaline (**3f**)*

Recrystallisation from ethyl acetate/tetrahydrofuran (ca. 1:1) yielded 68% of light yellow needles, mp 253–258 °C.– $C_{17}H_{11}ClN_6S$ (366.83) Anal. C,H,N.– IR (KBr): 3448, 3124 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): δ = 14.25 (s, 1H, NH), 8.29–8.22 (m, 1H), 8.16–8.08 (m, 1H), 7.46–7.68 (m, 2H) (H-6, H-7, H-8, H-9), 7.65 (s, 1H, pyrazole-H-4), 7.43 (dd, *J* = 4.8 Hz, *J* = 1.5 Hz, 1H, thiophene-H-5), 6.99–6.92 (m, 2H, thiophene-H-3, -H-4), 5.21 (s, 2H, CH_2).– EI MS (70 eV): *m/z* = 366 [M^+].

*4-[3(5)-Chloro-1H-pyrazol-5(3)-yl]-1-(2-furyl)-[1,2,4]triazolo[4,3-a]-quinoxaline (**3d**)*

Compound **7d** (crude product) (0.250 g, 0.70 mmol) was suspended in a mixture of dry *N,N*-dimethyl formamide and dry 1,4-dioxane (*ca.* 10 mL, ratio ca. 2:1) and the mixture was heated until a solution was obtained. Then the solution was cooled to room temperature and poured into ice water (100 mL). The crystals thus obtained were filtered, washed with water and light petroleum and dried in vacuo. Recrystallisation from ethyl acetate/tetrahydrofuran (ca. 3:1) yielded 0.160 g (68%) of light beige needles, mp 320–327 °C.– $C_{16}H_9ClN_6O$ (336.74) Anal. C,H,N.– IR (KBr): 3120 cm^{-1}.– ^{1}H-NMR ([D$_6$]DMSO): δ = 14.30 (s, 1H, NH), 8.21–8.14 (m, 2H), 8.14–8.09 (m, 1H), 7.80–7.66 (m, 3H), 7.38–7.33 (m, 1H), (H-6, H-7, H-8, H-9, pyrazole-H-4, furan-H-5), 7.29 (dd, *J* = 3.4 Hz, *J* = 0.8 Hz, 1H, furan-H-3), 6.94 (dd, *J* = 3.4 Hz, *J* = 1.8 Hz, 1H, furan-H-4).– EI MS (70 eV): *m/z* = 336 [M^+].

Benzodiazepine and Adenosine Receptor Binding Assays

[^{3}H]Diazepam (NET564, 3071 GBq/mmol, 83 Ci/mmol) and [^{3}H]CCPA (NET1026, 1110 GBq/mmol, 30 Ci/mmol) were obtained from New England Nuclear (NEN) Life Science Products (Köln, Germany). The 7-desmethyl precursor of [^{3}H]MSX-2 was synthesised in our laboratory [11] and radiolabelled by American Radiolabeled Chemicals Inc. (St. Louis, Minnesota, USA) through Biotrend (Köln, Germany) (ART691, 3145 GBq/mmol, 85 Ci/mmol).

Drug Solutions

The compounds were dissolved in DMSO and further diluted with tris(hydroxymethyl)aminomethane- (Tris-) HCl buffer (50 mM, pH 7.4) (final DMSO concentrations: 2.5% for A_1- and A_{2A}-binding assays and 1% for benzodiazepine-binding assays, respectively).

Benzodiazepine Binding Assays

Frozen rat brains were obtained from Pel-Freez, Rogers, Arkansas, USA. The cortex was dissected and inhibition of binding of [^{3}H]diazepam to rat brain cortical membranes was determined as previously described [9, 13]. In a final volume of 1 mL, each test tube contained 790 µL of Tris-HCl buffer (50 mM, pH 7.4), 10 µL of drug solution (see above), 100 µL of rat cerebral cortical membrane preparation with a protein concentration of ca. 100 µg per tube, and 100 µL of [^{3}H]diazepam solution, to give a final concentration of 1 nM. DMSO (final concentration: 1%) was necessary since the compounds showed a rather low water-solubility. Higher concentrations of DMSO, however, were not tolerated by the receptors. DMSO without test compound served as a control.

Incubations were performed at 2 °C for 1 h and were terminated by rapid filtration through glass fiber filters (Schleicher & Schüll GF51) using a Brandel cell harvester M-24 (Brandel, Gaithersburg, Maryland, USA). Three 5 mL washes with ice-cold Tris-HCl buffer were performed. Unlabelled diazepam (5 µM) was used to define non-specific binding. All compounds were initially tested in a single concentration (10 µM). For compound **3a** a full inhibition curve was determined in triplicate. K_i values were calculated from IC_{50} values, determined by the non-linear regression program Prism version 1.0 (Graphpad, San Diego, California, USA), using the Cheng-Prusoff equation [14] and a K_D value of 4 nM for diazepam [15].

Adenosine Binding Assays

Inhibition of binding of [^{3}H]2-chloro-N^6-cyclopentyladenosine (CCPA) [10] to A_1-adenosine receptors of rat brain cortical membranes and inhibition of [^{3}H]3-(3-hydroxypropyl)-8-(3-methoxystyryl)-7-methyl-1-propargyl-xanthine (MSX-2) [11] to adenosine receptors in rat brain striatal membranes were assayed in analogy to published procedures [16]. As buffer Tris-HCl, 50 mM, pH 7.4 (at room temp.) was used for all experiments. The incubation tubes for the A_1-assay contained 25 µL of test compound dissolved in DMSO, or DMSO alone as a control, respectively, 0.775 mL of buffer, 100 µL of radioligand solution in buffer to obtain a final concentration of 0.5 nM and 100 µL of membrane suspension (50 µg protein per tube) treated with adenosine deaminase, to give a final volume of 1 mL.

A_{2A}-assay tubes contained 25 µL of compound dissolved in DMSO, or DMSO as a control, respectively, 0.775 mL of buffer, 100 µL of radioligand solution in buffer to obtain a final concentration of 1 nM and 100 µL of membrane suspension (70 µg protein per tube) treated with adenosine deaminase, to give a final volume of 1 mL. 2-Chloroadenosine (50 µM) was used to define nonspecific binding. DMSO concentration was 2.5% (V/V) in all experiments. Incubation was performed at 23 °C for 1.5 h (A_1-assay), or at 23 °C for 30 mins. (A_{2A}-assay). Incubation was terminated by rapid filtration through glass fiber (Schleicher & Schüll GF51) filters using a cell harvester. In order to minimise nonspecific binding of the radioligand [^{3}H]MSX-2 on the filters, those were soaked in aqueous polyethylenimine solution (0.3%) at least 1 h before use. Filters were washed twice with 5 mL of ice-cold buffer. The wet filter papers were incubated with scintillation cocktail for at least 6 h before radioactivity was counted. Inhibition of the receptor-radioligand binding was determined by a range of 5 to 6 concentrations of the compounds. The Cheng-Prusoff equation [14] and K_D of 0.2 nM for [^{3}H]CCPA [10] and 8 nM for [^{3}H]MSX-2 [11] were used to calculate the K_i values from the IC_{50} values, determined by the non-linear curve fitting program PRISM™ (GraphPad, San Diego, California, USA).

References and Notes

☆ Dedicated to Professor Dr. Gottfried Heinisch, Innsbruck, on the occasion of his 60th birthday.

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Received: December 15, 1997 [FP268]