Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 421–437
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Derivatives of 4,6-diamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-2*H*-1-one: potential antagonist ligands for imaging the A_{2A} adenosine receptor by positron emission tomography (PET)

Marcus H. Holschbach [a], Dirk Bier [a], Walter Wutz [a], Wiebke Sihver [a], M. Schüller [a], Ray A. Olsson [a,b,*]

[a] *Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany*
[b] *Department of Internal Medicine, MDC Box 19, University of South Florida, Tampa, FL 33612-4799, USA*

Received 30 November 2004; received in revised form 20 December 2004; accepted 21 December 2004

Available online 25 February 2005

Abstract

The importance of the brain A_{2A} adenosine receptor (A_{2A}AR) in movement disorders urges the development of radiolabeled ligands for imaging those receptors by positron emission tomography (PET). This study evaluated one class of A_{2A}AR antagonists, derivatives of 4-amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-2*H*-1-one, **10a**, as agents for imaging brain A_{2A}ARs by PET.. Modifications of a literature synthesis of **10a** efficiently generated analogs **10b–s** for pharmacological evaluation. Radioligand binding experiments showed affinities for the rat brain A_{2A}AR in the low nanomolar range but similar affinities for the A_1AR and substantial unspecific binding. Autoradiography employing [^{3}H]**10a**, showing that high unspecific binding obscured specific binding to both the A_1AR and A_{2A}AR. Thus, compounds **10b–s** are unsuitable as ligands for imaging brain A_{2A}ARs by PET.

Keywords: PET; A_{2A} adenosine receptor antagonists; Triazolo[4,3-*a*]quinoxoline; Radioligands

1. Introduction

Several potent, highly selective antagonists for the A_{2A} adenosine receptor (A_{2A}AR) have appeared in the past decade [1–7]. In addition to their potential as drugs [8], those antagonists, if radiolabeled, could be useful ligands for medical imaging by means of positron emission tomography (PET). Indeed, labeling of several A_{2A}AR antagonists with carbon-11 has provided promising radioligands for PET [9–12]. Labeling with fluorine-18 offers some advantages over carbon-11. The appreciably longer physical half-life of fluorine-18 over that of carbon-11, 110 vs. 20 min, allows extended syntheses and imaging protocols and obviates the need to carry out imaging close to a cyclotron. The decay of fluorine-18 produces positrons having the lowest energy —and, thereby, the shortest range in tissue and greatest spatial resolution—of all the medically useful positron emitters. These reasons have guided our program for the synthesis and evaluation of A_{2A}AR antagonists that might be suitable for no-carrier-added (n.c.a.) radiofluorination and brain imaging.

Of the available A_{2A}AR antagonists, SCH 58261 (Fig. 1) has many desirable pharmacological properties [13], and a carbon 11-labeled congener has been developed for PET [14,15]. However, neither that compound nor its congeners are particularly well suited for our purposes because the nitrogen heterocycle is difficult to prepare, n.c.a. radiofluorination would necessarily occur several steps before the end of

Abbreviations: CPDPX, 8-cyclopentyl-3,4,5,6-tetrahydro-1,3-dipropyl-1*H*-purine-2,6-dione; ZM241385, 4-[2-(7-amino-2-(furan-2-yl)triazolo[1,5-*a*][1,3,5]triazin-5-ylamino)ethyl)phenol; CGS21680, 4-[2-[[6-amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl]benzenepropanoic acid.

* Corresponding author. Tel.: +1 813 974 4067; fax: +1 813 974 2189.
E-mail address: rolsson@hsc.usf.edu (R.A. Olsson).

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2004.12.005

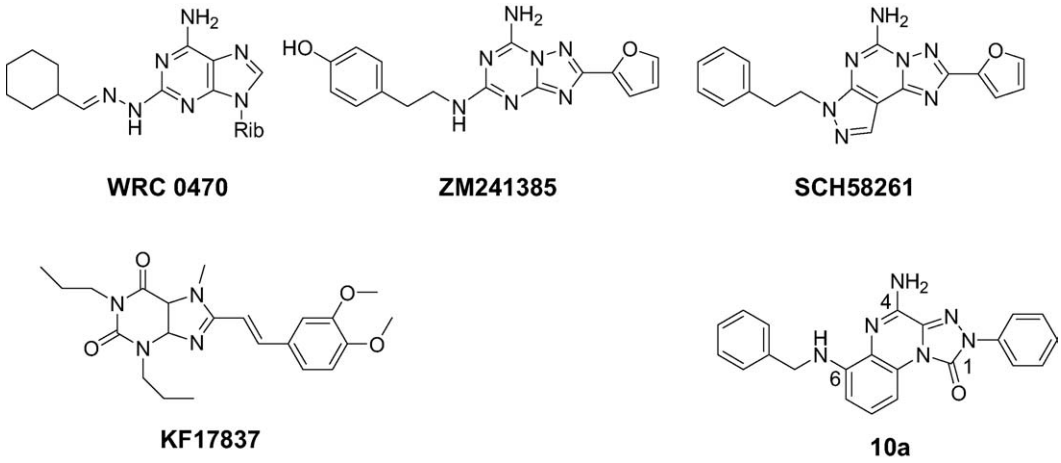


Fig. 1. *. Structures of the agonist and antagonists mentioned in the text. KF17837 and SCH58261 have been radiolabeled with carbon-11.

the synthesis, and neither of the exocyclic substituents favors a regioselective nucleophilic fluorination. Similar considerations apply to another high-affinity A_{2A}AR antagonist, ZM241385. Some other A_{2A}AR antagonists, the 8-styrylcaffeines [16], have been labeled with carbon-11 for PET [17], but their photolability [18] and poor penetration into the CNS are drawbacks. However, it seemed that modifications of the recently described synthesis [7] of 4-amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one, **10a**, might provide congeners suitable for radiofluorination.

A three-atom spacer separating an aromatic, alkyl or cycloalkyl group from C-2 of the adenine (or other heterocyclic) base is a motif common to a number of potent A_{2A}AR agonists and antagonists (Fig. 1) [4–6,19–23]. For example, that spacer in the agonist WRC 0470 consists of the methylene residue and two hydrazine nitrogens linking the cyclohexyl group to adenine C-2. Other three-atom linkers in WRC 0470 isosteres include ethoxy, ethylamino and 1-propyn-1-yl groups. In the case of antagonists such as SCH58261, the spacer linking the benzene ring consists of a two-carbon ethyl bridge and pyrazole N-7, and in ZM241385 an ethylamino group links the aryl moiety to the heterocyclic base. The linker in **10a** consists of the benzylic carbon, the nitrogen of the 6-amino group and C-6 of the quinoxaline moiety.

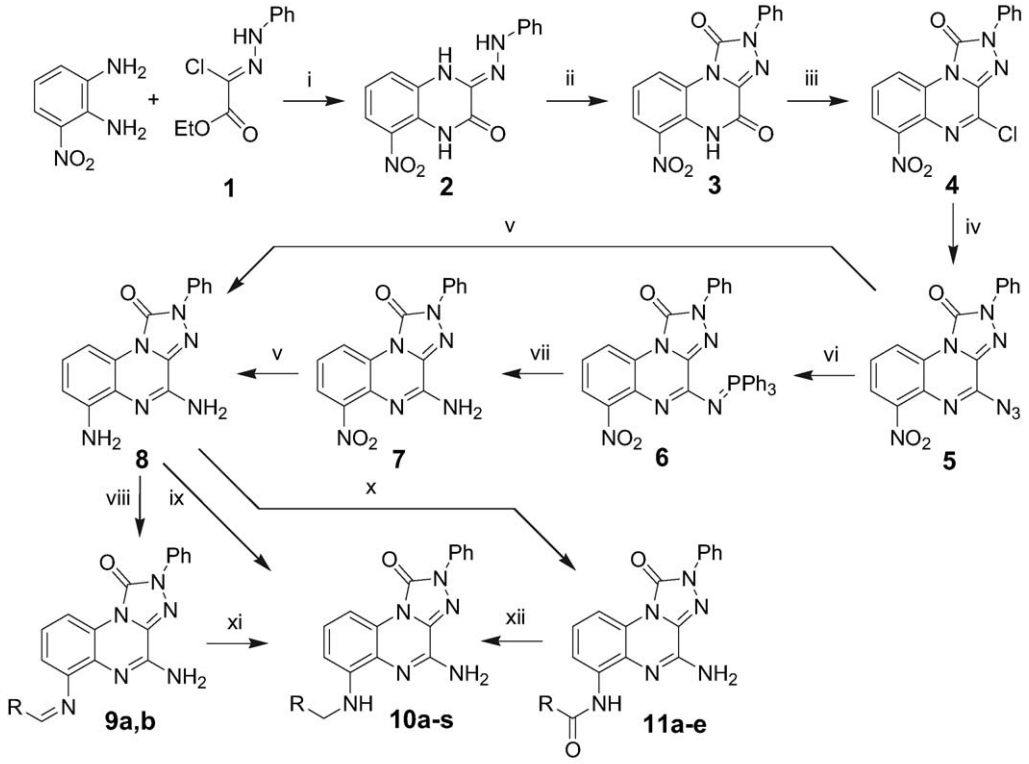
Here we report the synthesis of analogues of **10a**, with some modifications of the approach used by Colotta et al. [7]. As in our earlier study of antagonists for the A_1AR [24], the primary objective was to assess the impact of fluorination on pharmacological activity. Consistent with the intended use, we designed the synthetic pathways to meet the needs of n.c.a. radiofluorination. The series also included compounds with *O*-methoxy groups to evaluate ligands with the potential for labeling with carbon-11. Finally, we used in vitro autoradiography to assess the suitability of these fluorinated analogues to image A_{2A}ARs in brain by means of PET.

2. Chemistry

The design of the synthetic route anticipated the need for an electron-withdrawing group to activate the phenyl ring for aromatic nucleophilic radiofluorination and the need, in the production of a radioligand, for fluorination at the latest possible step to preserve a high radiochemical yield (RCY). The carbonyl group of benzaldehyde met the first criterion; changing the order of the individual steps in the Colotta synthesis met the second.

Scheme 1 shows the synthetic pathway, which employed the same intermediate, **3**, used by Colotta et al. [7] in their first report, but differed in subsequent steps. Colotta reduced the nitro group of **3**, then formed and reduced the benzaldimine to yield the 6-aralkylamino compound, and, finally, introduced the 4-amino group by chlorination and reaction with ammonia. Our strategy called for introducing the 4-amino group, protecting it, reducing the 6-nitro group and finally introducing the 6-aralkylamino group. We evaluated two alternative routes for introducing the 4-amino group. Both began by chlorinating **3** and the displacement of the chloride of **4** by azide. The azido group of **5** then underwent Staudinger reduction [25] to form the 4-amino-6-nitro compound, **7**. We reasoned that it would be necessary to protect the 4-amino group of **7** to insure the chemoselective reaction of the 6-amino group that reduction would generate from the 6-nitro group in a subsequent step. However, protection proved unnecessary; **7** failed to react with either Boc_2O, trifluoroacetic anhydride or benzaldehyde/$ZnCl_2$. The unreactivity of the 4-amino group of **7** had two important consequences. First, it insured that the selective derivatization of the 6-amino group of diamine **8** was feasible. Second, it simplified the synthesis of **8**. The simultaneous reduction [26] of the 4-azido and 6-nitro groups of **5** by $SnCl_2$ gave **8** in a single step. This diamine represents a versatile intermediate for the synthesis of variously substituted N-6 derivatives of **8**.

Preliminary studies (Table 1) assessed the feasibility of various approaches to 4-amino-6-aralkylaminoquinazolines **10a**–**s**. Only one method, reductive alkylation of **8** with $NaBH(OAc)_3$, gave relatively low yields. The reaction of **8** with either benzaldehyde or 4-(2-fluoroethoxy)benzaldehyde generated imines **9a**, **b**, which then underwent reduction by $NaBH_4$ to benzylamines **10a**, **b** in satisfactory overall yields. The reductive amination of aldimines by decaborane [27],



Scheme 1.

which avoided the isolation of the intermediate aldimine, proved the most efficient and convenient path to compounds **10b**–**s**. In keeping with the need to know whether fluorination would affect pharmacological activity, the panel of aldehydes reacted with **8** were mainly fluorobenzaldehydes, but also included furan-2-carboxaldehyde and its 5-bromo- and 5-iodo derivatives. Neither pyrrole-2-carboxaldehyde nor its N-methyl derivative reacted with **8**.

We also explored an alternative way to introduce the 6-benzylamino substituent, namely, the reaction of **8** with acyl halides rather than aldehydes to form amides **11a**–**e**, followed by reduction of the amides to benzylamines **10a**–**e**. That approach offered two potential advantages. First, amides tend to be more stable than aldimines, especially (ar)aliphatic aldimines, which would make the products easier to handle. Second, it would broaden the panel by adding aliphatic amino substituents. Reductants for **11a**–**e** included borane-THF [28], $NaBH_4$/acetic acid [29], sodium triacetoxyborohydride [30,31] and borane-dimethyl sulfide complex [32]. An attempt to reduce a carboxamide with $NaBH_4$/titanium isopropoxide

Table 1
Synthesis of 6-aralkylaminoquinoxalines. Comparison of methods

Compound	Method	Time (h)	Yield (%)
10a	$NaBH_4$ reduction of imine **9a**	8[a]	94
	Gabriel synthesis from **11e**	18[b]	90
10c	BH_3-THF reduction of amide **11d**	2[b]	91
	BMS	2[b]	63
	$NaBH_4$/AcOH "	8[b]	76
	$NaBH(OAc)_3$ reductive alkylation of **8**	1.5	60
	$B_{10}H_{14}$	18	90
	$NaBH_4/Ti(OiPr)_4$	No reaction	
10d	BMS reduction of amide **11b**[b]	2	58
	$NaBH(OAc)_3$ reductive alkylation	24	48
	$B_{10}H_{14}$	18	90

[a] Includes time necessary to make **9a**.
[b] Does not include reaction time generating and purifying the carboxamide.

[33] failed. Despite the success of these "proof of principle" preparations, reductive amination of an aldimine seemed best suited to a radiosynthesis, so we did not expand the carboxamide series. Trifluoroacetamide **11e** served as the starting material for a modified Gabriel synthesis [34]; alkylation with benzyl bromide followed by alkaline workup gave a good yield of 6-benzylamino compound **10a**. Table 2 lists the characteristics of novel compounds.

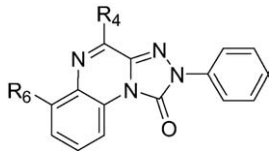
For the pharmacological evaluation of this series of compounds the known derivative **10a** was radiolabeled with tritium. When **9a** was reduced with [^{3}H]$NaBH_4$ under carefully controlled reaction conditions, [^{3}H]**10a** was obtained with a RCY of 12.5%, a radiochemical purity of > 98% and a specific activity of 5.6 Ci/mmol.

3. Pharmacology

Table 3 summarizes the studies of the affinity of rat A_1 and A_{2A}ARs for **10a**–**s**, and also measurements of an index of hydrophobicity. The compounds competed at both the A_1AR and the A_{2A}AR with K_is in the low-nanomolar range, but selectivity for the A_{2A}AR was low, at best just 3.6-fold (compound **10s**). While this work was in progress Colotta et al. [35] described the synthesis of compounds **10a**, **10c**, **10e**, **10g**, **10j**, **10m** and **10q** and their affinities for the bovine A_1AR and A_{2A}AR. That study showed that **10a** exhibited low-nanomolar affinity for the A_{2A}AR and > 110-fold less affinity at the A_1AR. Our measurements on rat brain receptors confirm that estimate of affinity for the A_{2A}AR, but we found that **10a** had a similar affinity for the A_1AR and, so, little selectivity. Species difference might account for that divergence. However, our results confirm their report of the low selectivity for the A_{2A}AR of the other compounds. In agreement with their high lipophilicities, the solubilities of **10a**–**s** in water were low, ~ 200 nM.

Autoradiographic studies employing [^{3}H]**10a** (Fig. 2) confirmed the lack of selectivity and the high unspecific binding seen in radioligand binding assays (data not shown). The uptake of [^{3}H]**10a** did not reflect the typical distribution in

Table 2
Characteristics of new compounds



Compounds	R_4	R_6	Yield (%)	Recryst	M.p. (°C)	Analytical formula
5	N_3	NO_2	92	THF	>300	$C_{15}H_8N_6O_3$ (C,H,N,O)
6	N=PPh_3	NO_2	94	THF	>300	$C_{33}H_{23}N_6O_3P$ (C,H,N,O)
7	NH_2	NO_2	92	THF/water	290–1	$C_{15}H_{10}N_6O_3$ (C,H,N,O)
8	NH_2	NH_2	100	MeOEtOH	222–3	$C_{15}H_{12}N_6O$ (C,H,N,O)
9a	NH_2	PhCH=N	100	MeCN	>300	$C_{22}H_{16}N_6O$ (C,H,N,O)
9b	NH_2	4-(F-EtO)PhCH=N	93	THF	>300	$C_{24}H_{19}N_6O_2$ (C,H,N,O)
10a	NH_2	$PhCH_2NH$	94	EtOH	213	$C_{22}H_{18}N_6O$ (C,H,N,O)
10b	NH_2	4-(F-EtO)$PhCH_2NH$	74	THF	201–2	$C_{24}H_{21}FN_6O_2$ (C,H,N,O)
10c	NH_2	$Ph(CH_2)_2NH$	90	MeCN	208–9	$C_{23}H_{20}N_6O$ (C,H,N,O)
10d	NH_2	$PhCHFCH_2NH$	90	MeCN	187–9	$C_{23}H_{19}FN_6O$ (C,H,N,O)
10e	NH_2	2-$FPhCH_2NH$	90	MeCN	210–3	$C_{22}H_{17}FN_6O$ (C,H,N,O)
10f	NH_2	3-$FPhCH_2NH$	82	MeCN	207–9	$C_{22}H_{17}FN_6O$ (C,H,N,O)
10g	NH_2	4-$FPhCH_2NH$	90	MeCN	202–4	$C_{22}H_{17}FN_6O$ (C,H,N,O)
10h	NH_2	2-$OHPhCH_2NH$	81	MeCN	198–9	$C_{22}H_{18}FN_6O_2$ (C,H,N,O)
10i	NH_2	3-$OHPhCH_2NH$	85	MeCN	201–2	$C_{22}H_{18}FN_6O_2$ (C,H,N,O)
10j	NH_2	4-$OHPhCH_2NH$	83	MeCN	213–5	$C_{22}H_{18}FN_6O_2$ (C,H,N,O)
10k	NH_2	2-$MeOPhCH_2NH$	87	MeCN	207–8	$C_{23}H_{20}N_6O_2$ (C,H,N,O)
10l	NH_2	3-$MeOPhCH_2NH$	91	MeCN	190–1	$C_{23}H_{20}N_6O_2$ (C,H,N,O)
10m	NH_2	4-$MeOPhCH_2NH$	90	MeCN	186–7	$C_{23}H_{20}N_6O_2$ (C,H,N,O)
10n	NH_2	2-F,4-$OHPhCH_2NH$	74	MeCN	219–21	$C_{22}H_{17}FN_6O_2$ (C,H,N,O)
10o	NH_2	2F,4MeO$PhCH_2NH$	84	MeCN	179–81	$C_{23}H_{19}FN_6O_2$ (C,H,N,O)
10p	NH_2	2F,5MeO$PhCH_2NH$	71	MeCN	173–5	$C_{23}H_{19}FN_6O_2$ (C,H,N,O)
10q	NH_2	2-$FurCH_2NH$	88	MeCN	204–5	$C_{20}H_{16}N_6O_2$ (C,H,N,O)
10r	NH_2	5-Br-2-$FurCH_2NH$	92	MeCN	193–4	$C_{20}H_{15}BrN_6O_2$ (C,H,N,O)
10s	NH_2	5-I-2-$FurCH_2NH$	90	MeCN	173–4	$C_{20}H_{15}IN_6O_4$ (C,H,N,O)
11a	NH_2	$PhCH(OCOCH_3)CONH$	95	MeOH	242	$C_{25}H_{20}N_6O_4$ (C,H,N,O)
11b	NH_2	PhCHFCONH	91	MeOH	219–21	$C_{23}H_{17}FN_6O_2$ (C,H,N,O)
11c	NH_2	CH_3CONH	83	THF/water	207–8	$C_{17}H_{14}N_6O_2$ (C,H,N,O)
11d	NH_2	$PhCH_2CONH$	93	THF-water	213–4	$C_{23}H_{18}N_6O_2$ (C,H,N,O)
11e	NH_2	CF_3CONH	87	EtOAc-Hex	222	$C_{17}H_{11}F_3N_6O_2$ (C,H,N,O)

Table 3
Adenosine receptor binding and hydrophobicity data

Compounds	K_i (nM)[a]			
	rA_1AR	$rA_{2A}AR$	$A_{2A}AR/A_1AR$	log k'w
8	19 (15–23)	8 (7–9)	0.42	3.37
9a	15 (12–19)	ND	ND	ND
10a	20 (10–39)	17 (12–23)	0.83	5.08
10b	20 (16–22)	19 (11–33)	0.99	5.02
10c	32 (22–47)	28 (21–37)	0.87	5.43
10d	33 (24–45)	25 (19–34)	0.76	5.31
10e	12 (9–15)	32 (24–42)	2.72	5.25
10f	22 (15–33)	24 (14–43)	1.10	5.17
10g	20 (15–280	30 (24–38)	1.47	4.90
10h	54 (42–69)	20 (14–29)	0.37	4.39
10i	12 (9–15)	20 (13–25)	1.75	4.46
10j	15 (12–18)	20 (13–31)	1.34	4.47
10k	22 (18–26)	33 (25–45)	1.54	5.10
10l	18 (13–24)	38 (20–67)	2.17	5.02
10m	22 (14–35)	29 (21–40)	1.30	5.01
10n	34 (21–55)	35 (28–45)	1.05	4.77
10o	33 (25–42)	87 (49–153)	2.66	5.29
10p	18 (9–36)	53 (23–121)	2.90	5.19
10q	8 (7–10)	17 (14–21)	2.14	4.73
10r	16 (12–20)	25 (18–33)	1.57	5.22
10s	14 (11–18)	51 (49–51)	3.65	5.46

[a] Mean and 95% confidence limits of three assays, each in triplicate. ND, not determined.

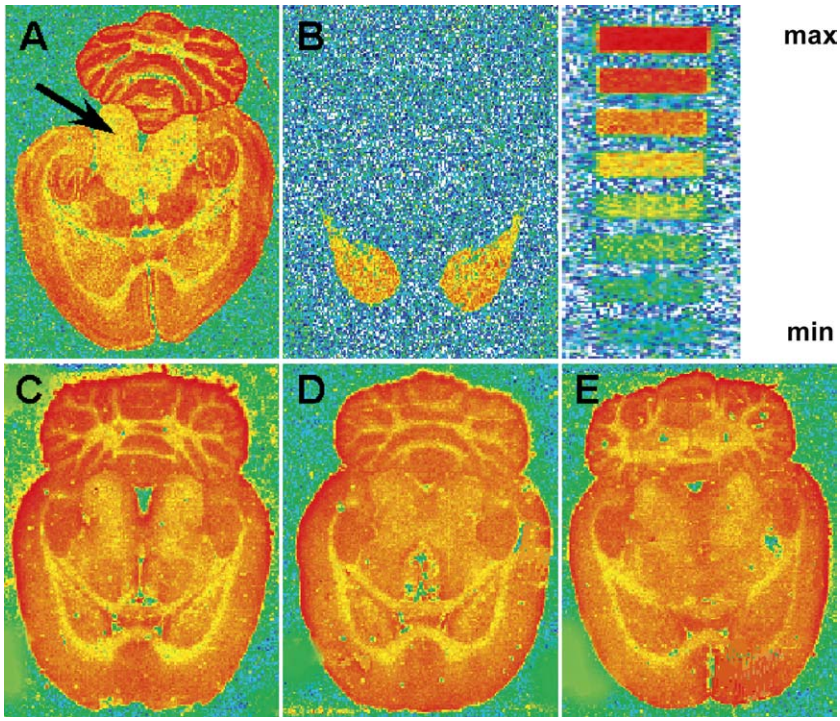


Fig. 2. Autoradiographs of consecutive horizontal sections of a rat brain incubated with: **A**, the A_1AR antagonist [^{3}H]DPCPX, which shows the expected distribution of tracer specifically bound to that receptor; **B**, the $A_{2A}AR$ antagonist [^{3}H]ZM241385, which shows the expected high density of this receptor in basal ganglia; **C**, [^{3}H]**10a**; **D**, [^{3}H]**10a** plus the $A_{2A}AR$ agonist CGS21680 (5 μM), and **E** [^{3}H]**10a** plus the A_1AR antagonist CPDPX (5 μM). The arrow in Panel **A** points to the inferior colliculus, an area of low A_1AR density. Note that in panels **C–E** this area is heavily labeled, indicative of a high degree of unspecific binding. Note also that neither CPDPX nor CGS21680 appreciably displaced [^{3}H]**10a** from areas of known high A_1AR or $A_{2A}AR$ density, respectively, additional evidence that unspecific binding obscured specific binding. The range of ligand density represented by the color scale at top right differs from figure to figure. Maximum density is 260 fmol/mg in panel **A**, 1700 fmol/mg in panel **B** and 5600 fmol/mg in panels **C–E**.

brain of either the A_1AR [36] or the A_{A2}AR [37]. Rather, there was a massive tracer accumulation in all brain regions having a high density of cell bodies. Likewise, neither the selective A_{A2}AR agonist CGS21680 nor the selective A_1AR antagonist DPCPX significantly displaced bound [^{3}H]**10a**. Similar results were obtained by blocking A_{2A}ARs or A_1ARs with either CGS21680 or DPCPX before incubation with [^{3}H]**10a**.

5. Conclusion

Although 2-phenyl-4-amino-6-arylamino-triazoloquinoxalines **10a–s** are potent antagonists at the A_{2A}AR, they lack selectivity, have poor water solubility and bind strongly to brain structures other than the A_{2A}AR. Thus, they are unsuited for imaging of the brain A_{2A}AR by PET. Aside from their unsuitability as PET ligands, the low selectivity of this class of compounds suggests that they might have important A_1AR-mediated side effects that could curtail their usefulness as drugs.

6. Experimental protocols

6.1. Chemistry

Melting points were measured on an Electrothermal™ apparatus and are uncorrected. The Zentralabteilung für chemische Analysen, Forschungszentrum Juelich, performed the elemental analyses, which were within ± 0.4% of the calculated composition. Thin layer chromatography (TLC) employed precoated silica sheets (4 × 8 cm, Polygram™, Macherey-Nagel, Düren) developed with one of the following mixtures (v/v) of ethyl acetate/hexane: 25:75 (Solvent A); 35:65 (Solvent B), or 50:50 (Solvent C). Mass spectra (MS), ESI, were obtained on a Finnigan Automass III mass spectrometer (Thermo Quest, Egelsbach). ^{1}H, ^{13}C and ^{19}F NMR spectra were obtained at 200.13, 50.32 and 188.31 MHz, respectively, by means of a Bruker DPX-200 spectrometer (Avance 200) in ≈ 5% solution in DMSO-d_6 at 25 °C. Chemical shifts are given in δ ppm using the residual proton and carbon resonances of DMSO-d_6 at 2.52 and 50.32 MHz, respectively, as references. The multiplicity symbols s, d, t and m refer to singlet, doublet, triplet and multiplet, respectively.

Solvents and reagents, which were of the highest grade available, were from either Sigma-Aldrich, Deisenhofen, Germany or Lancaster Synthesis, Muelheim am Main, Germany. *N*,*N*-Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled under argon and stored in lightproof containers over 4 Å molecular sieves. Dichloromethane (DCM) and 1,2-dichloroethane (DCE) were dried over 4 Å molecular sieves. Dry methanol, *n*-propanol and DMF (puriss., abs., over molecular sieves) were from Fluka, Buchs, Switzerland. Tocris-Cookson provided ZM241385, CGS21680, and CPDPX. GPP(NH)p and adenosine deaminase (AD) were from Sigma-Aldrich. Phosphate buffer (No. 82561) was from Fluka. Sep-Pak Plus C18 cartridges, Long Body, 360 mg, Part No. WAT023635, were obtained from Millipore Waters, Eschborn, Germany and were preconditioned with acetonitrile (20 ml) and water (20 ml) prior to use. Other solvents and reagents were used as supplied by the vendor. [^{3}H]$NaBH_4$ (150 mCi, 85 Ci/mmol) was from Biotrend, Cologne, Germany. [^{3}H]CPDPX and [^{3}H]CGS 21680 were from New England Nuclear, and [^{3}H]ZM241,385 was from Tocris. The synthesis of 5-iodofuran-2-carboxaldehyde was as described [38].

6.1.1. N-*Phenyl-C-ethoxycarbonylformohydrazidoyl chloride (**1**)*

Sodium acetate trihydrate (26 g, 191 mmol) was added to a solution of ethyl-2-chloroacetoacetate (27.6 ml, 200 mmol) in ethanol (500 ml) and the solution was cooled in an ice/salt bath to between –4 and 0 °C. A parallel reaction consisted of the addition of an ice-cold aqueous solution of 4 M $NaNO_2$ (13.8 g, 200 mmol), in H_2O (50 ml) to a solution of aniline (18.24 ml, 200 mmol) in 6 N HCl (120 ml), cooled in an ice/salt bath to –4 to 0 °C. A slow rate of addition kept the temperature below 0 °C. The cold solution of phenyldiazonium chloride was then added to the ester at a rate that kept the temperature between –4 and 0 °C. When the addition was complete, the yellow suspension was refrigerated for 3 h, diluted with ice/water (1 l) and again refrigerated for 3 h The product precipitated as glistening white crystals, pure by TLC (Solvent A), R_f (product) 0.65; (Solvent C), R_f (product) 0.91). Yield 44 g, 98%. M.p. 79–80 °C. Ref. [7] 79 °C.

*6.1.2. 8-Nitro-3-(phenyl-hydrazono)-3,4-dihydro-1H-quinoxalin-2-one (**2**)*

Compound **1** (22.6 g, 100 mmol) in EtOH (250 ml) was added to a mixture of 2,3-diamino nitrobenzene- (15.32 g, 100 mmol) and triethylamine (16.8 ml, 120 mmol) in EtOH (500 ml) and the dark red mixture was stirred at reflux for 4 h TLC (Solvent C) R_f (product) 0.00, extended spot, R_f (diamine) 0.52, R_f (chloride) 0.91. The dark crystals formed during storage overnight at –2 °C were filtered off, washed with water and ethanol and dried at 120 °C. Yield 22.2 g, 75%, m.p. 204 °C (EtOH/H_2O). Ref. [7] 205–7 °C (AcOH).

*6.1.3. 6-Nitro-2-phenyl-2H,5H-[1,2,4]triazolo[4,3-a]quinoxaline-1,4-dione (**3**)*

Under efficient stirring triphosgene (14.8 g, 50 mmol) was added in portions to a solution of **2** (14.87 g, 50 mmol) in anhydrous THF (250 ml). After 2 min the dark solution lightened and became turbid. The mixture was refluxed for 3 h, cooled to room temperature and then was held for 3 h at –2 °C. Decanting the supernatant into vigorously stirred ice/water (600 ml) destroyed unreacted triphosgene. The residual dark yellow solid was mixed with THF/water (50:50, v/v, 100 ml), filtered by suction, washed with ethanol and dried for 2 h at 120 °C. TLC (Solvent C) R_f (educt) 0.0, R_f (product) 0.72).

Yield 16 g, 100%. Recrystallization from CH_3CN/DMF (1/1) gave an analytical sample. M.p. 251 °C (CH_3CN/DMF); Ref. [7] 250–2 °C (EtOAc).

*6.1.4. 4-Chloro-6-nitro-2-phenyl-2H-[1,2,4]triazolo[4,3-a]-quinoxalin-1-one (**4**)*

PCl_5 (5.2 g, 25 mmol) was added to a suspension of **3** (8 g, 25 mmol) in $POCl_3$ (150 ml) under argon. Pyridine (1.5 ml, 18.75 mmol) was added and the mixture was stirred for 4 h in a 125–130 °C oil bath. In the first 30 min starting material completely dissolved and the solution turned light brown. Stirring continued as the mixture cooled to room temperature and it was set aside at 2 °C overnight. The canary-yellow crystals of product were filtered off, taken up in ethanol (200 ml) (CAUTION! exothermic!), filtered and oven-dried at 120 °C for 2 h TLC showed a single spot (Solvent C), R_f (starting material) 0.74, R_f (product) 0.82; (Solvent B), R_f (product) 0.51. Yield 7.6 g, 95%, m.p. > 300 °C. Recrystallization from acetone gave an analytical sample.

*6.1.5. 4-Azido-6-nitro-2-phenyl-2H-[1,2,4]triazolo[4,3-a]-quinoxalin-1-one (**5**)*

Adding NaN_3 (1.6 g, 24 mmol) in one portion to a suspension of **4** (6.83 g, 20 mmol) in dry DMF (150 ml) and stirring at room temperature dissolved starting material almost completely over 30 min. TLC (Solvent B), R_f (starting material) 0.51; R_f (product) 0.35; (Solvent C), R_f (educt) 0.81, R_f (product) 0.68). The product spot became yellow on heating. Pouring the reaction mixture into water (200 ml), precipitated the faintly yellow product, which was filtered off and air-dried, yield 6.7 g, 92%. Recrystallization from THF gave an analytical sample. M.p. > 300 °C (dec). ^{1}H NMR, δ: 7.47, m, 1H, H-9; 7.65, m, 2H, phenyl H; 8.08, M, 3H, phenyl H; 8.27, dd, 1H, H-8; 9.24, dd, 1H, H-7. ^{13}C NMR, δ: 113.88, 119.21, 120.46, 122.12, 127.05, 128.18, 130.52, 131.90, 132.02, 137.20, 141.02, 142.16, 144.88, 148.26. MS Calc. for $C_{15}H_8N_8O_3$, molecular weight 348.28: *m/z* 348 (M^+), 349 $(M + 1)^+$.

*6.1.6. 4-Amino-6-nitro-2-phenyl-2H- [1,2,4]triazolo[4,3-a]-quinoxalin-1-one (**7**). Method A, via an intermediate iminophosphorane (**6**)*

Triphenylphosphine (1.33 g, 5.06 mmol, 1.1 eq) was added in one portion to a suspension of compound **5** (1.61 g, 4.6 mmol) in THF (46 ml). Starting material dissolved during stirring at room temperature for 2 h and then at 50 °C for 30 min. After cooling to room temperature water (46 ml) was added, the mixture was stirred for 1.5 h, stored overnight at –30 °C, the yellow precipitate filtered off and oven-dried for 2 h at 120 °C. The material was pure by TLC (Solvent B), R_f (starting material) 0.35, R_f (product) 0.57). HRMS indicated formation of the iminophosphorane **6**, MW 582.55 g/mol. The yield was 2.51 g, 94%, m.p. > 300 °C. ^{1}H NMR, δ: 7.25–7.43, m, 2H, H-8 and H-9; 7.55–7.72, m, 12H, phenyl H; 7.83–7.94, m, 6H, phenyl H; 8.13, m, 2H, phenyl H; 7.79, dd, 1H, H-7. ^{13}C NMR, δ: 117.32, 119.84, 120.54, 126.24, 126.56, 127.25, 128.57, 129.68, 129.93, 129.96, 130.13, 133.61, 133.82, 136.09, 138.25, 144.88, 145.20, 149.41, 153.47, 153.56. MS Calc. for $C_{33}H_{23}N_6O_3P$, molecular weight 582.55: *m/z* 582 (M); 583 $(M + 1)^+$.

For conversion to **7**, compound **6** was dissolved in THF (10 ml/mmol) and 3 N HCl (2 ml/mmol) was added. The mixture was stirred at room temperature for 1 h; after 20 min a yellow precipitate formed. TLC (Solvent B) showed that starting material and product had the same R_f but different colors, R_f (starting material): 0.57, yellow spot, R_f (product): 0.57, colorless spot). The mixture was set aside overnight at –30 °C to give, after filtration and air-drying, **7** in quantitative yield as orange crystals, pure by TLC, m.p. 290–1 °C. Yield 1.19 g, 92%. ^{1}H NMR, δ: 7.29–7.57, m, 5H, H-9 and 4 phenyl H; 7.74, dd, 1H, H-8; 8.03, d, 1H, phenyl H; 8.29, br s, 2H, $–NH_2$; 8.74, dd, 1H, H-7. ^{13}C NMR, δ: 117.65, 120.15. 120.42, 123.21, 125.83, 127.36, 129.60, 130.04, 131.44, 137.84, 145.29, 148.81, 148.98. MS Calc. for $C_{15}H_{10}N_6O_3$, molecular weight 322.28: *m/z* 322 (M^+).

*6.1.7. 4-Amino-6-nitro-2-phenyl-2H-[1,2,4]triazolo[4,3-a]-quinoxalin-1-one (**7**). Method B, one pot procedure*

Triphenylphosphine (1.45 g, 5.5 mmol, 1.1 eq) was added in one portion to a suspension of compound **5** (1.74 g, 5 mmol) in THF (50 ml). The canary-yellow suspension was stirred at room temperature for 2 h and then at 50 °C for 30 min, during which the mixture became clear. After cooling to room temperature 3 N HCl (10 ml) was added. A yellow precipitate formed within 20 min during stirring at room temperature. Workup as in Method A gave orange crystalline product, 1.2 g, 75%, pure by TLC. Recrystallization from THF gave an analytical sample, m.p. 290–1 °C.

6.1.8. Attempted synthesis of 4-(tert-butoxycarbonyl)-amino-6-nitro-2-phenyl-2H-[1,2,4]triazolo[4,3-a]-quinoxalin-1-one

Treatment of **7** with 2.2 eq of Boc_2O and 10 mol% of DMAP in THF (6 h, 85 °C) or acetonitrile (20 h, 85 °C) gave only unreacted starting material.

6.1.9. Attempted synthesis of 4-benzylideneamino-6-nitro-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one

Refluxing **7** in trifluoroacetic anhydride or the $ZnCl_2$-catalyzed reaction of **7** with benzaldehyde in refluxing EtOH gave only starting material.

*6.1.10. 4,6-Diamino-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**8**). Method A. Reduction of **5***

Finely ground **5** (8.58 g, 24 mmol) suspended in 95% ethanol (200 ml) was stirred during the addition of a solution of $SnCl_2 \cdot 2H_2O$ (60 g, 264 mmol) in ethanol (150 ml). Heating to 105 °C (oil-bath temperature), gave a clear solution after 15 min. Stirring continued for 1.5 h, completing the reaction, as monitored by TLC in two systems. For TLC analysis aliquots of the reaction mixture were poured into THF. The addition of 32% NH_4OH precipitated $Sn(OH)_2$ as a fluffy white

solid; the yellow supernatant contained the product. Analysis by TLC (Solvent C), R_f (starting material) 0.68, R_f (product) 0.44; (Solvent B), R_f (starting material) 0.35, R_f (product) 0.16. Evaporation of the reaction mixture left an oily residue that was taken up in THF (250 ml). The addition of 32% ammonia (100 ml) precipitated $Sn(OH)_2$. The yellow organic phase was decanted and the inorganic salt was washed with THF (100 ml). The pooled organic phases were evaporated to a yellow solid; TLC revealed a trace of UV-absorbing fluorescent impurity of R_f 0.00. Taking up the solid residue in 10 M HCl (50 ml) and ethanol (50 ml) and stirring vigorously for 15 min dissolved the tin salts and precipitated **8**·HCl as a cream-colored solid. TLC (Solvent B), R_f: 0.00, fluorescent in UV light). Exposure to air and light changed the color of the salt from white to red. The precipitate was collected by centrifugation (10 min at 2600 × *g*) and suspended in aqueous 32% ammonia (150 ml). THF (250 ml) was added, the mixture was stirred in a beaker for 10 min, the phases were separated and the organic layer was washed with brine (2 × 100 ml). The clear yellow organic phase was dried over Na_2SO_4, filtered and taken to dryness. Trituration with hot methanol (40 ml), cooling overnight, filtration and oven drying for 1 h at 120 °C gave the product as dark yellow crystals in quantitative yield (6.9 g). Recrystallization from methoxyethanol (ca. 10 ml/g) gave an analytical sample, m.p. 222–3 °C; Ref. [7] 220–2 °C. ^{1}H NMR, δ: 5.37, br s, 2H, $-NH_2$; 6.67, d, 1H, H-9; 6.98, t, 1H, H-8; 7.35, m, 3H, $-NH_2$ and phenyl H; 7.55, t, 2H, phenyl H; 7.88, d, 1H, H-7; 8.17, m, 2H, phenyl H. ^{13}C NMR, δ: 102.67, 111.01, 120.27, 122.92, 124.69, 124.88, 127.07, 129.99, 132.33, 138.26, 143.58, 144.99, 149.39. MS, Calc. for $C_{15}H_{12}N_6O$, molecular weight 292.30: *m*/*z* 292 (M^+), 293 $(M + 1)^+$.

*6.1.11. 4,6-Diamino-2-phenyl-2H-[1,2,4]triazolo[4,3-a]-quinoxalin-1-one (**8**). Method B. Reduction of **7***

Compound **7** (483 mg, 1.5 mmol) and $SnCl_2 \cdot 2H_2O$ (1.7 g, 7.5 mmol) in dry EtOH (15 ml) was stirred in an oil bath heated to 90–100 °C. In 2 min starting material dissolved and by 5 min TLC (Solvent B), R_f (starting material) 0.57, R_f (product) 0.15) showed the reaction was over. Evaporation gave a yellow solid that was taken up in THF (10 ml). Dilution with water (30 ml) initiated precipitation of product, which continued overnight in the refrigerator.

*6.1.12. 4-Amino-6-(benzylidene-amino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**9a**)*

Benzaldehyde (112 μl, 1.1 mmol) and a catalytic amount of dry $ZnCl_2$ (ca. 100 mg) were added to a solution of **8** (292 mg, 1 mmol) in dry THF (15 ml). The suspension was stirred at 95–100 °C for 8 h; after 1 h product began to precipitate. TLC (Solvent B), R_f (educt) 0.15, R_f (product) 0.05. Cooling to room temperature gave a yellow solid that was filtered off, suspended in THF, filtered again and air-dried for 1 h, giving product as a light yellow powder in quantitative yield (380 mg, 100%, m.p. > 300 °C (dec). The product, which is a mixture of the *E*- and *Z*-isomers, can be recrystallized from CH_3CN.

*6.1.13. 4-Amino-6-{[4-(2-fluoro-ethoxy)benzylidene]-amino}-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**9b**)*

The reaction of 4-(2-fluoro-ethoxy)-benzaldehyde (370 mg, 2.2 mmol) and **8** (584 mg, 2 mmol) catalyzed by $ZnCl_2$ (ca. 100 mg) gave product. TLC (Solvent B), R_f (starting material) 0.15, R_f (product) 0.05. The orange solid was filtered off, suspended in THF and sonicated. Filtration and air-drying for 1 h gave the product as yellow crystals. Yield 830 mg, 93%, m.p. > 300 °C (dec). ^{1}H NMR, δ: 4.24–4.46, m, 2H, $-OCH_2-$; 4.63–4.98, m, 2H, $-CH_2F$; 7.06, d, 1H, H-9; 7.15, d, 2H, 2 phenyl H; 7.25–7.44, m, 2H, H-8 and phenyl H; 7.54–7.62, m, 4H, $-NH_2$ and 2 phenyl H; 7.94, m, 2H, 2 phenyl H; 8.09, m, 2H, H-7 and phenyl H; 8.63, m, 2H, –C*H*N– and phenyl H. ^{13}C NMR, δ: 68.56 (benzylidene), 81.53, 84.69, 102.72, 111.09, 115.58, 115.86, 120.30, 120.35, 122.96, 124.69, 124.89, 125.41, 127.11,130.02, 130.07, 130.77, 132.33, 132.70, 138.19, 138.25, 143.48, 145.02, 149.40, 163.95, 192.22. ^{19}F NMR, δ: –222.57, –222.76. MS, Calc. for $C_{24}C_{19}FN_6O_2$, molecular weight 442.45: compound decomposed to **8** during analysis.

*6.1.14. 4-Amino-6-benzylamino-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10a**). Method A. $NaBH_4$ reduction of **9a***

A suspension of **9a** (190 mg, 0.5 mmol) in dry MeOH (10 ml) containing $NaBH_4$ (57 mg, 1.5 mmol) was stirred for 5 min in an 80–90 °C oil bath, when TLC showed the reaction was complete (Solvent B), R_f (starting material) 0.05, R_f (product) 0.55. After cooling to room temperature the mixture was refrigerated for 1 h Filtration of the yellow solid, washing with MeOH and oven drying for 1 h at 120 °C gave the product, 180 mg (94%), m.p. 218 °C (EtOH). Ref. [7] 215–7 °C (ethanol/ethyl acetate). ^{1}H NMR, δ: 4.45, d, 2H, $-CH_2-$; 6.03, t, 1H, –NH–; 6.56, d, 1H, H-9; 7.06, t, 1H, H-8; 7.26–7.43, m, 8H, $-NH_2$ and 6 phenyl H; 7.57, m, 2H, phenyl; 7.92, d, 1H, H-7; 8.09, d, 2H, phenyl. ^{13}C NMR, δ: 47.63 (benzylic), 102.60, 106.94, 120.29, 122.95, 124.40, 124.83, 127.13, 127.83, 128.28, 129.36, 130.02, 132.31, 138.25, 140.62, 143.09, 144.88, 145.34, 149.39. MS, Calc. for $C_{22}H_{18}N_6O$, molecular weight 382.42: *m*/*z* 382 (M^+).

*6.1.15. 4-Amino-6-benzylamino-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10a**). Method B. Alkylation of **11e** (modified Gabriel synthesis)*

Under argon, NaH (60%, 21 mg, 0.525 mmol) was suspended in dry DMF (2 ml). A solution of **11e** (194 mg, 0.5 mmol) in dry DMF (2 ml) was added dropwise by syringe and the clear brownish solution was stirred for 1 h at room temperature. Benzyl bromide (66 μl, 0.55 mmol) in dry DMF (1 ml) was added dropwise and the mixture was stirred for 18 h at 70–80 °C. MeOH (4 ml) and NaOH (2 M, 1 ml, 2 mmol) was added and the mixture was stirred at 70–80 °C for 10 min longer. Dropwise addition of water (2–3 ml) to the hot, well stirred solution caused the precipitation of a yellow solid. After cooling to room temperature the solid was fil-

tered off, washed with MeOH (2–3 ml) and triturated twice with hot MeOH (4–5 ml). Filtration, air-drying and crystallization from CH_3CN yielded 172 mg, 90%, of yellow crystals, m.p. 216–8 °C (CH_3CN).

*6.1.16. 4-Amino-6-[4-(2-fluoro-ethoxy)benzylamino]-2-phenyl-2H- [1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10b**)*

Reduction of **9b** (442 mg, 1 mmol) in dry MeOH (20 ml) with 3 equiv. $NaBH_4$ for 10 min at 80–90 °C then cooling on ice gave product. TLC (solvent B) R_f (starting material) 0.05, R_f (product) 0.40. Yield 330 mg, 74%, m.p. 201–2 °C. ^{1}H NMR, δ: 4.13–4.88, M, 6H, –CH_2CH_2F and –CH_2–; 5.91, t, 1H, –*NH*–; 6.57, d, 1H, H-9; 6.89–7.10, m, 4H, H-8 and 3 phenyl H; 7.35, m, 5H, –NH_2 and H-8 + 2 phenyl H; 7.57, t, 2H, phenyl H; 7.88, d, 1H, H-7; 8.07, d, 2H, phenyl H.. ^{13}C NMR, δ: 47.09 (benzylic), 67.08, 68.08, 81.40, 84.72, 102.55, 106.90, 115.34, 120.25, 122.90, 124.36, 124.82, 127.09, 129.70, 130.00, 132.28, 132.77, 138.25, 143.09, 144.88, 145.30, 149.36, 158.12. ^{19}F NMR, δ: – 222.45. MS, Calc. for $C_{24}H_{19}FN_6O_2$, molecular weight 444.46: *m*/*z* 444 (M^+).

*6.1.17. 4-Amino-6-(2-phenyl-ethylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10c**).*
*Method A. Borane-THF reduction of **11d***

Under argon and at room temperature, BH_3-THF (5.1 ml of a 1 M solution of BH_3 in THF, 5.1 mmol) was added over 5 min to a slurry of **11d** (410 mg, 1 mmol) in dry THF. Educt dissolved during the addition, giving a clear brown solution. The mixture was stirred for 2 h, when TLC indicated the complete disappearance of educt. Water (600 µl) was carefully added (gas evolution!) followed by 10% HCl (1100 µl). After stirring for another 15 min the volatiles were evaporated and the aqueous residue was made alkaline by the addition of 2 M NaOH. The mixture was extracted with ethyl acetate (2 × 30 ml), the pooled organic phases washed with brine (2 × 30 ml), dried over anhydrous Na_2SO_4 and evaporated to give a yellow semisolid residue. Coevaporation with MeOH (20 ml) left a yellow solid which was triturated twice with hot MeOH and recrystallized twice from CH_3CN (~8 ml) by refluxing and cooling to –30 °C). Yield 362 mg (91%) of golden yellow crystals, m.p. 209–10 °C (CH_3CN). ^{1}H NMR, δ: 2.93, t, 2H, –CH_2–; 3.38, m, 2H, –CH_2NH–; 5.66, t, 1H, –CH_2N*H*–; 7.11–7.61, m, 11 H, –NH_2, H-9, H-8 and 8 phenyl; 7.94–8.10, m, 3H, H-7, phenyl H. ^{13}C NMR, δ: 45.29 (benzylic), 102.36, 106.55, 119.10, 120.30, 122.80, 124.40, 125.02, 127.01, 127.10, 129.27, 129.60, 130.01, 132.28, 138.25, 140.43, 143.09, 144.88, 145.18, 149.38. MS, Calc. for $C_{23}H_{20}N_6O$, molecular weight 396.44: *m*/*z* 396 (M^+).

*6.1.18. 4-Amino-6-(2-phenyl-ethyamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10c**).*
*Method B. Borane-dimethyl sulfide (BMS) reduction of **11d***

At 0 °C under argon, BMS (10 M, 210 µl, 2.1 mmol, 2.1 eq) was added dropwise to a slurry of **11d** (410 mg, 1 mmol) in dry toluene (4 ml). The suspension was stirred at 0 °C for 15 min and at reflux (oil bath 125–130 °C) for 2 h. The oil bath was removed and saturated Na_2CO_3 (6 ml) was added carefully. After cooling to room temperature the reaction mixture was stirred for another 30 min and poured into MeOH (40 ml). Precipitated product was filtered off. Three triturations with hot methanol (10 ml) removed a lipophilic yellow impurity. After filtration the solid was extracted twice into DMF (15 ml), insoluble impurities filtered off and product precipitated by the addition of water (20–30 ml). The amine collected by centrifugation was recrystallized from CH_3CN as yellow crystals. Yield 250 mg, 63%, m.p. 208–10 °C.

*6.1.19. 4-Amino-6-(2-phenyl-ethylamino)-2-phenyl-2H-1,2,4-triazolo[4,3-a]quinoxalin-1-one (**10c**).*
*Method C. $NaBH_4$/acetic acid reduction of **11d***

Under ice cooling acetic acid (148 µl, 2.5 mmol) in dry THF (1 ml) was added dropwise over 2 min to a slurry of **11d** (205 mg, 0.5 mmol) and sodium borohydride (95 mg, 2.5 mmol) in dry THF (4 ml). The suspension was stirred at 0 °C for 5 min and at reflux (85–95 °C oil bath) for 8 h. After the reaction mixture cooled to room temperature, poured into 0.1 M NaOH (25 ml) and extracted with DCM (2 × 50 ml). The organic phase was washed with water (2 × 50 ml), dried over Na_2SO_4 and DCM was rotary evaporated. Crystals formed when the yellow solid residue was triturated with methanol (5 ml). The solid was triturated twice more with hot methanol (2 × 5 ml), product was filtered off and air dried to give 150 mg, 76% of product as yellow crystals, m.p. 208–10 °C (CH_3CN).

*6.1.20. 4-Amino-6-(2-fluoro-2-phenyl-ethylamino)-2-phenyl-2H-1,2,4-triazolo[4,3-a]quinoxalin-1-one (**10d**)*

A slurry of **11b** (428 mg, 1 mmol) in dry toluene (4 ml) was treated with boron-methyl sulfide complex (10 M, 210 µl, 2.1 mmol, 2.1 eq). The suspension was stirred at 0 °C for 15 min and at reflux (125–130 °C oil bath) for 2 h. The oil bath was removed and sat. Na_2CO_3 (6 ml) was added carefully. The reaction mixture was cooled to room temperature and the orange suspension was stirred for 30 min longer. The mixture was poured into MeOH (60 ml) and precipitated salts were filtered off. Concentration of the methanolic solution to dryness gave a solid that was purified by recrystallization from CH_3CN. Yield 378 mg, 58%, m.p. 187–8 °C (CH_3CN). ^{1}H NMR, δ: 3.57–3.83, m, 2H, CHFCH_2–; 5.69, t, 1H, –NH–; 5.90, t, 1H, –C*H*F–; 6.67, d, 1H, H-9; 7.12, t, 1H, H-8; 7.37–7.62, m, 10H, –NH_2– + 8 phenyl; 7.96, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 60.64 (benzylic), 120.31, 123.07, 124.46, 126.79, 126.92, 129.18, 129.38, 130.03, 138.24, 145.39. ^{19}F NMR, δ: – 180.04. MS, Calc. for $C_{23}H_{19}FN_6O$, molecular weight 414.43: *m*/*z* 415 $(M + 1)^+$.

*6.1.21. Reductive alkylation of **8** using $NaBH(OAc)_3$. General method*

Diamine **8** (292 mg, 1 mmol) was suspended in dry solvent (10 ml), a solution of the aldehyde (1.05 mmol) in solvent (5 ml) was added, followed by solid $NaBH(OAc)_3$ (300 mg, 1 mmol). The mixture was stirred at room tempera-

ture for the specified time. Reaction monitoring by TLC employed Solvent B.

*6.1.22. 4-Amino-6-[4-(2-phenylethylamino]-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10c**)*

Phenylacetaldehyde reacted with **8** in THF for 1.5 h according to the general method. TLC: R_f 0.61. Adding ethanol (10 ml) to the mixture precipitated an impurity that centrifugation removed. Evaporation gave a solid that was purified by MPLC (silica, solvent B). Evaporation of the fraction that contained product gave yellow crystals for recrystallization from CH_3CN. Yield: 240 mg, 60%, m.p. 208–9 °C.

*6.1.23. 4-Amino-6-[4-(2-fluoro-2-phenylethylamino]-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10d**)*

The reaction of **8** with (±)-α-fluorophenylacetaldehyde in 1,2-DCE required 24 h TLC: R_f (product) 0.54, R_f (aldehydes) 0.69. The turbid yellow reaction mixture was centrifuged and the supernatant evaporated. The semi-solid residue was purified by MPLC (silica, solvent B), Evaporation of the fraction containing the product gave a solid residue for recrystallization from CH_3CN. Yield: 300 mg, 48%, m.p. 187–9 °C (CH_3CN).

*6.1.24. Attempted synthesis of 4-amino-6-[4-(2-phenylethylamino]-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10c**) using $Ti(O^iPr)_4/NaBH_4$*

Diamine **8** (72 mg, 0.25 mmol), phenylacetaldehyde (33 μl, 0.25 mmol) and $Ti(O^iPr)_4$ (148 μl, 0.5 mmol) in dry EtOH (2 ml) were stirred under argon at room temperature for 3 h $NaBH_4$ (28 mg, 0.75 mmol) was added and the mixture was stirred for 3 h more. After hydrolysis with water TLC showed only **8**.

*6.1.25. Reductive amination of **8** using decaborane in methanol. General method*

Under argon at room temperature **8** (292 mg, 1 mmol) was suspended in dry MeOH (15 ml). The aldehyde (1.2 mmol) in MeOH (1–2 ml) was added followed by solid decaborane (36 mg, 0.3 mmol). The mixture was stirred at room temperature for the specified time. In every instance TLC employed Solvent B (R_f (diamine) 0.16). In order to decompose excess borane, acetone (5 ml) and water (5 ml) were added and stirring continued until gas evolution stopped (2 h). The solvents were decanted, the crude product was suspended in MeOH (10 ml) and the MeOH evaporated. The text below describes the work-up of individual preparations. Unless noted, MeCN was the recrystallization solvent.

*6.1.26. 4-Amino-6-[4-(2-phenylethylamino]-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10c**)*

The general method reacted **8** with phenylacetaldehyde for 18 h (TLC: R_f 0.61). Product was triturated twice with hot MeOH and filtered. The yellow solid product was recrystallized from CH_3CN. Yield: 356 mg, 90%, m.p. 208–9 °C.

*6.1.27. 4-Amino-6-[4-(2-fluoro-2-phenylethylamino]-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10d**)*

The general method reacted **8** with (±)-α-fluorophenylacetaldehyde for 18 h (TLC: R_f 0.54). The solid residue was triturated twice with CH_3CN (2 × 5 ml), filtered and recrystallized from CH_3CN. Yield: 372 mg, 90%, m.p. 187–9 °C.

*6.1.28. 4-Amino-6-(2-fluorobenzylamino-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10e**)*

Compound **8** reacted with 2-fluorobenzaldehyde for 3 h (TLC: R_f 0.48). The crude product was triturated with hot MeOH (5 ml), cooled to 0 °C for 6 h and the yellow solid filtered and air-dried. Yield: 360 mg, 90%, m.p. 201–3 °C. ^{1}H NMR, δ: 4.52, d, 2H, –CH_2–; 6.01, t, 1H, –NH–, 6.59, d, 1H, H-9; 7.07–7.61, 10 H, –NH_2, H-8, 7 phenyl H; 7.94, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 46.97 (benzylic), 102.77, 106.99, 114.28, 114.47, 114.69, 114.89, 120.30, 124.03, 124.08, 124.81, 127.14, 130.03, 131.20, 131.37. ^{19}F NMR, δ: – 119.35. MS, Calc. for $C_{22}H_{17}FN_6O$, molecular weight 400.41: *m*/*z* 400 (M^+).

*6.1.29. 4-Amino-6-(3-fluorobenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10f**)*

The reaction of **8** with 3-fluorobenzaldehyde required 6 h (TLC: R_f 0.52). Yield: 328 mg, 82%, m.p. 207–9 °C. ^{1}H NMR, δ: 4.50, d, 2H, –CH_2–; 6.12, t, 1H, –NH–, 6.53, t, 1H, H-9; 7.05–7.58, 10 H, –NH_2, H-8, 7 phenyl H; 7.92, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 46.97 (benzylic), 102.77, 106.99, 114.28, 114.47, 114.69, 114.89, 120.30, 124.03, 124.08, 124.81, 127.14, 130.03, 131.20, 131.37. ^{19}F NMR, δ: 119.35. ^{19}F NMR, δ: – 113.80. MS, Calc. for $C_{22}H_{17}FN_6O$, molecular weight 400.41: *m*/*z* 400 (M^+), 401 $(M + 1)^+$.

*6.1.30. 4-Amino-6-(4-fluorobenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10g**)*

Compound **8** reacted with 4-fluorobenzaldehyde in 3 h (TLC: R_f 0.46). The solid residue was triturated with hot MeOH (5 ml), cooled to 0 °C for 6 h, the yellow solid filtered and air-dried. Yield: 360 mg, 90%, m.p. 202–4 °C. ^{1}H NMR, δ: 4.46, s, 2H, –CH2–; 6.07, br s, 1H, –NH–; 6.54, d, 1H, H-9; 7.12, t, 1 h H-8; 7.21, t, 2H, phenyl H; 7.40, m, 5H, –NH_2 + 3 phenyl H; 7.57, t, 2H, phenyl H; 7.94, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 46.76 (benzylic), 102.68, 106.98, 115.86, 116.28, 120,30, 122.98, 124.42, 124.82, 127.15, 130.4, 130.21, 132.30, 136.80, 136.86, 138.24,142.92, 145.35, 149.39. ^{19}F NMR, δ: – 116.48. MS, Calc. for $C_{22}H_{17}FN_6O$, molecular weight 400.41: *m*/*z* 400 (M^+).

*6.1.31. 4-Amino-6-(2-hydroxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10h**)*

The reaction of **8** with 2-hydroxybenzaldehyde went to completion in 4 h (TLC: R_f 0.31). Yield: 323 mg, 81%, m.p. 198–9 °C. ^{1}H NMR, δ: 4.30, m, 2H, –CH_2–; 5.89, t, 1H, –NH–; 6.61, m, 3H, H-9 and 2 phenyl H; 6.77–6.90, m, 3H, H-8 and 2 phenyl H; 7.03–7.38, m, 7H, –NH_2 and 5 phenyl

H; 7.92, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 63.68 (benzylic), 102.34, 106.76, 115.97, 119.74, 120.27, 122.92, 124.39, 124.60, 124.89, 126.09, 127.09, 128.96, 129.79, 129.99, 130.97, 132.31, 138.13, 138.26, 143.35, 144.88, 145.26, 149.39, 156.26, 157.09. MS, Calc. for $C_{22}H_{18}N_6O_2$, molecular weight 398.42. *m/z* 398 (M^+), 399 $(M + 1)^+$.

*6.1.32. 4-Amino-6-(3-hydroxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10i**)*

The reaction of 3-hydroxybenzaldehyde with **8** required 4 h (TLC: R_f 0.33). Yield: 338 mg, 85%, m.p. 201–2 °C. ^{1}H NMR, δ: 4.33,t, 2H, –CH_2–; 5.97, t, 1H, –NH–; 6.54, d, 1H, H-9; 6.83, m, 1H, phenyl H; 7.01–7.16, m, 2H, phenyl H, 7.36, m, 3H, H-8 and 2 phenyl H; 7.56, m, 2H, phenyl H; 7.92, d, 1H, H-7; 8.09, d, 2H, phenyl H; 9.40, s, 1H, –OH. ^{13}C NMR, δ: 49.49 (benzylic), 102.53, 106.90, 114.80, 114.95, 118.87, 120.28, 122.90, 124.38, 124.84, 127.22, 130.01, 130.35, 132.31, 138.26, 142.10, 143.13, 145.32, 149.39, 158.42. MS, Calc. for $C_{22}H_{18}N_6O_2$, molecular weight 398.42: *m/z* 398 (M^+), 399 $(M + 1)^+$.

*6.1.33. 4-Amino-6-(4-hydroxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10j**)*

The reaction of p-hydroxybenzaldehyde with **8** required 4 h (TLC: R_f 0.28). The solid residue was triturated with hot MeOH (5 ml), cooled to 0 °C for 6 h and the yellow solid filtered and air-dried. Yield: 330 mg, 83%, m.p. 213–5 °C. ^{1}H NMR, δ: 4.29, d, 2H, –CH_2–; 5.80, t, 1H, –NH–; 6.58, d, 1H, H-9; 6.76, m, 2H, H-8; 7.03–7.61, m, 11 H, –NH_2 + 9 phenyl H; 8.09, d, 1H, H-7; 9.38, s, 1H, –OH. ^{13}C NMR, δ: 47.37 (benzylic), 102.46, 106.88, 115.80, 116.12, 120.31, 124.85, 127.14, 129.83, 130.03,130.25. MS, Calc. for $C_{22}H_{18}N_6O_2$, molecular weight 398.42: *m/z* 398 (M^+), 399 $(M + 1)^+$.

*6.1.34. 4-Amino-6-(2-methoxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10k**)*

The reaction of 2-methoxybenzaldehyde with **8** required 4 h (TLC: R_f 0.48). Yield: 359 mg, 87%, m.p. 207–8 °C. ^{1}H NMR, δ: 3.87, s, 3H, –OCH_3; 4.40, t, 2H, –CH_2–; 5.96, br s, 1H, –NH–; 6.55, d, 2H, H9 and phenyl H; 6.90, t, 1H, H-8; 7.06, t, 2H, phenyl H; 7.23–7.40, m, 5H, –NH_2 and 3 phenyl H; 7.57, t, 2H, phenyl H; 7.92, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 56.28 (benzylic), 102.47, 106.84, 111.67, 120.29, 121.10, 122.99, 124.41 12487, 127.12, 127.76, 129.19, 129.36, 130.02, 132.32, 138.26, 143.21, 144.88, 145.30, 149.40, 158.03. MS, Calc. for $C_{23}H_{20}N_6O_2$, molecular weight 412.44: *m/z* 412 (M^+), 413 $(M + 1)^+$.

*6.1.35. 4-Amino-6-(3-methoxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10l**)*

The reaction of 3-methoxybenzaldehyde with **8** required 2 h (TLC: R_f 0.51). Yield: 375 mg, 91%, m.p. 190–1 °C. ^{1}H NMR, δ: 3.71, s, 3H, –OCH_3; 4.44, m, 2H, –CH_2–; 6.03, t, 1H, –NH–; 6.58, d, 1H, H-9; 6.88, m, 1H, H-8; 7.03, m, 3H, phenyl H; 7.24–7.40, m, 4H, –NH_2 and 2 phenyl H; 7.57, m, 2H, phenyl H; 7.93, m, 1H, H-7; 809, m, 2H, phenyl H. ^{13}C NMR, δ: 55.85 (benzylic), 113.05, 113.94, 120.31, 122.97, 124.40, 130.03, 130.45, 138.26, 142.35, 143.11, 145.36, 149.41, 160.32. MS, Calc. for $C_{23}H_{20}N_6O_2$, molecular weight 412.44: *m/z* 412 (M^+), 413 $(M + 1)^+$.

*6.1.36. 4-Amino-6-(4-methoxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10m**)*

Compound **8** required 4 h to react with 4-methoxybenzaldehyde (TLC: R_f 0.44). The solid residue was triturated with hot MeOH (5 ml), cooled to 0 °C for 6 h and the yellow solid was filtered and air-dried. Yield: 370 mg, 90%, m.p. 186–7 °C. ^{1}H NMR, δ: 3.75, s, 3H, –OCH_3; 4.37, t, 2H, –CH_2–; 5.90, t, 1H, –NH–; 6.59, m, 1H, H-9; 6.93, m, 2H, phenyl H; 7.06, t, 1H, H-8; 7.35, m, 5H, –NH_2 and 3 phenyl H; 7.57, m, 2H, phenyl H; 7.92,d, 1H, H-7, 8.10,d, 2H, phenyl H. ^{13}C NMR, δ: 55.92 (benzylic), 102.53, 106.93, 114.75, 120.28, 122.90, 124.37, 124.83, 127.12, 129.66, 130.01, 132.30, 132.31, 138.25, 143.12, 144.88, 145.31, 149.39, 155.30. MS, Calc. for $C_{23}H_{20}N_6O_2$, molecular weight 412.44: *m/z* 412 (M^+), 413 $(M + 1)^+$.

*6.1.37. 4-Amino-6-(2-fluoro-4-hydroxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10n**)*

The reaction of **8** and 2-Fluoro-4-hydroxybenzaldehyde required 7 h (TLC: R_f 0.28). The residue was triturated with hot MeOH (5 ml), cooled to 0 °C for 6 h and the yellow solid was filtered and air-dried. Yield: 308 mg, 74%, m.p. 219–21 °C. ^{1}H NMR, 413, m, 2H, –CH_2–; 580, t 1H, –NH–; 661, m, 3H, H-9 and 2 phenyl H; 709, t, 1H H-8; 733, m, 4H, –NH2 and 2 phenyl; 757, m, 2H, phenyl H, 791, d, 1H, H-7; 809, d, 2H, phenyl H; 987, s, 1H, –OH. ^{13}C NMR, δ: 41.24 (benzylic) 102.70, 103.24, 103.71, 106.72, 112.27, 112.32, 116.73, 117.04, 120.27, 122.94, 124.38, 124.84, 127.10, 129.99, 131.53, 131.66, 132.28, 138.24, 142.87, 145.36, 149.36, 158.96, 159.19, 159.51, 164.35. ^{19}F NMR, δ: – 118.04; MS, Calc. for $C_{22}H_{17}FN_6O_2$, molecular weight 416.41: *m/z* 416 (M^+).

*6.1.38. 4-Amino-6-(2-fluoro-4-methoxybenzylamino)-2-phenyl-2H- [1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10o**)*

Compound **8** reacted with 2-fluoro-4-methoxybenzaldehyde in 4 h (TLC: R_f 0.46). The solid residue was triturated with hot MeOH (5 ml), cooled to 0 °C for 6 h, the yellow solid filtered and air-dried. Product was recrystallized from CH_3CN. Yield: 362 mg, 84%, m.p. 179–81 °C. ^{1}H NMR, δ: 3.25, s, 3H, –OCH_3; 4.39, t, 2H, –CH_2–; 5.88, t, 1H, –NH–; 6.60, d, 1H, H-9; 6.73, m. 2H, phenyl H; 7.07, t, 1H, H-8; 7.35, m, 4H, –NH_2 and 2 phenyl H; 7.55, m, 2H, phenyl H; 7.92, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 56.43 (benzylic), 102.18, 102.69, 102.75, 106.76, 110.95, 111.01, 118.52, 118.83, 120.26, 120.40, 124.82, 127.11, 130.00, 131.32, 131.45, 132.25, 132.28, 138.24, 124.79, 145.33, 14540, 149.37, 159.49, 160.60, 160.82, 164.33. ^{19}F NMR, δ: – 117.26. MS, Calc. for $C_{23}H_{19}FN_6O_2$, molecular weight 430.43: *m/z* 430 (M^+), 431 $(M + 1)^+$.

*6.1.39. 4-Amino-6-(2-fluoro-5-methoxybenzylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10p**)*

Compound **8** reacted in 6 h with 2-fluoro-5-methoxybenzaldehyde (TLC: R_f 0.46). Yield: 305 mg, 71%, m.p. 173–5 °C. ^{1}H NMR, δ: 3.68, s, 3H, –OCH_3; 4.46, t, 2H, 1-CH_2–; 6.00, t, 1H, –NH–; 6.58, d, 1H, H-9; 6.56–7.14, m, 5H, –NH_2 and 3 phenyl H; 7.34–7.59, m, 4H, H-8 and 3 phenyl H; 7.93, d, 1H, H-7; 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 56.20 (benzylic), 102.91, 106.84, 113.72, 113.88, 115.77, 115.86, 116.44, 116.90, 120.24, 123.09, 124.43, 124.81, 127.08, 127.96, 128.29, 129.98, 132.28, 138.24, 142.74, 145.35, 145.40, 149.35, 153.23, 156.28, 156.32, 157.92. ^{19}F NMR, δ: – 130.24, MS, Calc. for $C_{23}H_{19}FN_6O_2$, molecular weight 430.43: *m/z* 430 (M^+), 431 $(M + 1)^+$.

*6.1.40. 4-Amino-6-furan-2-ylmethylamino-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10q**)*

The reaction of furan-2-carboxaldehyde with **8** required 2 h (TLC: R_f 0.50). Yield: 328 mg, 88%, m.p. 204–5 °C. ^{1}H NMR, δ: 4.46, m, 2H, –CH_2–; 5.88, t, 1H, –NH–; 6.22–6.43, M, 2H, H-9 and furyl H; 6.72, d, 1H, furyl H-; 7.10, t, 1H, H-8; 7.34, m, 3H, furyl H and 2 phenyl H; 7.68,m, 3H, –NH_2 and phenyl H; 7.94, d, 1H, H-7; 8.09, d, 2H, phenyl. ^{13}C NMR, δ: 40.75 (benzylic); 102.94, 107.06, 108.20, 111.32, 120.30, 123.09, 124.40, 124.76, 127.13, 130.14, 132.29, 138.24, 142.65, 143.20, 145.41, 149.38, 153.64. MS, Calc. for $C_{20}H_{16}N_6O_2$, molecular weight 372.38: *m/z* 372 (M^+).

*6.1.41. 4-Amino-6-(5-bromofuran-2-ylmethylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10r**)*

The reaction of 5-bromofuran-2-carboxaldehyde with **8** required 2 h (TLC: R_f 0.53). Yield: 415 g, 92%, m.p. 193–4 °C. ^{1}H NMR, δ: 4.46, t 2H, –CH_2–; 5.92, br s, 1H, –NH–; 6.45, m, 2H, H-9 and furyl H; 6.69, d, 1H. furyl H; 708, t, 1H, H-8; 7.35, m, 3H, –NH_2 and phenyl H; 7.58, m, 2H, phenyl; 7.94, d, 1H, H-7; 8.08, d, 2H, phenyl H. ^{13}C NMR, δ: 40.61 (benzylic), 103.08, 107.04, 111.27, 113.18, 120.28, 120.89, 123.13, 124.43, 124.72, 127.11, 130.00, 132.27, 138.23, 142.36, 144.88, 145.42, 149.35, 156.25. MS, Calc. for $C_{20}H_{15}BrN_6O_2$, molecular weight 451.28: *m/z* 451 (M^+).

*6.1.42. 4-Amino-6-(5-iodofuran-2-ylmethylamino)-2-phenyl-2H-[1,2,4]triazolo[4,3-a]quinoxalin-1-one (**10s**)*

The reaction of 5-iodofuran-2-carboxaldehyde [38] with **8** required 2 h (TLC: R_f 0.55). Yield: 448 mg, 90%, m.p. 173–4 °C. ^{1}H NMR, δ: 4.48, m, 2H, –CH_2–; 5.92, br s, 1H, –NH–; 6.35, t, 1H, furyl H; 6.65, d, 2H, H-9 and furyl H; 7.09, t, 1H, H-8; 7.38, m, 3H, –NH_2 and phenyl H; 7.67, m, 2H, phenyl H; 7.93, d, 1H, H-7, 8.09, d, 2H, phenyl H. ^{13}C NMR, δ: 40.61 (benzylic), 91.11, 103.03, 107.01, 111.43, 120.28, 121.45, 123.09, 124.42, 124.77, 127.11, 130.00, 132.27, 138.22, 142.40, 145.41, 149.35, 156.24, 159.15. MS, Calc. for $C_{20}H_{15}IN_6O_2$, molecular weight 498.28: *m/z* 498 (M^+).

*6.1.43. Acetic acid (4-amino-1-oxo-2-phenyl-1,2-dihydro-[1,2,4]triazolo[4,3-a]quinoxalin-6-ylcarbamoyl)-1-phenyl-methyl ester (**11a**). General method C*

O-Acetyl mandelic acid chloride (226 μl, 1 mmol) in dry THF (2 ml) was added over 5 min to an ice-cold slurry of **8** (292 mg, 1 mmol) in dry THF (10 ml) containing triethylamine (237 μl, 1.575 mmol). After stirring at 0 °C for 5 min and room temperature for 10 min, TLC (Solvent B), R_f (educt) 0.20, R_f (product) 0.25 showed a quantitative reaction. Water (50 ml) was added, the aqueous solution extracted with ethyl acetate (2 × 50 ml), the pooled organic phases washed with 1 M $NaHCO_3$ (2 × 50 ml) and brine (2 × 50 ml), dried over anhydrous Na_2SO_4 and the solvent evaporated. Boiling the solid yellow residue in methanol (10 ml) and cooling gave an analytically pure product that was collected by filtration and dried at 120 °C. Yield 440 mg, 95%, m.p. 242 °C. ^{1}H NMR, δ: 2.34, t, 3H, –COCH_3; 6.18, s, 1H, PhC*H*(OAc)CO–; 7.23–8.37, m, 15H, –NH_2, H-7, H-8, H-9 and 10 phenyl H; 10.00, s, 1H, –CONH–. ^{13}C NMR, δ: 21.67, 76.52, 110.05, 115.71, 120.31, 123.89, 124.54, 126.07, 127.27, 128.22, 129.64, 129.80, 130.07, 131.77, 132.11, 136.34, 138.10, 146.69, 149.25, 167.08, 170.54. MS, Calc. for $C_{25}H_{20}N_6O_4$, molecular weight 468.46: *m/z* 468 (M^+).

6.1.44. N*-(4-Amino-1-oxo-2-phenyl-1,2dihydro-[1,2,4]triazolo[4,3-a]quinoxalin-6-yl)-2-fluoro-2-phenyl-acetamide (**11b**)*

Method C applied to **8** (1.02 g, 3.5 mmol) in dry THF (30 ml), triethylamine (700 μl, 5 mmol) and (±)-α-fluorophenylacetic acid chloride (634 mg, 3.675 mmol) in dry THF (2 ml), with stirring at 0 °C for 5 min and then at room temperature for 20 min, gave a quantitative yield of product by TLC (ethyl acetate/hexane 35:65, v/v), R_f (starting material) 0.20, R_f (product) 0.51. Workup gave analytically pure product. Yield 1.36 g, 91%, m.p. 219–21 °C. ^{1}H NMR, δ: 6.28, d, 1H, –C*H*(F)–, *J* (H,F) 47 Hz; 7.18–7.60, m, 11 H, –NH_2, –NH–, H-8, H-9, –CH_2–, 4 phenyl H; 787, S, 2H, phenyl H; 8.08, d, 2H, phenyl H; 8.31, m, 2H, phenyl, 10.16, d, 1H, H-7. ^{13}C NMR, δ: 110.28, 115.75, 120.25, 123.81, 124.51, 126.25, 127.24, 128.05, 128.16, 129.70, 130.04, 131.40, 132.05, 135.82, 138.10, 146.78, 149.20, 166.76. ^{19}F NMR, δ: – 172.9. MS, Calc. for $C_{23}H_{17}FN_6O_2$, molecular weight 428.42: *m/z* 428 (M^+).

6.1.45. N*-(4-Amino-1-oxo-2-phenyl-1,2-dihydro-[1,2,4]triazolo[4,3-a]quinoxalin-6-yl)- acetamide (**11c**)*

Method C applied to **8** (584 mg, 2 mmol), triethylamine (420 μl, 3 mmol) and acetyl chloride (149 μl, 2.1 mmol) in dry THF (2 ml) gave a quantitative reaction by TLC (Solvent B), R_f (starting material) 0.20, R_f (product) 0.00, blue fluorescence in UV light). The addition of water (20 ml) and centrifugation (10 min at 2600 × *g*) separated a pale yellow solid that was dried at 120 °C overnight. Yield 550 mg, 83%, m.p. 207–8 °C. ^{1}H NMR, δ: 2.20, s, 3H, –COCH_3; 7.24, t, 1H, H-9; 7.40, m, 1H, H-8; 7.57, m, 2H, phenyl H; 7.72, br s, 2H, –NH_2; 809, d, 2H, phenyl H; 8.23–8.46, m, 2H, H-7 and phe-

nyl H; 9.34, s 1H, –CON*H*–. ^{13}C NMR, δ: 109.41, 120.38, 123.83, 124.50, 127.29, 130.07, 132.05, 133.03, 138.13, 144.88, 146.47, 149.30, 168.93. MS, Calc. for $C_{17}H_{14}N_6O_2$, molecular weight 334.33: *m/z* 334 (M^+).

6.1.46. N-*(4-Amino-1-oxo-2-phenyl-1,2-dihydro-[1,2,4]triazolo[4,3-a]quinoxalin-6-yl)-2-phenyl-acetamide (**11d**)*

Method C employed **8** (584 mg, 2 mmol) in dry THF (20 ml), triethylamine (420 µl, 3 mmol) and phenylacetic acid chloride (278 µl, 2.1 mmol) in dry THF (2 ml). TLC showed a quantitative reaction (Solvent B), R_f (starting material) 0.20, R_f (product) 0.35, blue fluorescence in UV light). Workup yielded 804 mg, 93%, of **11d**, m.p. 213–4 °C. ^{1}H NMR, δ: 3.83, t, 2H, PhC*H*$_2$–; 7.16–7.68, m, 10 H, –NH$_2$, C-9, C-8, 6 phenyl H; 8.06–8.33, m, 5H, H-7 and 4 phenyl H; 9.50, s, 1H, –CON*H*–. ^{13}C NMR, δ: 44.79, 109.55, 116.25, 120.36, 123.87, 124.51, 125.97, 127.29, 127.64, 129.44, 130.07, 130.26, 132.04, 132.82, 136.45, 138.12, 146.42, 149.27, 169.69. MS, Calc. for $C_{23}H_{18}N_6O_2$, molecular weight 410.43, *m/z* 410 (M^+).

6.1.47. N-*(4-Amino-1-oxo-2-phenyl-1,2-dihydro-[1,2,4]triazolo[4,3-a]quinoxalin-6-yl)-2,2,2-trifluoro-acetamide (**11e**)*

Method C employed **8** (292 mg, 1 mmol) in dry THF (10 ml), triethylamine (210 µl, 1.5 mmol) and trifluoroacetic acid anhydride (149 µl, 1.05 mmol) in dry THF (2 ml). TLC (Solvent B): R_f (starting material) 0.20, R_f (product) 0.59, fluorescent in UV light). Workup gave, after air-drying, analytically pure product as pale cream-colored crystals, 340 mg, 87%, m.p. 222 °C. ^{1}H NMR, δ: 7.30, m, 2H, H-9 and H-8; 7.56, m, 2H, phenyl H, 7.76–8.08, m, 4H, –NH$_2$, H-7 and phenyl H; 8.42, d, 2H, phenyl H; 10.37, s, 1H, CF$_3$CON*H*–. ^{13}C NMR, δ: 112.20, 118.20, 120.30, 123.71, 124.76, 127.33, 128.10, 129.54, 130.07, 131.95, 138.05, 147.16, 149.19, 171.23. ^{19}F NMR, δ: – 74.90. MS, Calc. for $C_{17}H_{11}F_3N_6O_2$, molecular weight 388.37, *m/z* 388 (M^+).

*6.1.48. Radiosynthesis of [^{3}H]**10a***

[^{3}H]NaBH$_4$ reduction of the Schiff base **9a** generated [^{3}H]**10a**. A 2 ml vial containing a magnetic stirring bar sealed with a rubber septum was charged with a solution of [^{3}H]NaBH$_4$ (150 mCi, 85 Ci/mmol, 66 µg, 1.8 µmol) in dry *n*-propanol (75 µl). To this solution was added under stirring a mixture of **9a** (2 mg, 5.3 µmol) in dry DMF (200 µl) followed by dry methanol (500 µl). After stirring for 8 min at room temperature, the reaction was stopped by the addition of acetonitrile/water, 30:70 (v/v). The reaction mixture was transferred to a semipreparative HPLC column in several 400 µl portions, the fractions containing product were collected, pooled (total volume 26 ml) and diluted with water (150 ml). The aqueous solution was passed through a Sep-Pak cartridge (flow ± 5 ml/min), the cartridge was washed with water (40 ml) and the product was eluted with ethanol (10 ml).

Semi-preparative radio-HPLC used a C-18 column (Multospher 120 5 µm, 250 × 8 mm) eluted with acetonitrile/water, 30:70 (v/v) at a flow rate of 4 ml/min. In this system the k'-values of the precursor Schiff base and the tritiated ligand were 2.61 and 5.83, respectively. For continuous measurement of radioactivity the outlet of the UV detector was connected to a flow scintillation analyzer (Radiomatic™ 515 TR Series, Packard, Dreieich, Germany) and the collected data were processed by an integrated software system.

Quality control of the product employed a Supersphere Si-60 column (250 × 4 mm) eluted with ethyl acetate/hexane, 50/50 (v/v) containing 0.6% triethylamine at a flow rate of 1 ml/min. Under these conditions the k'-value for the tritiated ligand was 2.88 and that of the educt 4.22. Measurement of the specific activity of the product used HPLC with UV monitoring at 254 nm. Integrating the UV absorption of the product peak and referring that area to a standard curve measured the mass of product. The product peak was collected and an aliquot counted in a liquid scintillation counter. The RCY of tritiated ligand was 12.5%, radiochemical purity was > 98% and specific activity was 5.6 Ci/mmol. When stored at –20 °C the product was stable for over 6 months.

7. Biological evaluations

7.1. Radioligand binding assays using rat brain membranes

Corpora striata (for A_{2A}AR assays) and frontal cortices (for A_1AR assays) from rat brains were homogenized for 1 min in 20 volumes of ice-cold 50 mM Tris–HCl buffer, pH 7.4 containing 10 mM $MgCl_2$, soybean trypsin inhibitor (20 µg/ml), bacitracin (200 µg/ml), and benzamidine HCl (160 µg/ml) by means of an Ultra Turrax at 20,000 rpm. The homogenate was centrifuged at 48,000 × *g* for 10 min at 4 °C (Beckmann Optima L, SW41Ti rotor). The pellet was suspended in 20 volumes of Tris–HCl, pH 7.4, containing AD (2 U/ml) and Trypsin Inhibitor (20 µg/ml), and then was incubated for 30 min at 37 °C. After centrifugation at 48,000 × *g* for 10 min at 4 °C the resulting pellet was diluted in 20 volumes of 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$. Aliquots of the homogenate (1 ml) were stored at –80 °C. The assays were performed in triplicate by incubating aliquots of the membrane fractions (65–120 µg protein per assay) in Tris–HCl, pH 7.4, containing AD (2 U/ml), [^{3}H]CPDPX (2 nM) and cortical homogenates for the A_1AR or striatal homogenates for A_{2A}AR, in a total assay volume of 200 µl. Incubation was carried out at 20 °C for 90 min. In saturation experiments the non-specific binding was defined as the binding in the presence of either 10 µM CPDPX or 50 µM 2-chloroadenosine for studies of the A_1AR and A_{2A}AR, respectively. In A_{2A}AR competition experiments [^{3}H]CGS21680 was used in a concentration of 1.5 nM. Centrifugation at 48,000 × *g* for 6 min at 8 °C separated bound from free ligand. Supernatants were discarded, the pellets

washed with 1 ml ice cold buffer and dissolved by incubating in Solvable™ (500 μl, Canberra-Packard) for 120 min at 50 °C. Aliquots of 450 μl were placed in scintillation vials with scintillation cocktail (10 ml, Ultima Gold XR, Canberra-Packard). Radioactivity was measured in a liquid scintillation counter. Protein estimation was performed with a commercial assay (Bio-Rad DC Protein Assay) after solubilization in 15% NH_4OH containing 2% SDS (w/v); human serum albumin served as a standard. A computer-assisted curve-fitting program (Graph Pad Prism, version 3.0) calculated IC_{50}, K_i and K_D.

7.2. Autoradiography

Frozen rat brains (–20 °C) were sectioned at a thickness of 20 μm in the horizontal plane (Leica AG Microsystems, Germany), mounted on gelatine-coated microscope slides (Laboroptik GmbH, Germany), dried at 4 °C and stored until use at –80 °C. Tissue sections were preincubated for 15 min at 4 °C in 50 mM Tris–HCl containing 1 mM $MgCl_2$ and 2 U/ml AD, pH 7.4, and thereafter for 90 min at 23 °C in the same buffer containing either 0.9 nM [^{3}H]CPDPX, 13 nM [^{3}H]**10a** or 2.2 nM [^{3}H]ZM241385, alone or with 200 nM **10a**. Some sections were incubated for 60 min with 5 μM CPDPX or CGS21680 before [^{3}H]**10a** incubation (blocking experiment), others were incubated with [^{3}H]**10a** followed by incubation with 5 μM CPDPX or CGS21680 for 60 min (displacement experiment). The sections were washed twice for 5 min in 50 mM Tris–HCl at 4 °C, rinsed in deionized water, dried in a stream of cold air and placed on a phosphor image plate (Fujifilm) and exposed for 4 days. Laser scanning of the plates employed a phosphor imager BAS 5000 (Fujifilm) controlled with software provided by the vendor (Version 2.11a, Raytest Isotopenmeßgeräte, Germany). The advanced image analyzer program (AIDA 3.10, Raytest Isotopenmeßgeräte, Germany) analyzed data from regions of interest (ROIs). Analysis of binding data arrayed in Microsoft Excel 2000 employed a non-linear, curve-fitting analysis program (Graph Pad Prism 3.00).

7.3. Measurement of an index of lipophilicity

A method employing HPLC [39] estimated of the lipophilicities of compounds **8**, **9a** and **10a**–**s**. Briefly, that method called for measurements of the retention times of the test compounds on a 4.6 × 250 mm column of 5 μm Kromasil RP18 eluted at a rate of 1 ml/min with each of the following proportions of water/methanol: 22.5:77.5; 20:80; 15:85, or 10:90, with monitoring the effluent at 254 nm. Converting each datum to a capacity factor k' by the formula $k' = (t - t_o)/t_o$, where t and t_o are the retention time and void time, respectively, and solving the linear regression of log k' on methanol: water ratio for methanol = 0 gave the lipophilicity parameter log k'w.

7.4. Determination of water solubility

A saturated solution of the test compounds in water was prepared. After the undissolved material was separated by centrifugation (20,000 × g, 10 min), the supernatant was diluted with water to a defined volume and the UV absorption of aliquots ($n = 3$) of the aqueous solution was determined at λ_{max}. The concentration of the solution was calculated using a previously determined standard calibration curve for each compound.

7.5. Data analysis

Radioligand binding data are the mean and 95% confidence limits of three separate experiments, each in triplicate.

Acknowledgements

R.A.O. was supported in part by the Ed C. Wright Chair in Cardiovascular Research, University of South Florida.

Appendix

Elemental analyses of new compounds

Compounds	Formula	Molecular weight	C	H	N	O	Calculated
			C	H	N	O	Found
5	$C_{15}H_8N_6O_3$	348.28	51.73	2.32	32.17	13.78	
			51.71	2.36	32.21	13.74	
6	$C_{33}H_{23}N_6O_3P$	582.55	38.04	3.98	14.43	8.24	
			37.97	4.04	14.39	8.20	
7	$C_{15}H_{10}N_6O_3$	322.28	55.90	3.13	26.08	14.89	
			55.92	3.06	26.13	14.87	
8	$C_{15}H_{12}N_6O$	292.30	61.64	4.14	28.75	5.47	
			61.65	4.08	28.81	5.42	
9a	$C_{22}H_{16}N_6O$	380.40	69.46	4.24	22.09	4.21	
			69.41	4.27	22.14	4.18	
9b	$C_{24}H_{19}N_6O_2$	442.45	65.15	4.33	18.99	7.23	
			65.09	4.30	19.03	7.26	
10a	$C_{22}H_{18}N_6O$	382.42	69.10	4.74	21.98	4.18	
			69.08	4.70	22.02	4.14	
10b	$C_{24}H_{21}FN_6O_2$	444.46	64.86	4.76	18.91	7.20	
			64.81	4.79	18.88	7.24	
10c	$C_{23}H_{20}N_6O$	396.44	69.68	5.08	21.20	4.04	
			69.70	5.11	21.17	4.07	
10d	$C_{23}H_{19}FN_6O$	414.43	66.66	4.62	20.28	3.86	
			66.68	4.58	20.27	3.88	
10e	$C_{22}H_{17}FN_6O$	400.41	65.99	4.28	20.99	4.00	
			65.95	4.31	21.03	4.04	
10f	$C_{22}H_{17}FN_6O$	400.41	65.99	4.28	20.99	4.00	
			66.03	4.30	20.94	3.96	
10g	$C_{22}H_{17}FN_6O$	400.41	65.99	4.28	20.99	4.00	
			65.96	4.29	21.03	4.01	
10h	$C_{22}H_{18}FN_6O_2$	398.42	66.32	4.55	21.09	8.03	
			66.30	4.53	21.13	7.99	
10i	$C_{22}H_{18}FN_6O_2$	398.42	66.32	4.55	21.09	8.03	
			66.28	4.54	21.05	8.07	
10j	$C_{22}H_{18}FN_6O_2$	398.42	66.32	4.55	21.09	8.03	
			66.33	4.58	21.12	8.01	
10k	$C_{23}H_{20}N_6O_2$	412.44	66.98	4.89	20.38	7.76	
			67.03	4.84	20.41	7.72	
10l	$C_{23}H_{20}N_6O_2$	412.44	66.98	4.89	20.38	7.76	
			66.99	4.88	20.34	7.78	
10m	$C_{23}H_{20}N_6O_2$	412.44	66.98	4.89	20.38	7.76	
			66.95	4.92	20.40	7.73	
10n	$C_{22}H_{17}FN_6O_2$	416.41	63.46	4.11	20.18	7.68	
			63.42	4.14	20.20	7.66	
10o	$C_{23}H_{19}FN_6O_2$	430.43	64.18	4.45	19.52	7.43	
			64.19	4.43	19.56	7.39	
10p	$C_{23}H_{19}FN_6O_2$	430.43	64.18	4.45	19.52	7.43	
			64.20	4.47	19.49	7.44	
10q	$C_{20}H_{16}N_6O_2$	372.38	64.51	4.33	22.57	8.59	
			64.48	4.36	22.61	8.60	
10r	$C_{20}H_{15}BrN_6O_2$	451.28	53.23	3.35	18.62	7.09	
			53.19	3.33	18.60	7.13	
10s	$C_{20}H_{15}IN_6O_4$	498.28	8.21	3.03	16.87	6.42	
			48.18	2.99	16.91	6.38	
11a	$C_{25}H_{20}N_6O_4$	468.46	64.10	4.30	17.94	13.66	
			64.11	4.31	17.91	13.69	
11b	$C_{23}H_{17}FN_6O_2$	428.42	64.48	4.00	19.62	7.47	
			64.52	4.02	19.65	7.50	
11c	$C_{17}H_{14}N_6O_2$	334.33	61.07	4.22	25.14	9.57	
			61.04	4.20	25.16	9.61	
11d	$C_{23}H_{18}N_6O_2$	410.43	67.31	4.42	20.48	7.80	
			67.28	4.41	20.51	7.83	
11e	$C_{17}H_{11}F_3N_6O_2$	388.30	52.58	2.86	21.64	8.24	
			52.62	2.90	21.61	8.27	

References

[1] J. Shimada, K. Suzuki, H. Nonaka, A. Ishii, S. Ishikawa, *E*)-1,3-Dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthines: potent and selective adenosine A_2 antagonists, J. Med. Chem. 35 (1992) 2342–2345.

[2] K.A. Jacobson, C. Gallo-Rodriguez, N. Melman, B. Fischer, M. Maillard, A. van Bergen, P.J.M. Van Galen, Structure–activity relationship of 8-styrylxanthines as A_2-selective antagonists, J, Med. Chem. 36 (1993) 1333–1342.

[3] H. Nonaka, M. Ichimura, M. Takeda, Y. Nonaka, J. Shimada, F. Suzuki, KF 17387, (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine, a potent and selective adenosine A_2 receptor antagonist, Eur. J. Pharmacol. 267 (1994) 335–341.

[4] S.M. Poucher, J.R. Keddie, P. Singh, S.M. Stoggall, P.W.R. Caulkett, G. Jones, et al., The In vitro pharmacology of ZM 241,385, a potent, non-xanthine A_{2A}-selective adenosine receptor antagonist, Br. J. Pharmacol. 115 (1995) 1096–1102.

[5] P.G. Baraldi, B. Cacciari, G. Spalluto, M.J. Pinedas de las Infantas y Villatoro, C. Zocchi, S. Dionisotti, E. Ongini, Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives: potent and selective A_{2A} adenosine antagonists, J. Med. Chem. 39 (1996) 1164–1171.

[6] P.G. Baraldi, B. Cacciari, M.J. Spalluto, M. Bronson, S. Dionisotti, E. Ongini, et al., Design, synthesis, and biological evaluation of a second generation of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as potent and selective A_{2A} adenosine receptor antagonists, J. Med. Chem. 41 (1998) 2126–2133.

[7] V. Colotta, D. Catarzi, F. Varano, L. Cecchi, G. Filacchioni, C. Martini, et al., 4-Amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-*a*]-quinoxalin-1-one: a new A_{2A} adenosine receptor antagonist with high selectivity versus A_1 receptors, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 39–41.

[8] P.J. Richardson, H. Kase, P.G. Jenner, Adenosine A_{2A} receptor antagonists as new agents for the treatment of Parkinson's disease, Trends Pharmacol. Sci. 18 (1997) 338–344.

[9] M.H. Holschbach, R.A. Olsson, Applications of adenosine receptor ligands in medical imaging by positron emission tomography, Curr. Pharm. Des. 8 (2002) 99–110.

[10] K. Ishiwata, J. Noguchi, H. Toyama, Y. Sakiyama, N. Koike, S. Ishii, et al., Synthesis and preliminary evaluation of [^{11}C]KF17837, a selective adenosine A_{2A} antagonist, Appl. Radiat. Isot. 47 (1996) 507–511.

[11] J. Noguchi, K. Ishiwata, S. Wakayabashi, T. Nariai, S. Shumiya, S. Ishii, et al., Evaluation of carbon-11-labeled KF 17837: a potential CNS adenosine A_{2a} receptor ligand, J. Nucl. Med. 39 (1998) 498–503.

[12] S. Stone-Elander, J.O. Thorell, L. Erikkson, B.B. Fredholm, M. Ingvar, In vivo biodistribution of [N-^{11}C-methyl]KF 17837 using 3-D-PET: evaluation as a ligand for the study of adenosine A_{2A} receptors, Nucl. Med. Biol. 24 (1997) 187–191.

[13] E. Ongini, S. Dionisotti, S. Gessi, E. Irenius, B.B. Fredholm, Comparison of CGS15493, ZM241385 and SCH58261 as antagonists at adenosine receptors, Naunyn Schmiedebergs Arch. Pharmacol. 359 (1999) 7–10.

[14] S. Todde, R.M. Moresco, P. Simonelli, P.G. Baraldi, B. Cacciari, G. Spalluto, et al., Radiosynthesis and biodistribution of [^{11}C]-5-amino-7-[3-(4-methoxy)-phenylpropyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine, a new potent and selective ligand for the in vivo imaging of adenosine A_{2A} receptor system using positron emission tomography (PET), J. Med. Chem. 43 (2000) 4359–4362.

[15] R.M. Moresco, S. Todde, S. Belloli, P. Simonelli, A. Panzacchi, M. Rigamonte, M. Galli-Kienle, F. Fazio, In vivo imaging of adenosine A2A receptors in rat and primate brain using [11C]SCH442416, Eur. J. Nucl. Med. Mol. Imag. Published online 9 Nov (2004) DOI 10.1007/s00259-004-1688-5).

[16] J. Shimada, F. Suzuki, H. Nonaka, A. Ishii, S. Ichikawa, *E*)-1,3-Dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthines: potent and selective adenosine A2 antagonists, J. Med. Chem. 35 (1992) 2342–2345.

[17] K. Ishiwata, J. Noguchi, S. Wakayabashi, J. Shimada, N. Ogi, T. Nariai, et al., 11C-labeled KF18446: a potential central nervous system adenosine A2a receptor ligand, J. Nucl. Med. 41 (2000) 345–354.

[18] Y. Nonaka, J. Shimada, H. Nonaka, N. Koike, N. Aoki, H. Kobayashi, et al., Photoisomerization of a potent and selective adenosine A2 antagonist, (*E*)-1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl) xanthine, J. Med. Chem. 36 (1993) 3731–3733.

[19] M. Ueeda, R.D. Thompson, L.H. Arroyo, R.A. Olsson, 2-Alkoxyadenosines. Potent, selective agonists at the coronary artery A_{2A} adenosine receptor, J. Med. Chem. 35 (1992) 1334–1339.

[20] J.E. Francis, W.D. Cash, S. Psychoyos, G. Ghai, P. Wenk, R.C. Friedman, G.A. Stone, M. Desai, M. Williams, et al., Struture–activity profile of a series of novel triazoloquinazoline adenosine antagonists, J. Med. Chem. 31 (1988) 1014–1020.

[21] A. Matsuda, M. Shinozaki, T. Yamaguchi, H. Homma, R. Nomoto, T. Miyasaka, Nucleosides and nucleotides. 103. 2-alkynyladenosines: a novel class of selective adenosine A_2 receptor agonists with potent antihypertensive effects, J. Med. Chem. 35 (1992) 241–252.

[22] K. Niiya, R.A. Olsson, R.D. Thompson, S.K. Silvia, M. Ueeda, 2-*N*′-alkylidenehydrazino)adenosines: potent and selective coronary vasodilators, J. Med. Chem. 35 (1992) 4557–4561.

[23] G. Cristalli, A. Eleuteri, S. Vittori, R. Volpini, M.J. Lohse, K.-N. Klotz, 2-Alkynyl derivatives of adenosine and adenosine-5′-*N*-ethyluronamides as selective agonists at A_2 adenosine receptors, J. Med. Chem. 35 (1992) 2363–2368.

[24] M.H. Holschbach, T. Fein, C. Krummeich, R.G. Lewis, W. Wutz, U. Schwabe, et al., A_1 adenosine receptor antagonists as ligands for positron emission tomography (PET) and single photon emission tomography (SPET), J. Med. Chem. 41 (1998) 556–563.

[25] M. Vaultier, N. Knouzi, R. Carrié, Reduction d'azides en amines primaires par une methode generale utilisant la reaction de Staudinger, Tetrahedron Lett. 24 (1983) 763–764.

[26] F.D. Bellamy, K. Ou, Selective reduction of aromatic nitro compounds with stannous chloride in acidic and non-aqueous medium, Tetrahedron Lett. 25 (1984) 839–842.

[27] W.B. Bae, S.H. Lee, Y.J. Cho, C.M. Yoon, A reductive amination of carbonyls with amines using decaborane in methanol, J. Chem. Soc., Perkin Trans 1 (2000) 145–146.

[28] C.S. Schneider, J. Mierau, Dopamine receptor agonists: Resolution and pharmacological activity of 2,6-diaminotetrahydrobenzothiazole and an aminothiazole analogue of apomorphine, J. Med. Chem. 30 (9) (1987) 494–498.

[29] S. Kano, Y. Tanaka, E. Sugino, S. Hibino, Reduction of some functional groups with titanium (IV) chloride/sodium borohydride, Synthesis (Mass.) (1980) 695–697.

[30] N. Umino, T. Iwakuma, N. Itoh, Sodium acetoxyborohydride as new reducing agents. I. Reduction of carboxamides to the corresponding amines, Tetrahedron Lett. 10 (1976) 763–766.

[31] A.F. Abdel-Magid, K.G. Carson, B.H. Harris, C.A. Maryanoff, R.D. Shah, Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. Studies on direct and indirect reductive amination procedures, J. Org. Chem. 61 (1996) 3849–3862.

[32] M. Bonnat, A. Hercouet, M. Le Corre, Effect of the temperature on the stoichiometry of borane-dimethyl sulfide reduction of secondary and tertiary amines, Synth. Commun. 21 (1991) 1579–1582.

[33] S. Battacharyya, Titanium (IV) isopropoxide and sodium borohydride: a reagent of choice for reductive amination, Tetrahedron Lett. 35 (1994) 2401–2404.

[34] P.A. Harland, P. Hodge, W. Maughan, E. Wildsmit, Synthesis of primary amines via alkylation of the sodium salt of trifluoroacetamide: an alternative to the Gabriel synthesis, Synthesis (Mass.) (1984) 941–943.

[35] V. Colotta, D. Catarzi, F. Varano, G. Filacchioni, C. Martini, L. Trincavelli, et al., Synthesis of 4-amino-6-(hetero)alkylamino-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives as potent A_{2A} adenosine receptor antagonists, Bioorg. Med. Chem. 11 (2003) 5509–5518.

[36] J. Fastbom, A. Pazos, A. Probst, J.M. Palacios, The Distribution of adenosine A_1 receptors and 5′-nucleotidase in the brain of some commonly used experimental animals, Neuroscience 22 (1987) 813–826.

[37] P. Svenningsson, C. Le Moine, G. Fisone, B.B. Fredholm, Distribution, biochemistry, and function of striatal adenosine A_{2A} receptors, Prog. Neurobiol. 59 (1999) 355–396.

[38] Z.N. Nazarova, Synthesis of iodo derivatives of the furan series, 5-iodofuraldehyde, J. Gen. Chem. USSR 25 (1955) 509–513.

[39] D.J. Minnick, D.A. Brent, J. Frenz, Modeling octanol–water partition coefficients by reversed-phase liquid chromatography, J. Chromatogr. 461 (1989) 177–191.