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Tricyclic oxazolo[2,3-*f*]purinediones: potency as adenosine receptor ligands and anticonvulsants

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Abstract—Synthesis and physicochemical properties of 7-mono- and 6,7-disubstituted dihydrooxazolo-[3,2-*f*]purinediones are described. Oxazolo[2,3-*f*]purinediones were synthesized by cyclization of 8-bromotheophylline with oxiranes. The obtained compounds (**1**–**22**) were evaluated for their affinity at adenosine A_1 and A_{2A} receptors. They showed mainly adenosine A_{2A} receptor affinity at low micromolar concentrations and A_{2A} selectivity, for example, compound **9** with an octyl substituent at the oxazole ring displayed adenosine A_{2A} receptor affinity ($K_i = 0.998\,\mu M$) and at least 25-fold A_{2A} versus A_1 selectivity. This compound was less selective (5-fold) towards human recombinant A_{2B} and A_3 adenosine receptors. In this group of compounds active adenosine A_1 receptor antagonists were also identified. Oxazolopurinediones were evaluated in vivo as anticonvulsants in MES and ScMet tests and examined for neurotoxicity in mice (ip). Compounds with long alkyl chains showed anticonvulsant activity in both tests (in 100 and 300 mg/kg doses), accompanied by significant neurotoxicity. The anticonvulsant activity in rats (po) was higher and without signs of neurotoxicity. SAR and QSAR studies stressed the importance of lipophilic 7-substituents for both types of pharmacological activity. The volume of the substituent is, however, limited at the A_{2A} AR, an *n*-octyl group being optimal.

1. Introduction

The adenosine receptor (AR) family consists of four subtypes designated A_1, A_{2A}, A_{2B} and A_3.[1] The responses of these four ARs are mediated by receptor-coupled G proteins, which may activate several different effector systems including adenylate cyclase, potassium and calcium channels, phospholipase A2 or C, and guanylate cyclase.

On the basis of the widespread effects attributed to the accumulation of endogenously released adenosine it has been considered that regulation of ARs has substantial therapeutic potential.[2–5] Specificity in its central actions appears to depend on the distribution of AR subtypes in the brain. The need for the search of subtype-selective AR ligands has been recognized. Currently, selective A_1 AR antagonists are developed as potassium-saving diuretics with kidney protective properties for the treatment of acute renal failure. Adenosine A_{2A} receptors offer an attractive target for CNS diseases, for example, adenosine A_{2A} receptor antagonists have therapeutic potential in neurodegenerative disorders: Parkinson's and Huntington's disease, as drugs controlling motor functions and exhibiting neuroprotective properties.[4,6–9] The involvement of ARs interaction in seizure disorders was discussed.[8–11] Stimulation of adenosine A_1 and A_{2A} receptors was described as playing role in the suppression of seizures (pentylenetetrazole induced convulsions and audiogenic seizures in DBA/2 mice), while the ARs antagonists increased the incidence of both clonic and tonic seizures.[4,10,11]

In our research we have been dealing with tricyclic xanthine derivatives[12,13] conceptually designed as potential

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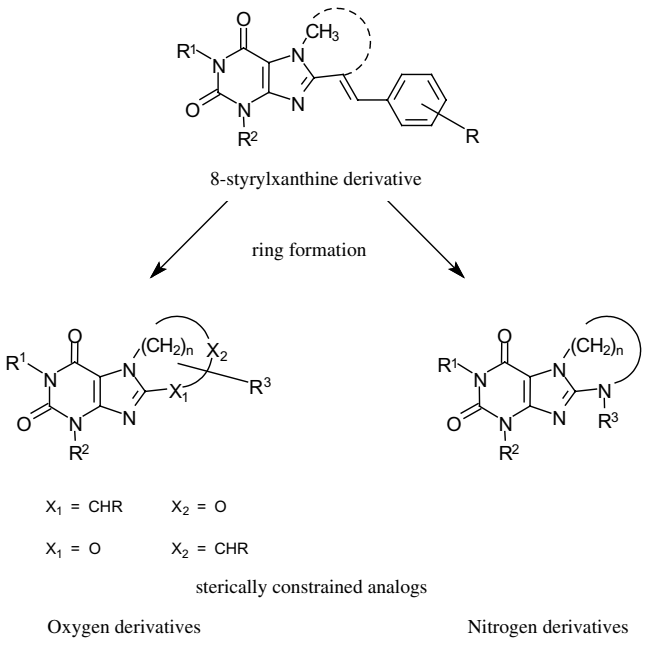


Figure 1. Oxygen- or nitrogen-containing annelated theophylline analogs.

A_1 or A_{2A} adenosine receptor antagonists. Compounds with oxygen or nitrogen containing fused rings were obtained as constrained analogs of 8-styrylxanthines, which are potent and selective A_{2A} AR antagonists (Fig. 1).[14]

Such tricyclic structures exhibited A_1 or A_{2A} adenosine receptor antagonist properties, in some cases selectivity to one of the above mentioned subtypes of receptors. The investigated compounds have shown anticonvulsant activity in the in vivo tests, however the mechanism of this action was not clear.

As a continuation of our studies, the systematic examination of oxazolo[2,3-*f*]purinediones was performed mainly of those that were mono- or disubstituted at the oxazole ring. Syntheses, structure elucidation by means of X-ray analysis, in vitro evaluation of the affinity to ARs and in vivo examination of anticonvulsant properties are described.

2. Chemistry

The synthesis of 7- or 6,7-disubstituted tetrahydro[1,3]oxazolo[2,3-*f*]purinediones was accomplished as shown in Figure 2. 8-Bromotheophylline[15] reacted with oxiranes in the presence of a catalytic amount of pyridine in anhydrous propanol, butanol or acetonitrile (Fig. 2). Intermediate hydroxyalkyl derivatives were not isolated and subsequently cyclization took place. Structures, physicochemical properties of the synthesized compounds and reaction conditions are summarized in Tables 1 and 2. Compounds **2**, **3**, **4** and **5** were described previously[16–18] with incomplete spectroscopic data and without pharmacological investigations. Compound **1** had been obtained by de Martiis et al.[16] in two steps. In our approach it was synthesized as described above. The structures of the compounds were confirmed by crystallographic studies of two selected compounds (see below) and by UV spectral analyses, showing a slight bathochromic shift of λ_{max} (276–280 nm) typical[19] for non-8-amino xanthines. In the IR spectra they presented typical absorption bands[20] for cyclic ethers and carbonyl groups of xanthines and in the ^{1}H NMR spectra, the expected chemical shifts were observed. Spectroscopic data are presented in Table 3. The existence of isomeric structures **1a**–**22a** must be taken into consideration, especially in the case of the disubstituted derivative **22**. The structure of **22** was assigned on the basis of its ^{1}H NMR spectral data. Crucial for the confirmation of the structure of **22** was the chemical shift of 7-H (absent in isomer **22a**), which is observed for all the structures in the range of 5.13–5.75 ppm and similarly at 5.29 ppm for the structure **22**. Compounds **2**–**11**, **13**–**22** possessing at C-7 and **12** at C-6 and C-7 centres of asymmetry were obtained as racemates.

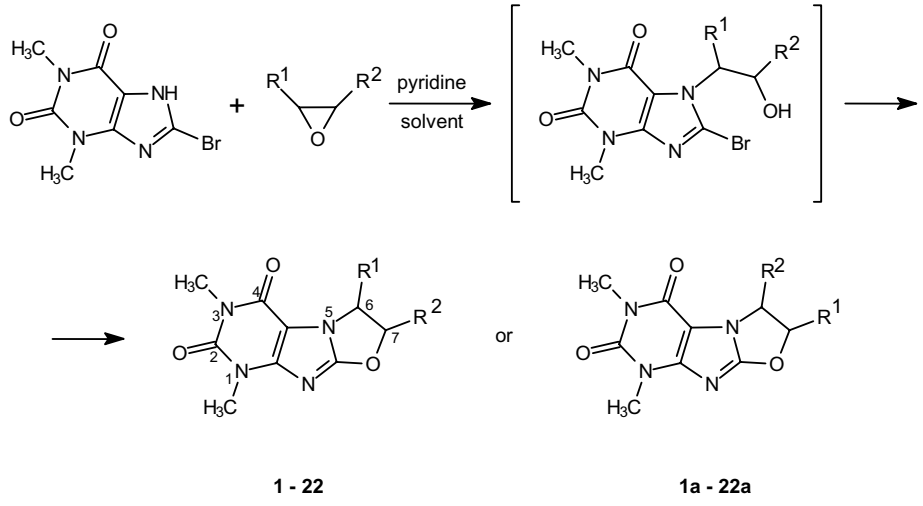
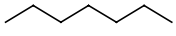
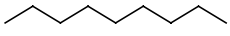
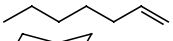
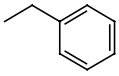
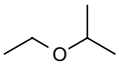
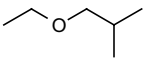
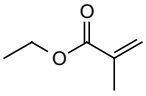
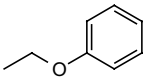
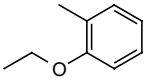
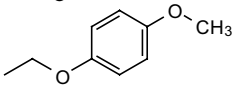
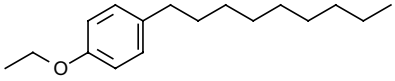
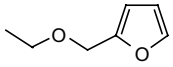


Figure 2. Synthesis of 7-mono or 6,7-disubstituted dihydrooxazolo[2,3-*f*]purinediones.

Table 1. Structures and elemental analysis of dihydro-1,3-oxazolo[2,3-*f*]purinediones

No.	R^1	R^2	Mol. form., m. wt.	Calculated (%)			Found (%)		
				C	H	N	C	H	N
1	H	H	$C_9H_{10}N_4O_3$, 222.20	48.81	4.83	25.10	48.88	4.93	25.02
2	H	CH_3	$C_{10}H_{12}N_4O_3$, 236.21	Ref. 17					
3	H		$C_{10}H_{11}N_4O_3Cl$, 270.67	Ref. 17					
4	H		$C_{11}H_{14}N_4O_3$, 250.26	Ref. 18					
5	H		$C_{10}H_{12}N_4O_4$, 252.23	Ref. 16					
6	H		$C_{12}H_{16}N_4O_3$, 264.29	54.54	6.11	21.20	54.54	5.92	21.06
7	H		$C_{13}H_{18}N_4O_3$, 278.31	56.11	6.53	20.13	56.15	6.65	20.15
8	H		$C_{15}H_{22}N_4O_3$, 306.36	58.80	7.24	18.29	58.52	6.96	18.05
9	H		$C_{17}H_{26}N_4O_3$, 334.41	61.07	7.85	16.75	61.42	7.82	16.70
10	H		$C_{13}H_{16}N_4O_3$, 276.29	56.51	5.84	20.28	56.42	5.96	20.30
11	H		$C_{15}H_{20}N_4O_3$, 304.34	59.21	6.63	18.41	59.16	6.89	18.11
12			$C_{13}H_{16}N_4O_3$	56.51	5.84	20.28	56.58	5.93	20.41
13	H		$C_{16}H_{16}N_4O_3$, 312.32	61.54	5.17	17.94	61.25	4.92	17.90
14	H		$C_{13}H_{18}N_4O_4$, 294.31	53.05	6.12	19.03	53.06	6.19	19.01
15	H		$C_{14}H_{20}N_4O_4$, 308.33	52.83	6.55	18.17	53.20	6.73	17.81
16	H		$C_{14}H_{16}N_4O_5$, 320.30	52.50	5.04	17.49	52.58	5.18	17.70
17	H		$C_{16}H_{16}N_4O_4$, 328.32	58.54	4.92	17.06	58.49	5.03	16.85
18	H		$C_{17}H_{18}N_4O_4$, 336.36	60.72	5.40	16.66	60.57	5.27	16.77
19	H		$C_{17}H_{18}N_4O_5$, 358.35	56.98	5.07	15.63	56.62	4.94	16.00
20	H		$C_{25}H_{34}N_4O_4$, 454.55	66.08	7.55	12.32	65.95	7.41	11.94
21	H		$C_{15}H_{16}N_4O_5$, 332.31	54.22	5.14	16.82	53.91	4.99	16.90
22	CH_3, CH_3		$C_{12}H_{16}N_4O_4$, 280.28	51.42	5.76	19.99	51.49	5.92	19.61

3. X-ray structure analysis of compounds 12 and 13

As it is clear from Figure 3, both structures of **12** and **13** contain the tricyclic oxazolo[2,3-*f*]purinedione moiety. Although nonplanarity of the whole skeleton has been observed, the purinedione rings are coplanar with a dihedral angle between both rings equaling only about 2°. In contrast two carbon atoms of the oxazole ring (C5a, C9a in **12** and C6, C7 in **13**) are in sp^3 hybridization and such rings in both molecules are in screw conformation (Table 4). But the bending of the oxazole ring in both molecules is different. Thus in **12**, due to the fact that the atoms C5a and C9a are part of a cyclohexane chair, the asymmetry parameter ΔC_2 in the oxazole ring is much higher than for **13** (see Table 4). In structure **13**, the phenyl ring of the benzyl substituent is inclined to the tricyclic oxazolo[2,3-*f*]purinedione skeleton at 42.8°. The tetracyclic molecule **12** possesses a semi-circular shape. Mainly because of this shape, an atypical structural disorder has been observed in the crystal. During structure refinement it was established that 30% of the molecules rotate about the O2···C8 line and have been located as it is shown in Figure 3.

4. Pharmacology

All compounds were tested in vitro in radioligand binding assays for affinity to A_1 and A_{2A} adenosine receptors in rat cortical membrane and rat striatial membrane preparations, respectively. The A_1 selective agonist [^{3}H]2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) and the A_{2A}-selective antagonist [^{3}H]1-propargyl-3-(3-hydroxypropyl)-7-methyl-8-(*m*-methoxystyryl)xanthine

Table 2. Physical properties and reaction conditions of dihydro-1,3-oxazolo[2,3-*f*]purinediones

No.	Mp °C	Yield (%)	Reaction medium	Reaction time (h)	Crystal. solvent	TLC R_f
1	265–268	50	Butanol	10	Methoxyethanol	0.22
2			Ref. 17			0.31
3			Ref. 17			0.35
4			Ref. 18			0.47
5			Ref. 16			0.10
6	187–188	91	Butanol	4	Ethanol	0.47
7	185–186	69	Butanol	4	Ethanol	0.65
8	175–176	96	Butanol	3	Ethanol	0.65
9	155–156	96	Butanol	4	Ethanol	0.59
10	176–178	82	Butanol	2	Ethanol	0.50
11	152–153	97	Butanol	2	Ethanol + H_2O	0.67
12	245–248	85	Butanol	10	Ethanol	0.65
13	190–191	75	Butanol	3	Ethanol	0.50
14	173–175	83	Butanol	2	Ethanol	0.39
15	141–142	92	Propanol	5	50% Ethanol	0.58
16	188–190	78	Butanol	3	Propanol	0.45
17	181–182	78	Propanol	2	Ethanol	0.41
18	172–174	56	Butanol	4	Ethanol	0.45
19	173–175	74	Butanol	3	Methoxyethanol	0.36
20	141–142	80	Butanol	3	Ethanol	0.55
21	157–160	74	Butanol	3	Ethanol	0.46
22	156–158	45	Acetonitrile	2	Ethanol	0.60

([^{3}H]MSX-2) were used as radioligands.[21,22] The results are presented in Table 5.

Six selected compounds **9**, **12**, **13**, **15**, **17** and **20** were further tested for their affinity to human recombinant A_{2B} and A_3 receptors recombinantly expressed in Chinese hamster ovary (CHO) cells. 4-(2-[7-Amino-2-(2-furyl)-[1,2,4]-triazolo[2,3-*a*]triazin-5-[1,3,5](4-(amino)-ethyl)phenol ([^{3}H]ZM241385) was used as a radioligand in A_{2B} binding studies[32] and [^{3}H]2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purine-5-one ([^{3}H]PSB-11) was used as A_3-selective receptor ligand[23,33] (Table 6).

All compounds were also tested in vivo as anticonvulsants, following the Anticonvulsant Drug Development (ADD) Program at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) in Bethesda, MD, using their protocols.[24–26]

Compounds were injected intraperitoneally into the mice and evaluated in the preliminary anticonvulsant screening with at least three dose levels (30, 100 and 300 mg/kg). The profile of anticonvulsant activity was established by maximal electroshock (MES) pattern test and subcutaneous pentylenetetrazole (ScMet) seizure test. The MES assays have predictive value for agents as potential therapeutics in the management of grand mal epilepsy, whereas the ScMet test is for those likely to be effective against petit mal.[24] Minimal motor impairment was measured by the rotorod toxicity test. The results are given in Table 7. Some compounds (**6**, **7**, **10**, **11**, **12**, **13**, **14**) were administered orally to rats and examined in the MES, ScMet screen and rotorod test (Table 8). For one compound (**4**) a quantitative test was performed (ED_{50} and TD_{50} determination). Results of this test compared with the literature data available for valproate[27] are reported in Table 9.

5. Biological results and conclusions

5.1. In vitro tests

The results of the binding assays towards adenosine A_1 and A_{2A} receptors (Table 5) showed that oxazolopurine derivatives had moderate affinity towards these two receptor subtypes mostly with selectivity for A_{2A} receptors. The most active substances had long alkyl, alkenyl (**7**, **8**, **9**, **10**), benzyl (**13**) or isosteric isobutoxymethyl (**15**) and phenoxymethyl (**17**) substituents in position 7 of the oxazole ring. Steric properties of the substituent are crucial for the affinity to the adenosine A_{2A} receptors: the replacement of the isobutoxymethyl group in active compound **15** with an isopropoxymethyl substituent in **14** caused a significant decrease in affinity. Shortening of the alkyl chain and its substitution with chlorine or hydroxyl (**2**–**5**), or introduction of substituted arylethers (**18**–**21**) decreased or even abolished affinity. Also annelation of a fourth ring (**12**) or additional substitution in position 6 of the oxazole ring (compound **22**) was disadvantageous. On the other hand, it is worth noticing that in one case (benzyl derivative **13**) A_1 selectivity (4-fold A_1-selective over A_{2A}) was observed. The best A_{2A} receptor ligand of the present series was **9** with a long lipophilic octyl substituent in position 7 of the oxazole ring (K_i $A_{2A} = 0.988\,\mu M$ at least, 25-fold A_{2A}-selective versus A_1). This compound also showed some selectivity towards human recombinant A_{2B} and A_3 adenosine receptors. However, among the six selected compounds additionally tested at A_{2B} and A_3 ARs, representing different structures (**9**, **12**, **13**, **15**, **17**, **20**), compound **9** was the best ligand for A_{2B} and A_3 ARs as well. The other investigated compounds caused only low inhibition of [^{3}H]ZM241385 binding to the A_{2B} receptor, without showing any adenosine A_3 affinity (Table 6). The most potent selective ligands from the previously examined class of nitrogen analogs (Fig. 1) of oxazolopurinediones

Table 3. Spectroscopic data of compounds **1**–**22**

Compd	IR ν (cm^{-1})	UV λ_{max}, $\log\varepsilon$	^1H NMR δ (ppm)
1	2954-Alkyl, 1709-CO (pos. 2), 1658-CO (pos. 4), 1209-COC, 742-CH_2	280 5.02	3.17 (s, 3H, N_3CH_3), 3.31 (s, 3H, N_1CH_3), 4.30 (t, J = 7.98 Hz, 2H, N_5CH_2), 5.13 (t, J = 8.02 Hz, 2H, CH_2)
2	2955-Alkyl, 1710-CO (pos. 2), 1661-CO (pos. 4), 1141-COC, 744-CH_2	280 5.09	Ref. 18
3	2955-Alkyl, 1711-CO (pos. 2), 1657-CO (pos. 4), 1208-COC, 744-CH_2	278 4.92	Ref. 18
4	2952, 2884-alkyl, 1713-CO (pos. 2), 1656-CO (pos. 4), 1052-COC, 742-CH_2	280 4.99	Ref. 18
5	3407-OH, 2956-alkyl, 1710-CO (pos. 2), 1655-CO (pos. 4), 1081-COC, 746-CH_2	280 4.98	3.16 (s, 3H, N_3CH_3), 3.30 (s, 3H, N_1CH_3), 3.62–3.84 (2m, 2H, N_5CH_2), 4.07–4.39 (2m, 2H, CH_2OH), 5.30 (t, J = 5.5 Hz, 1H, OH), 5.46–5.61 (m, 1H, CH)
6	2959, 2876-alkyl, 1715-CO (pos. 2), 1655-CO (pos. 4), 1126-COC, 743-CH_2	280 5.06	1.01 (t, J = 7.43 Hz, 3H, $(CH_2)_2CH_3$), 1.45–1.52 (m, 2H, CH_2–CH_2CH_3), 1.7–2.02 (2m, 2H, $CH_2CH_2CH_3$), 3.37 (s, 3H, N_3CH_3), 3.50 (s, 3H, N_1CH_3), 3.99 (q, J = 7.7 Hz, 1H, N_5CH_2), 4.48 (q, J = 8.25 Hz, 1H, N_5CH_2), 5.34–5.44 (m, 1H, CH)
7	2958, 2932-alkyl, 1715-CO (pos. 2), 1655-CO (pos. 4), 1126-COC, 743-CH_2	280 4.97	0.91 (t, J = 7.15 Hz, 3H, $(CH_2)_3CH_3$), 1.35–1.53 (m, 4H, CH_2–$(CH_2)_2CH_3$), 1.81–2.02 (2m, 2H, $CH_2(CH_2)_2CH_3$), 3.34 (s, 3H, N_3CH_3), 3.47 (s, 3H, N_1CH_3), 3.95–4.01 (q, J = 7.7 Hz, 1H, N_5CH_2), 4.43–4.49 (q, J = 7.7 Hz, 1H, N_5CH_2), 5.32–5.41 (m, 1H, CH)
8	2956, 2929, 2858-alkyl, 1715-CO (pos. 2), 1655-CO (pos. 4), 1086-COC, 744-CH_2	280 5.06	0.88 (t, J = 6.74 Hz, 3H, $(CH_2)_5CH_3$), 1.29–1.54 (m, 8H, CH_2–$(CH_2)_4CH_3$), 1.81–2.01 (2m, 2H, $CH_2(CH_2)_4CH_3$), 3.37 (s, 3H, N_3CH_3), 3.50 (s, 3H, N_1CH_3), 3.95–4.02 (dd, J = 7.70 Hz, 1H, N_5CH_2), 4.45–4.50 (dd, J = 7.57 Hz, 1H, N_5CH_2), 5.38–5.42 (m, 1H, CH)
9	2956, 2926, 2856-alkyl, 1715-CO (pos. 2), 1654-CO (pos. 4), 1051-COC, 743-CH_2	280 5.06	0.87 (t, J = 6.74 Hz, 3H, $(CH_2)_7CH_3$), 1.26–1.54 (m, 12H, $CH_2(CH_2)_6CH_3$), 1.94–2.01 (m, 1H, $CH_2(CH_2)_6CH_3$), 1.94–2.06 (m, 1H, $CH_2(CH_2)_6CH_3$), 3.37 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 3.97–4.02 (q, J = 7.70 Hz, 1H, N_5CH_2), 4.45–4.51 (q, J = 8.11 Hz, 1H, N_5CH_2), 5.33–5.41 (m, 1H, CH)
10	3075-Alkenyl, 2954-alkyl, 1714-CO (pos. 2), 1655-CO (pos. 4), 1210-COC, 744-CH_2	280 5.10	1.92–2.34 (3×m, 4H, $(CH_2)_2$), 3.38 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 4.01 (q, J = 7.56 Hz, 1H, N_5CH_2), 4.49 (t, J = 8.94 Hz, 1H, N_5CH_2), 5.06–5.14 (m, 2H, CH_2=CH), 5.35–5.45 (m, 1H, CH_2=CH), 5.75–5.88 (m, 1H, CH)
11	3077-Alkenyl, 2991, 2859-alkyl, 1713-CO (pos. 2), 1659-CO (pos. 4), 1097-COC, 744-CH_2	280 5.01	1.44–2.12 (m, 8H, $(CH_2)_4$), 3.38 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 4.00 (q, J = 7.70 Hz, 1H, N_5CH_2), 4.45–4.50 (m, 1H, N_5CH_2), 4.95–5.04 (m, 2H, CH_2=CH), 5.33–5.43 (m, 1H, CH_2=CH), 5.61–5.84 (m, 1H, CH)
12	2955–2873-Alkyl, 1706-CO (pos. 2), 1658-CO (pos. 4), 1199-COC, 744-CH_2	280 4.93	1.38–2.00 (m, 6H, cyclohexyl), 2.39–2.45 (m, 1H, cyclohexyl), 2.87–2.92 (m, 1H, cyclohexyl), 3.38 (s, 3H, N_3CH_3), 3.52 (s, 3H, N_1CH_3), 3.65–3.74 (dt, 1H, N_5CH), 4.47–4.56 (dt, 1H, OCH)–
13	3025-Phenyl, 2948-alkyl, 1703-CO (pos. 2), 1653-CO (pos. 4), 1059-COC, 746-CH_2	280 4.97	3.14–3.21 (m, 1H, CH_2), 3.31 (d, J = 6.33 Hz, 1H, CH_2), 3.36 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 4.10 (q, J = 7.70 Hz, 1H, N_5CH_2), 4.39 (q, J = 8.25 Hz, 1H, N_5CH_2), 5.54–5.65 (m, 1H, CH), 7.37 (m, 5H, phenyl)
14	2971, 2934, 2867-alkyl, 1714-CO (pos. 2), 1655-CO (pos. 4), 1149-COC, 744-CH_2	280 4.76	1.12–1.15 (m, 6H, $2CH_3$), 3.38 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 3.61–3.73 (m, 1H, C$H$$(CH_3)_2$), 3.75–3.83 (m, 2H, N_5CH_2), 4.30–4.45 (m, 2H, CH_2O), 5.42–5.50 (m, 1H, CH)
15	2957, 2874-alkyl, 1714-CO (pos. 2), 1672-CO (pos. 4), 1120-COC, 742-CH_2	280 4.97	0.84 (q, J = 3.03 Hz, 6H, $2CH_3$), 1.80–1.87 (m, 1H, C$H$$(CH_3)_2$), 3.28 (d, J = 6.60 Hz, 2H, CH_2CH), 3.38 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 3.78 (m, 2H, N_5CH_2), 4.31–4.44 (m, 2H, CH_2O), 5.43–5.51 (m, 1H, CH)

(*continued on next page*)

Table 3 (*continued*)

Compd	IR ν (cm^{-1})	UV λ_{max}, $\log\varepsilon$	^1H NMR δ (ppm)
16	2955-Alkyl, 1719-CO (pos. 2), 1651-CO (pos. 4), 1294-ester, 1163-COC, 743-CH_2	280 5.01	1.88 (s, 3H, CH_3), 3.38 (s, 3H, N_3CH_3), 3.51 (s, 3H, N_1CH_3), 4.24–4.30 (m, 1H, N_5CH_2), 4.44–4.6 (m, 3H, $N_5CH_2+CH_2O$), 5.61–5.67 (m, 2H, $=CH_2$), 6.04 (s, 1H, CH)
17	3025-Phenyl, 2948-alkyl, 1703-CO (pos. 2), 1653-CO (pos. 4), 1059-COC, 746-CH_2	276 5.03	3.39 (s, 3H, N_3CH_3), 3.52 (s, 3H, N_1CH_3), 4.30 (dd, $J=3.99$ Hz, 1H, N_5CH_2), 4.40 (dd, $J=3.99$ Hz, 1H, N_5CH_2), 4.47–4.60 (m, 2H, CH_2O), 5.66–5.74 (m, 1H, CH), 6.98 (m, 2H, 2′6′phenyl), 6.95 (m, 1H, 4′phenyl), 7.26–7.32 (m, 2H, 3′5′phenyl)
18	3064-Phenyl, 2952-alkyl, 1704-CO (pos. 2), 1655-CO (pos. 4), 1240-O phenyl, 1127-COC, 750-CH_2	276 4.93	2.04 (s, 3H, CH_3), 3.40 (s, 3H, N_3CH_3), 3.53 (s, 3H, N_1CH_3), 4.29 (dd, $J=3.58$ Hz, 1H, N_5CH_2), 4.40 (dd, $J=3.58$ Hz, 1H, N_5CH_2), 4.49–4.62 (m, 2H, CH_2O), 5.68–5.75 (m, 1H, CH), 6.80 (d, $J=7.98$ Hz, 1H, 6′phenyl), 6.91 (t, $J=7.4$ Hz, 3′phenyl) 7.11–7.29 (m, 2H, 4′5′phenyl)
19	3064-Phenyl, 2951, 2888-alkyl, 1704-CO (pos. 2), 1654-CO (pos. 4), 1240-O phenyl, 744-CH_2	280 4.93	3.40 (s, 3H, N_3CH_3), 3.53 (s, 3H, N_1CH_3), 3.76 (s, 3H, OCH_3), 4.25 (dd, $J=3.85$ Hz, 1H, N_5CH_2), 4.34 (dd, $J=4.13$ Hz, 1H, N_5CH_2), 4.47–4.59 (m, 2H, CH_2O), 5.64–5.70 (m, 1H, CH), 6.83 (s, 4H, phenyl)
20	2959-Phenyl, 2929–2872-alkyl, 1709-CO (pos. 2), 1658-CO (pos. 4), 1243-O phenyl, 1123-COC, 743-CH_2	280 5.04	0.77–1.63 (3×m, 19H, $(CH_2)_8CH_3$), 3.40 (s, 3H, N_3CH_3), 3.53 (s, 3H, N_1CH_3), 4.28 (q, $J=3.71$ Hz, 1H, N_5CH_2), 4.38 (m, 1H, N_5CH_2), 4.47–4.59 (m, 2H, CH_2O), 5.64–5.72 (m, 1H, CH), 6.79–6.82 (m, 2H, 2′6′phenyl), 7.15–7.27 (m, 2H, 3′5′phenyl)
21	3121-Phenyl, 2952-alkyl, 1712-CO (pos. 2), 1655-CO (pos. 4), 1110-COC, 879-phenyl, 744-CH_2	280 5.03	3.37 (s, 3H, N_3CH_3), 3.50 (s, 3H, N_1CH_3), 3.81 (dd, $J=4.12$ Hz, 1H, N_5CH_2), 3.86 (dd, $J=4.04$ Hz, 1H, N_5CH_2), 4.26–4.58 (m, 4H, CH_2–O–CH_2), 5.41–5.49 (m, 1H, CH), 6.33 (m, 2H, 3′4′furan), 7.40 (s, 1H, 2′furan)
22	2955, 2851-alkyl, 1703-CO (pos. 2), 1656-CO (pos. 4), 1099-COC, 749-CH_2	278 5.02	1.58 (d, $J=9.08$ Hz, 6H, $2CH_3$), 3.39 (s, 3H, N_3CH_3), 3.52 (s, 3H, N_1CH_3), 3.66 (s, 3H, OCH_3), 5.29 (s, 1H, CH)

were investigated for their antagonistic properties in radioligand binding sodium shift assays and could be characterized as antagonists for A_{2A}ARs.[12] Taking into account the structural similarity of currently investigated group of compounds, we may presume that oxazolo[2,3-*f*]purinediones have an A_{2A}AR antagonistic profile of activity as well.

5.2. In vivo tests

The character of the substituent at the oxazole ring seems to have a significant influence on the profile and strength of the anticonvulsant activity (Table 7). Compounds with alkyl and alkenyl substituents (from 2–6 C atoms) and a benzyl moiety (comp. **4**, **6**, **7**, **8**, **10**, **11**, **13**) showed protective activity in both tests in short time (0.5 h). For compounds **7** and **10** the protective activity appeared within 0.25 h after injection and lasted for 4 h. Some compounds (**3**, **12**, **14**, **15**) showed protection only in chemical seizures. In the neurotoxicity test all of the substances showed toxicity at the active dose. Such signs as myoclonic jerks (**13**), loss of righting reflex (**6**, **10**, **11**), tonic extension (**11**), continuous seizure activity (**13**) and even death (**15**, **22**) appeared. Introducing a phenoxyalkyl moiety in position 7 (**17**–**21**) or a second substituent in position 6 of the oxazole ring (**22**) completely abolished anticonvulsant activity.

The most active compounds in mice tests were also examined for their activity in rats showing after oral administration even much better seizure protection (Table 8). In the MES test after administration of 30 mg/kg dose po anticonvulsant activity without neurotoxic symptoms was shown in all the tested compounds except compound **14**. Compound **12** active in mice only in the ScMet test showed, after oral administration in rats, activity in the MES test. Compound **6** active in both tests in mice, showed only protection in ScMet test in rats at a maximal dose of 50 mg/kg. Compound **4** was advanced to phase II evaluation for quantification of activities (ED_{50} and TD_{50}) against ScMet induced seizures. These pharmacological parameters were compared with data for valproate (Table 9). The time to peak effect (TPE) was comparable but the protective index (PI) was two times lower than that for valproate.

The anticonvulsant activity of the examined compounds was analyzed for correlation with ARs affinity. Different effects were observed. Compound **9** the most active and selective A_{2A} AR antagonist was deprived as well of anticonvulsant as neurotoxic activity. Structure **4** not active as A_1 or A_{2A} AR antagonist has shown short time lasting anticonvulsant activity in both used test (being classified to I ASP class). Contrary to that results compound **13** showing in some degree selective A_1 AR affin-

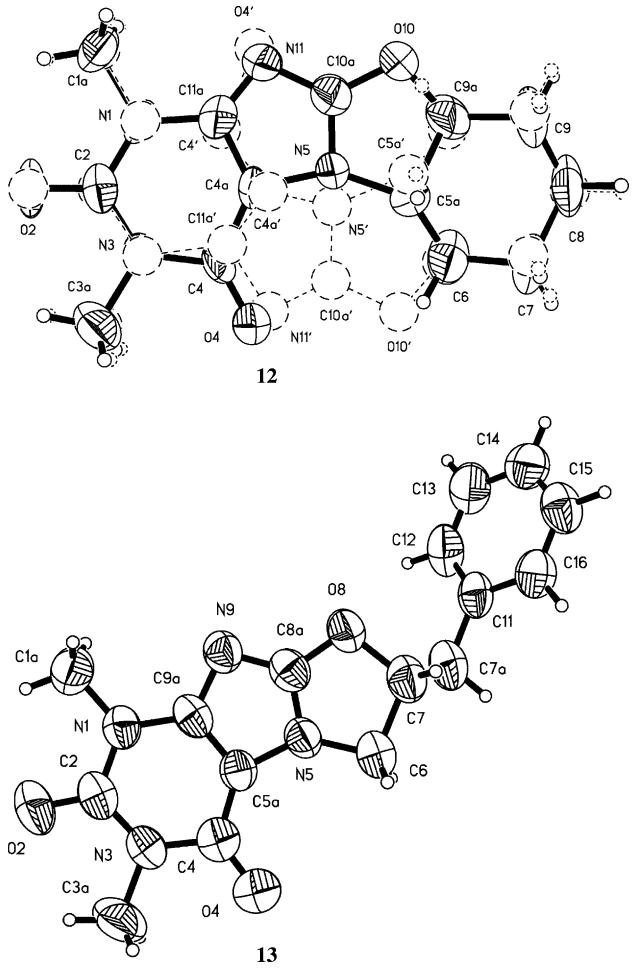


Figure 3. ORTEP drawings of **12** and **13**. For **12** molecule with sof = 0.3 has been designated by dotted lines.

ity in dose of 100 mg/kg protected against MES and ScMet induced seizures, but in the same dose presented toxicity. Similarly **7** and **10** showing although week A_1AR affinity were active in both tests at doses 100 mg/kg accompanied with neurotoxicity. As the conclusion it should be noticed that the apparent correlation of proconvulsant or anticonvulsant activity (in MES or ScMet induced seizures) with AR affinity was not observed.

5.3. SAR and QSAR studies

In preliminary structure and activity studies, the percent inhibition of oxazolopurinediones (%) of radioligand binding to both studied adenosine receptors (A_1, A_{2A}) has been examined as a function of three lipophilicity descriptors $\log P$, $\log D$ and $c\log P$ ($\log P$ and $\log D$ were calculated[34] $c\log P$ was obtained experimentally[35]). All indispensable numerical data are collected in Table 5 while results in graphical form are presented in Figure 4. For all three descriptors second-degree polynominal relations have been obtained but rather unsatisfying. However after omitting only one point (compound **20**) linear relations have been obtained with significantly higher correlation coefficient. The respective relations have suggested that the compounds with maximal inhibition should possess $c\log P$, $\log P$, and $\log D$ about 3.5.

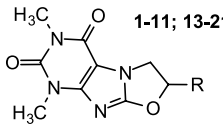
As a basis for subsequent QSAR analysis, the set of eight oxazolopurinediones with known K_i versus [^{3}H]MSX (Table 5) has been taken into account. The data finally selected as the descriptors are collected in Table 10. In the first stage, the relationship of $K_i = f(c\log P)$ has been established keeping, as before in the preliminary study, a second-degree polynominal character ($r^2 = 0.52$) with minimal K_i at $c\log P$ about 3 (Fig. 5a). It agrees well with the relationship from Figure 4 for compounds exhibiting the highest affinity to the receptor.

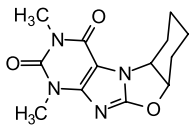
Due to the lack of suitable descriptors, molecular modeling[36] and geometry optimization[40] for all eight oxazolopurines were performed. In order to broaden our knowledge on this subject, the volumes of the substituents at the main tricyclic oxazolo[2,3-*f*]purinedione skeleton (ΔV) were calculated and selected as descriptors (Table 10). From the linear relationship (Fig. 5b) it

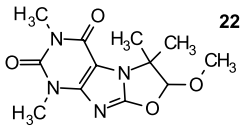
Table 4. Selected geometry details for **12** (for molecule with sof = 0.7 only) and **13**

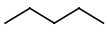
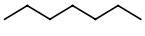
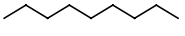
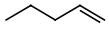
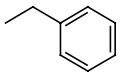
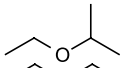
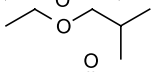
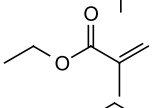
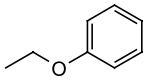
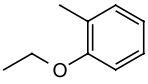
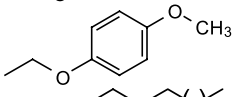
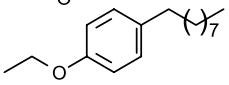
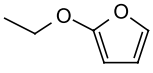
12		**13**	
(a) Torsion angles (°)			
N5–C5a–C9a–O10	−17.6	N5–C6–C7–O8	3.7
C5a–C9a–O10–C10a	22.7	C6–C7–O8–C8a	−1.9
C9a–O10–C10a–N5	4.3	C7–O8–C8a–N5	4.0
O10–C10a–N5–C5a	11.9	O8–C8a–N5–C6	−1.8
C10a–N5–C5a–C9a	−18.8	C8a–N5–C6–C7	−4.6
(b) Deviation of the selected atoms from purine ring plane (Å)			
C5a	0.327 (5)	C6	−0.153 (5)
C9a	−0.030 (5)	C7	−0.156 (5)
O10	0.021 (4)	O8	−0.099 (4)
(c) Random deviation purine ring atoms (Å)			
	0.033		0.171
(d) Asymmetry parameter (twofold-axis) (°)			
ΔC_2 (C2a… O10–C10a)	12.8°	ΔC_2 (N5… O8–C7)	2.0°

Table 5. Affinities of oxazolo[2,3-*f*]purinediones at A_1 and A_{2A} adenosine receptors and lipophilicity parameters







No.	R	A_1 versus [^{3}H]CCPA[a] % inhib. ± SEM concn 25 μM	K_i ± SEM versus [^{3}H]CCPA $n=3$ (μM)	A_{2A} versus [^{3}H]MSX-2[b] % inhib. ± SEM concn 25 μM	K_i ± SEM versus [^{3}H]MSX-2 $n=3$ (μM)	Log *P*	*c*log *P*	Log *D* (pH 7.4)
1	H	8 ± 2	>25	15 ± 2	>25	−0.95		−0.95
2	CH_3	11 ± 1	>25	0	>25	−0.42	−0.50	−0.42
3		7 ± 4	>25	0	>25	−0.36	−0.67	−0.20
4		11 ± 11	>25	20 ± 2	>25	0.09	0.34	0.09
5		6 ± 3	>25	27 ± 3	>25	−1.84	−0.80	−1.68
6		21 ± 6	>25	50 ± 2	26.0 ± 0.80	0.60	0.34	0.60
7		29 ± 8	>25	57 ± 4	16.1 ± 2.87	1.11	0.80	1.11
8		40 ± 10	⩾ 25	87 ± 6	3.70 ± 0.85	2.13	2.13	2.13
9		33 ± 7	>25	90 ± 4	0.998 ± 0.05	3.15	3.60	3.66
10		41 ± 9	⩾ 25	48 ± 9	22.7 ± 5.8	0.76	0.43	0.76
11		30 ± 3	>25	47 ± 6	⩾ 25	1.78		1.78
12		20 ± 2	>25	42 ± 7	⩾ 25			
13		69 ± 3	2.07 ± 0.15	80 ± 6	8.39 ± 1.76	1.26	0.51	1.26
14		6 ± 2	>25	8 ± 4	>25	−0.21	−0.08	−0.05
15		18 ± 2	>25	55 ± 7	2.67 ± 0.54	0.06	0.66	0.22
16		9 ± 3	>25	9 ± 1	>25	−0.04	−0.32	0.11
17		35 ± 6	>25	69 ± 2 ($n=4$)	12.9 ± 3.9	0.63	0.70	0.78
18		37 ± 12	>25	48 ± 3	⩾ 25	1.07	1.01	1.22
19		9 ± 8	>25	33 ± 11	>25	0.58	0.96	0.74
20		16 ± 8	>25	37 ± 4	>25	6.19	5.55	5.32
21		18 ± 6	>25	24 ± 1	>25	−0.04		−0.04
22		15 ± 7	>25	32 ± 3	>25	0.34		0.34

[a] At rat brain cortical membranes.
[b] At rat brain striatal membranes.

Table 6. Binding of oxazolo[2,3-*f*]purinediones to human recombinant A_{2B} and A_3 adenosine receptors

Compd	A_{2B} human versus [^{3}H]ZM241385 % inhibition ± SEM concn 10 μM ($n=2$)	K_i ± SEM (μM)	A_3 human versus [^{3}H]PSB-11 % inhibition ± SEM concn 10 μM ($n=2$)	K_i ± SEM (μM)
9	56 ± 1	5.2 ± 2.7	39 ± 2	12.3 ± 0.6[a]
12	24 ± 4	>10	0 ± 0	>10
13	17 ± 5	>10	16 ± 1	>10
15	29 ± 9	>10	0 ± 0	>10
17	39 ± 0	>10	22 ± 4	>10
20	31 ± 1	>10	2 ± 0	>10

[a] Estimated value by extrapolation; full curve could not be determined due to limited solubility of the compound.

Table 7. Anticonvulsant activity and neurotoxicity of oxazolo[2,3-*f*]purinediones

Compd[a]	MES[b]				ScMet[b]				Toxicity[b]			ASP class[c]
	0.25 h	0.5 h	1 h	4 h	0.25 h	0.5 h	1 h	4 h	0.25 h	0.5 h	4 h	
1		—		—		—		—		300	300[d]	3
2		—		—		—		—		—	—	3
3		—		—		300		—		300[e]	—	2
4		300		—		100		—		300[e]	—	1
5		—		—		—[g]		—		—	—	3
6		300		—		300		—		300[f]	—	2
7	100	300		—		100		300	100[f]	100[e]	—	1
8		300		—		300		—		300[e]	—	2
9		—		—		—		—		—	—	3
10	100	300		300		100		300	100[e]	100	300[f]	1
11		300		300	100	300		300		300[f]	—	1
12		—		—		300		—		—	—	2
13		100				100[i]				100		
				—				—		300[e]	—	1
14		—		—		300[j]		—		300[e]	—	2
15		—		—		300[e]		300		300[e]	—	2
16		—		—		—		—		300	—	3
17		—		—		—		—		—	—	3
18		—		—		—		—		—	—	3
19		—		—		—		—		—	—	3
20		—		—		—		—		—	—	3
21		—		—		—		—		300[f,d]	300	3
22		—		—		[h]		—		300[d]	300	3

Test results in mice after intraperitoneal injection.

[a] Suspension in 0.5% methylcellulose.

[b] Doses of 30, 100, 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby activity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4 h after injections were made. For compounds **7**, **10**, **11** biological response was after 0.25 h. The dash (—) indicates an absence of activity at the maximum dose administered (300 mg/kg).

[c] Classification is as follows: (1) Anticonvulsant activity was observed (even in less number than 50% of animals) at 100 mg/kg or less, (2) anticonvulsant activity at doses greater than 100 mg/kg, (3) compound inactive at 300 mg/kg.

[d] Death.

[e] Unable to grasp rotorod.

[f] Loss of righting reflex.

[g] Continuous seizure activity at dose 30 mg/kg.

[h] Death following tonic extension at dose 100 mg/kg.

[i] Myoclonic jerks.

[j] Death following continuous seizure at dose 30 mg/kg.

Table 8. Anticonvulsant activity and neurotoxicity of selected compounds after oral administration (30 mg/kg or 50 mg/kg) to rats

Compd[a]	MES					ScMet			Toxicity			
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	4 h	0.25 h	0.5 h	1 h	4 h
6							50				—[b]		
7	30		30				—				—		
10		30	30	30	30		—				—		
11			30				—				—		
12	30						—				—		
13	30		30	30			—				—		
14							—				—		

[a] Form—suspension in 0.5% methylcellulose.

[b] The dash (—) indicates an absence of activity and toxicity at the maximum dose administered (50 mg/kg).

has been deduced that the substituent volume ΔV should be limited to 400 cubic ($\AA^3$).

6. Conclusions

Tricyclic oxazolo[2,3-*f*]purinediones are adenosine receptor ligands with moderate affinity mostly exhibiting selectivity for A_{2A} versus A_1 receptors. These compounds were weak and neurotoxic anticonvulsants in mice tests, after intraperitoneal administration, showing much better activity in rats and lacking the neurotoxicity after oral administration. Although no apparent correlation between anticonvulsant activity and adenosine receptor affinity (the best A_{2A} ligand **9** was inactive as

Table 9. Quantitative anticonvulsant activity and neurotoxicity of **4** and valproate in mice ip

Compd	TPE[a] (h)	TD_{50}[b]	ED_{50} ScMet[c]	PI (TD_{50}/ED_{50})[d]
4	0.25/0.5	151.83 (108.02–180.07) [6.82]	132.96 (110.22–151.66) [10.53]	1.14
Valproate[e]	0.25/0.5	483	209	2.3

[a] Time to peak effect. The first value is for rotorod test (dose 200 mg/kg), the second is for the ScMet test (175 mg/kg). In the neurotoxicity assay, **4** was tested at 0.25–0.5 h, in ScMet at 0.25–1 h.
[b] Dose (mg/kg) eliciting evidence of minimal neurological toxicity in 50% of animals; 95% confidence interval is shown in parentheses; the slope of the regression line is shown in brackets.
[c] Dose (mg/kg) eliciting the ScMet protection in 50% animals.
[d] Protective index–neurotoxic dose/median effective dose.
[e] Data from Ref. 27.

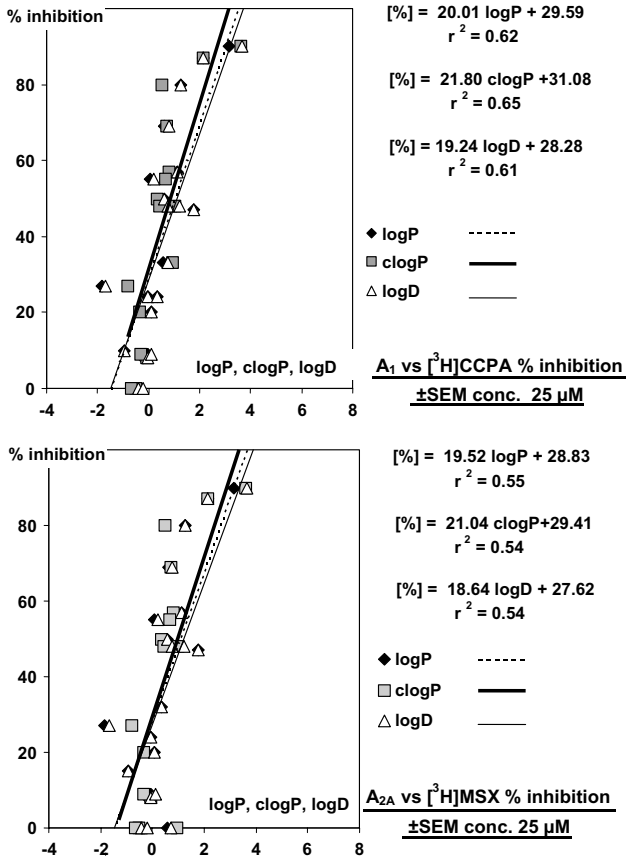


Figure 4. Relations between % inhibition of radioligand binding and lipophilicity descriptors.

anticonvulsant) was observed, it seems that a lipophilic group at the oxazole ring is necessary for both activities and the volume of the substituent being of the necessary optimal size is important for high adenosine A_{2A} receptor affinity.

7. Experimental

7.1. Chemistry

Melting points were determined on a MEL-TEMP II apparatus and are uncorrected. IR spectra were recorded as KBr discs on an FT Jasco IR 410 spectrometer. ^{1}H NMR spectra were performed with a Varian-Mercury 300 MHz spectrometer in DMSO-d_6 (**1** and **5**) or $CDCl_3$ (the remaining compounds given) in σ units, using TMS as an internal standard.

Table 10. Similarity descriptors used in QSAR analysis

Compd	K_i versus [^{3}H]MSX-2	$c\log P$	Volume of the substituent (Å^3)
6	26.00	0.34	159.51
7	16.10	0.80	212.53
8	3.70	2.13	321.57
9	1.00	3.60	430.99
10	22.70	0.43	197.25
13	8.39	0.51	267.81
15	2.67	0.66	352.60
17	12.90	0.70	309.75

TLC data were obtained with Merck silica gel 60F_{254} aluminum sheets using benzene/acetone (7:3) as developing system. The plates were visualized with UV light. UV spectra were recorded on a Jasco UV–vis V-530 apparatus in a concentration of 10^{-5} mol/L in methanol. Elemental analyses were performed on an Elemental Vario-EL III apparatus.

7.2. General procedure for the synthesis of 7-monosubstituted or 6,7-disubstituted 1,3-dimethyl-6,7-dihydro-1,3-oxazolo[2,3-*f*]purine-2,4(1*H*,3*H*)-dione

A mixture of 8-bromotheophylline (**1**, 2.58 g, 10 mmol) and 20 mmol of the corresponding oxirane was heated under reflux in anhydrous propanol, butanol or acetonitrile (see Tables 1, 2) in the presence of a catalytic amount of pyridine (0.3 mL) for 2–10 h. After dissolution of the starting substance (10 min to 2 h) the progress of the reaction was monitored by means of TLC showing three spots (starting material and probably ring-open and cyclic product). After further heating the spot of the open-chain product disappeared (2–10 h). Some main products showed only a little amount of starting material. After cooling the precipitate was collected by filtration and mixed with 10% NaOH to remove eventually the rest of unreacted 8-bromotheophylline and subsequently washed with water. A second crop (of worse quality) could be obtained by evaporating the solvent, mixing the residue with 10% NaOH and washing with

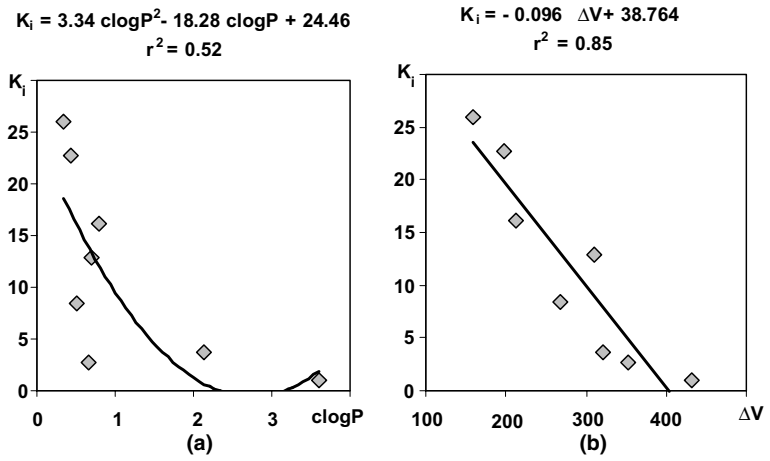


Figure 5. (a) Correlation between K_i-value at A_{2A} adenosine receptors and *c*log*P*. (b) Correlation between K_i-value at A_{2A} adenosine receptors and substituent volume.

water. Combined precipitates were purified by crystallization.

7.3. Analytical chromatographic measurements[35]

Analytical TLC was performed on 20×20 cm pre-coated RP-TLC Al sheets of RP-18, F_{254S} (Merck); samples of 2.5 μL of the solutes (0.1 mg/mL in methanol) were spotted with a Hamilton microliter syringe. The chromatograms were developed over a distance of 17.5 cm. The chambers were saturated with organic solvent vapor for 20 min. In the performed studies the water/methanol mixtures were used as mobile phases. The concentration of the organic modifier in the mobile phase ranged from 80–98%. All TLC measurements were performed at 21 °C. Spots were visualized under UV light at 254 nm.

8. Pharmacology

8.1. Adenosine receptor binding assays

Radioligand binding assays were performed as previously described using rat brain cortical membrane preparations for A_1 AR assays, and rat brain striatal membrane preparations for A_{2A} AR assays.[28–30] Frozen rat brains (unstripped) were obtained from Pel-Freez®, Rogers, Arkansas, USA. For assays at human A_3 ARs, CHO cell membranes containing the human A_3 AR were used as described.[28,31] [^{3}H]2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) was used as the A_1 radioligand, [^{3}H]3-(3-hydroxypropyl)-7-methyl-8-(*m*-methoxystyryl)-1-propargylxanthine ([^{3}H]MSX-2) as the A_{2A} ligand,[21] [^{3}H]4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolo-[2,3-*a*][1,3,5]-triazin-5-(4-(amino)-ethyl)phenol ([^{3}H]ZM-241385) as the A_{2B} receptor ligand,[32] and [^{3}H]2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo-[2,1-*i*]purine-5-one ([^{3}H]PSB-11) as the A_3 AR radioligand.[23,33] Initially, a single high concentration of compound (25 μM at A_1 and A_{2A}, 10 μM at A_{2B} and A_3 receptors) was tested in three (A_1, A_{2A}) or two (A_{2B}, A_3) independent experiments. For potent compounds, which showed greater than 50% inhibition of radioligand binding at the test concentration, curves were determined at the A_1 and A_{2A} ARs using 6–7 different concentrations of test compounds spanning 3 orders of magnitude. At least three separate experiments were performed each in triplicate. Data were analyzed using the PRISM program version 3.0 (GraphPad, San Diego, CA, USA).

8.2. Anticonvulsant screening

The anticonvulsant evaluation was carried out using reported procedures.[25,26] Male albino mice (CF-1 strain, 18–25 g), and male albino rats (Sprague-Dawley 100–150 g) were used as experimental animals. Groups of 1–5 mice were used in MES and ScMet tests, groups of 2–8 animals in rotorod tests. For the evaluation of activity after oral administration groups of 4 rats were used. The tested compounds were suspended in a 0.5% methyl–cellulose–water mixture.

In the preliminary screening each compound was administered as an ip injection at three dose levels (30, 100 and 300 mg/kg) with anticonvulsant activity and neurotoxicity assessed at 30 min and 4 h intervals after administration. For some compounds also intervals of 15 min and 1 h were used.

Anticonvulsant efficacy was measured by maximal electroshock (MES) and subcutaneous pentylenetetrazole (ScMet), neurological deficit was investigated in the rotorod tests; the data are presented in Table 7. Some selected derivatives were examined for oral activity in the rat MES, ScMet and neurotoxicity screen at 30 and 50 mg/kg doses. The results are summarized in Table 8.

The pharmacological parameters estimated in the preliminary screening were quantified for compound **4** (Table 9).

Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}) and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50}, groups of 8 mice were given

Table 11. Crystal data and structure refinement details for **12** and **13**

	12	**13**
Empirical formula	$C_{13}H_{16}N_4O_3$	$C_{16}H_{16}N_4O_3$
Formula weight	276.30	312.33
Temperature	293 (2) K	293 (2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$
Unit cell dimensions	$a = 10.238$ (2) Å	$a = 9.604$ (2) Å
	$b = 9.344$ (2) Å	$b = 9.604$ (2) Å
	$c = 13.547$ (3) Å	$c = 14.500$ (3) Å
	$\beta = 94.88$ (3)°	$\beta = 102.45$ (3)°
Volume	1291.3 (5) $Å^3$	1498.9 (5)
Z, Calculated density	4, 1.421 Mg/m^3	4, 1.384 Mg/m^3
Absorption coefficient	0.104 mm^{-1}	0.099 mm^{-1}
F (000)	584	656
Crystal size	0.2×0.2×0.4 mm	0.25×0.3×0.5 mm
Theta range for data	2.96°–25.0°	2.98°–23.0°
Index ranges	−12:*h*:12, −11:*k*:11, −16:*l*:15	−10:*h*:10, −12:*k*:12, −15:*l*:15
Diffractometer	KM-4 with CCD detector	KM-4 with CCD detector
Refl. collected/unique	6499/2208 [R(int) = 0.0994]	12953/2079 [R(int) = 0.1024]
Refinement method	Full-matrix-block least-squares on F^2	Full-matrix-block least-squares on F^2
Data/parameters	2208/263	2079/210
Goodness-of-fit on F^2	1.005	1.099
Final R [$I > 2\sigma$ (I)]	$R1 = 0.0686$, wR2 = 0.1529	$R1 = 0.0688$, wR2 = 0.1701
Largest diff. peak and hole	0.198 and −0.175 e $Å^{-3}$	0.285 and −0.184 e $Å^{-3}$

Table 12. Atomic coordinates (×10^4) and equivalent isotropic displacement parameters ($Å^2$×10^3) for (**12**) and (**13**)

12

	Molecule with sof = 0.7					Molecule with sof = 0.3			
N1	3964 (2)	2363 (3)	4768 (2)	57 (1)	N1′	4009 (6)	2420 (6)	4808 (5)	51 (2)
C1a	3902 (3)	3388 (3)	3968 (3)	69 (1)	C1a′	4123 (9)	3482 (9)	3990 (8)	88 (3)
C2	2871 (3)	1640 (3)	4949 (3)	57 (1)	C2′	2780 (8)	1608 (8)	4875 (6)	51 (2)
O2	1867 (2)	1800 (2)	4379 (2)	68 (1)	O2′	1753 (6)	1836 (7)	4408 (5)	83 (2)
N3	2946 (2)	692 (3)	5682 (2)	61 (1)	N3′	2880 (5)	605 (6)	5760 (4)	39 (2)
C3a	1690 (3)	−35 (4)	5786 (2)	81 (1)	C3a′	1790 (5)	−271 (6)	6106 (5)	29 (2)
C4	3982 (2)	296 (3)	6343 (3)	48 (1)	C4′	5129 (6)	1911 (6)	5343 (5)	28 (2)
O4	3977 (2)	−532 (2)	7029 (2)	60 (1)	O4′	6316 (5)	2856 (6)	5065 (4)	67 (2)
C4a	5065 (3)	1250 (3)	6089 (2)	56 (1)	C4a′	5291 (5)	1133 (5)	6257 (4)	14 (1)
N5	6408 (2)	1164 (2)	6558 (2)	55 (1)	N5′	6101 (5)	652 (6)	6803 (5)	50 (2)
C5a	7230 (3)	853 (4)	7480 (3)	76 (1)	C5a′	7388 (5)	1142 (5)	7311 (4)	17 (1)
C6	7400 (4)	−238 (3)	8066 (3)	93 (2)	C6′	7357 (6)	−580 (7)	7936 (5)	38 (2)
C7	8419 (4)	−324 (5)	8855 (3)	15 (2)	C7′	8464 (6)	−172 (7)	8821 (5)	29 (2)
C8	9732 (3)	401 (4)	8476 (3)	192 (2)	C8′	9679 (7)	165 (8)	8602 (6)	52 (2)
C9	9610 (3)	1580 (4)	7864 (3)	83 (2)	C9′	9658 (7)	1605 (8)	7818 (6)	48 (2)
C9a	8541 (4)	1513 (4)	7070 (4)	01 (2)	C9a′	8520 (5)	1174 (6)	7075 (5)	26 (2)
O10	8249 (2)	441 (2)	6339 (2)	171 (1)	O10′	6216 (4)	−852 (5)	8070 (4)	49 (1)
C10a	6998 (3)	2157 (3)	6034 (3)	69 (1)	C10a′	5501 (7)	−312 (8)	7298 (6)	54 (2)
N11	6226 (2)	2828 (3)	5315 (2)	59 (1)	N11′	4360 (6)	−659 (7)	6957 (6)	70 (2)
C11a	5148 (3)	2284 (3)	5377 (3)	56 (1)	C11a′	4264 (6)	597 (7)	6310 (5)	39 (2)

13

N1	5486 (3)	1860 (2)	−22 (2)	64 (1)	C7a	1997 (5)	−1033 (3)	2738 (3)	81 (1)
C1a	6895 (4)	2346 (4)	361 (3)	90 (1)	08	3468 (3)	708 (2)	2529 (2)	81 (1)
C2	4956 (4)	1877 (3)	−992 (3)	68 (1)	C8a	3802 (4)	921 (3)	1702 (3)	65 (1)
O2	5671 (3)	2303 (3)	−1518 (2)	94 (1)	N9	4994 (3)	1394 (3)	1514 (2)	66 (1)
N3	3619 (3)	1402 (3)	−1323 (2)	68 (1)	C9a	4621 (4)	1414 (3)	551 (2)	61 (1)
C3a	3073 (5)	1436 (5)	−2357 (3)	107 (2)	Cll	2524 (4)	−1079 (3)	3792 (3)	68 (1)
C4	2714 (4)	914 (3)	−789 (3)	70 (1)	C12	3875 (5)	−1444 (4)	4198 (3)	88 (1)
O4	1529 (3)	501 (3)	−1180 (2)	98 (1)	C13	4359 (5)	−1496 (4)	5160 (3)	97 (2)
C5a	3292 (4)	973 (3)	174 (2)	58 (1)	C14	3490 (5)	−1192 (4)	5746 (3)	85 (1)
N5	2783 (3)	635 (3)	968 (2)	63 (1)	C15	2130 (5)	−809 (3)	5368 (3)	83 (1)
C6	1480 (4)	226 (4)	1220 (3)	86 (1)	C16	1642 (4)	−759 (3)	4399 (3)	76 (1)
C7	1991 (4)	224 (4)	2319 (3)	77 (1)					

U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

a range of ip doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plot of this data, the respective ED_{50}, TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program written at NINCDS, NIH.

8.3. X-ray structure analysis of 12 and 13

The crystals of **12** and **13** were obtained by slow evaporation of ethanol solutions. All measurements of the crystals were performed on a Kuma4CCD κ-axis diffractometer with graphite-monochromated MoKα radiation at room temperature. The data were corrected for Lorentz and polarization effects. No absorption correction was applied. All crystallographic data and experimental details are presented in Table 11. The structures were solved by direct methods[37] and refined using SHELXL.[39] The full-matrix least-squares refinement was based on F^2. The anisotropic temperature factors for all non-H-atoms in structure **13** were applied. In the crystal of **12** structural disorder was found and for all non-H atoms in the molecule with structure occupation factor (sof) equaling 0.7 anisotropic temperature factors were used while for atoms from the molecule with sof of 0.3 only isotropic ones. The positions of all H-atoms were found from the electron density $\Delta\rho$ map and refined in a raiding model with the isotropic displacement parameters of 1.5 times the respective U_{eq} values for the parent atoms. Atomic scattering factors were those as in SHELXL.[38] Atomic coordinates are gathered in Table 12. Crystallographic data (excluding structural factors) for structures **12** and **13** reported in this paper have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EW, UK (Fax: +441223 336033; E-mail: deposit@ccdc.cam.ac.uk).

8.4. Computational procedures

The starting models of the molecules were based on crystallographic data for **12** and **13** using the PCMOD.6 program.[36] The geometries of the molecules were optimized with MOPAC 6.0 using AM1 Hamiltonians in an aqueous environment (dielectric constants equals 78.4).[40]

The values of $\log P$ and $\log D$ (at pH = 7.4) for the compounds investigated were calculated by means of PALLAS (version 1.2) program.[34] Multiple Linear Regression equations were computed by means of the QSAR-PC:PAR program written by R. C. Coburn.[37]

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