Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 397–402
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Impact of the aryl substituent kind and distance from pyrimido[2,1-*f*]purindiones on the adenosine receptor selectivity and antagonistic properties

Anna Drabczyńska [a], Britta Schumacher [b], Christa E. Müller [b], Janina Karolak-Wojciechowska [c], Barbara Michalak [a], Elżbieta Pękala [a], Katarzyna Kieć-Kononowicz [a,*]

[a] *Faculty of Pharmacy, Department of Chemical Technology of Drugs, Jagiellonian University Medical College, Medyczna 9, PL 30-688 Krakow, Poland*
[b] *Pharmaceutical Institute Poppelsdorf, University of Bonn, Kreuzbergweg 26, 53115 Bonn, Germany*
[c] *Institute of General and Ecological Chemistry, Technical University of Łódź, Żwirki 36, PL 30-924 Łódź, Poland*

Received 1 October 2001; received in revised form 29 December 2002; accepted 30 January 2003

Abstract

Adenosine receptor (AR) antagonists belong to two major groups of compounds: xanthines and non-xanthines. Recently several annelated xanthine derivatives have been described as selective A_1, A_{2A}, A_{2B} and A_3 ARs antagonists. Contrary to dipropyl derivatives, in the group of dimethyl (un)substituted arylalkyl pyrimido[2,1-*f*]purindiones selective mainly adenosine A_{2A} receptor antagonists were identified. Their activity depended on aryl substitution and its distance from pyrimido[2,1-*f*]purindione.

Keywords: Adenosine receptors antagonists; Adenosine A_{2A} receptors antagonists; Annelated xanthines; Pyrimido[2,1-*f*]xanthines

Adenosine regulates many physiological functions via specific cell membrane receptors. To date, four adenosine receptor (AR) subtypes have been cloned, A_1, A_{2A}, A_{2B} and A_3, each of which exhibits an unique tissue distribution, ligand affinity and signal transduction mechanism [1]. These G protein-coupled receptors are important physiological regulators in many tissues, coupled to different second messenger systems, such as adenylate cyclase, calcium or potassium channels (A_1) or phosholipase C (A_1, A_{2B} A_3) and D (A_3). Moreover, ARs are involved in interactions with receptors for other neurotransmitters and/or neuromodulators, such as receptors for neuropeptides (CGPR and VIP), ionotropic (NMDA) and metabotropic (mGlu I and III) glutamate receptors, GABA and nicotinic, muscarinic and dopamine receptors [1–4].

Selective interaction with AR subtypes offers very broad therapeutic potentials including CNS disorders, regulation of the electrophysiological properties of heart, immune system and inflammatory diseases, cell growth, asthma, kidney failure and ischemic injuries [5–9]. Since AR agonists may cause a wide spectrum of effects, AR-selective antagonists are generally believed to have more potential as drugs because fewer side-effects would be expected [10]. Although several antagonists of ARs were developed [see reviews [11,12]], only few are undergoing advanced clinical trials.

The main recent directions and problems of the AR antagonist research concerns development of selective AR subtype antagonists. It was shown that highly effective ARs ligands of commonly used laboratory animals have shown different affinity at human receptors [13,14]. Many ligands that have been previously characterised as highly selective for one subtype of AR were not investigated on the recently discovered A_{2B} and A_3 ARs [13–15] and should be re-evaluated. Frequently bioavailability of the recognised potent antagonists is low due to their hydrophobicity and low-water solubility [11,16]. The problem of classification of receptor antagonists as neutral antagonists or antagonists with inverse agonist activity is recently discussed [17]. The

* Corresponding author.
E-mail address: mfkonono@cyf-kr.edu.pl (K. Kieć-Kononowicz).

0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00051-5

existence of functional 'atypical' adenosine A_{2A} AR has been suggested [18]. The activity of allosteric enhancers of A_1 AR is currently examined and developed [19,20].

AR antagonists belong to two major groups of compounds: xanthines and non-xanthines. A large number of AR antagonists have been developed in the group of xanthine derivatives (Fig. 1). Structure–AR activity examination allowed to formulate general requirements necessary for the activity at each subtype of receptors.

A_1 AR antagonists are being developed as kidney protective diuretics, for the prevention of renal failure and as cognition enhancers. Important classes of A_1-selective antagonists comprise xanthine derivatives with bulky 8-substituents (e.g. **1**, **2**), additional requirements are connected with substituents in the 1,3 positions of the xanthine core. Contrary to the 1-position where no polar substituents are tolerated, hydrophilic substitution with large substituents at the 3-position is allowed. Unsubstituted position 7 is believed to be important for the activity [21]. A_{2A} selective AR antagonists are promising new drugs for the treatment of Parkinson's disease. An 8-styryl substituent in (*E*) configuration was recognised as crucial for A_{2A} AR antagonistic properties (e.g. **3**, **4**). Introduction of substituents at the 7 position was stated as possible for the active structures [22,23]. A_{2B} AR antagonists may have potential in the treatment of asthma, myocardial reperfusion injury, allergic reactions and non-insulin dependent diabetes and retinopathy. The first potent antagonists at human A_{2B} AR were found in the group of 1,3-dialkylxanthines [24]. More selective and potent A_{2B} AR antagonists (e.g. **5**, **6**) have been reported nowadays among 8-phenyl xanthine derivatives [25,26] and 3-unsubstituted xanthines bearing polar substituents enhancing their water solubility [27]. Recently xanthine ligands were found for the A_3 AR subtype (e.g. **7**, **8**), initially believed to be xanthine insensitive [28,29]. A_3 AR antagonists are being developed as anti-inflammatory and anti-asthmatic drugs, antidepressants, antiarrhythmics, immunomodulators, renal protective diuretics, antiparkinson and cognition enhancing drugs. Anticancer activity is also currently examined.

In subsequent studies, an interesting approach was pursued based on annelation of xanthine derivatives. Compounds possessing fused rings (types A–E) of the general structure presented in Fig. 2 were investigated and have shown different affinity to AR subtypes. Linear triazolopurinones (fused type A) were found [30,31] to display A_1 antagonistic properties at human A_1 ARs selective over rat A_{2A} ARs (Fig. 3, **9**, **10**). The same group of compounds allowed development of **11**, **12** with high affinities to A_3 ARs [30,31]. Angular triazolopurinones (fused type B) exhibited high affinities to human A_1 (**13**, **14**) and/or A_3 ARs (**15**, **16**) with high selectivities versus rat A_{2A} ARs [32,33]. Pyrimidopurinones **17**, **18** are examples of 3,9-substituted xanthines. Compounds possessing noradamantyl residues exhibited selective rat A_1 activity over rat A_{2A} and human A_{2B} ARs [34]. Imidazopurinones (fused type D) with increased water solubility are potent A_1 (**19**) [35], A_{2A} (**20**), A_3 (**21**) antagonists [36]. Compound **21** in a tritiated

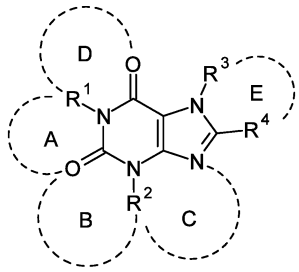


Fig. 2. General structures of annelated xanthines examined as AR antagonists.

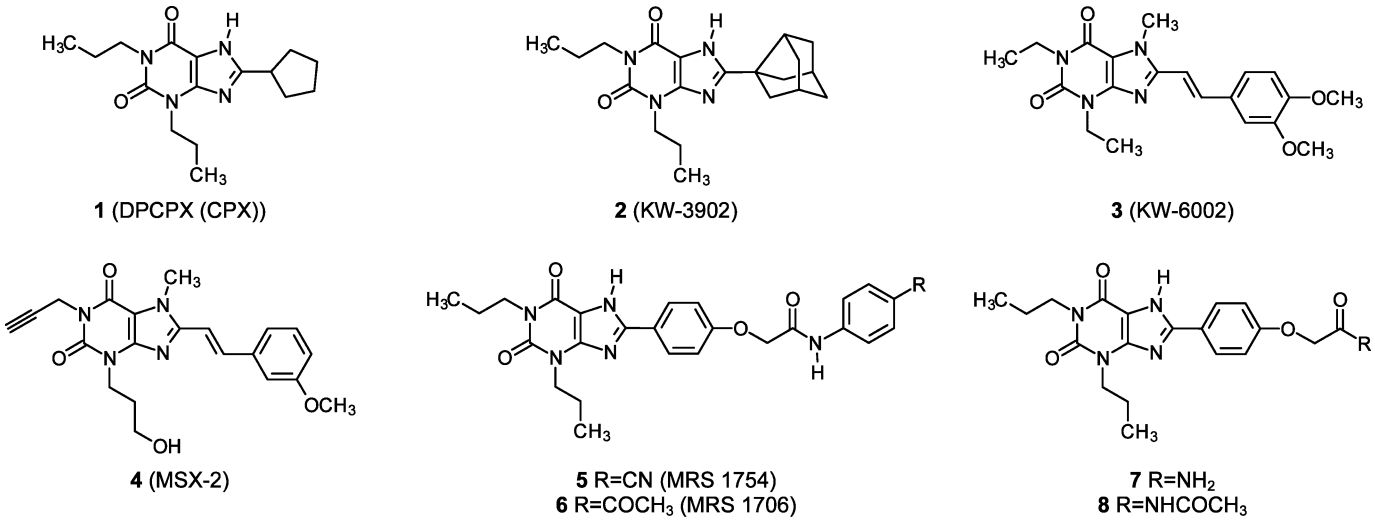


Fig. 1. Xanthine adenosine A_1 (**1**, **2**), A_{2A} (**3**, **4**), A_{2B} (**5**, **6**) and A_3 (**7**, **8**) receptor antagonists.

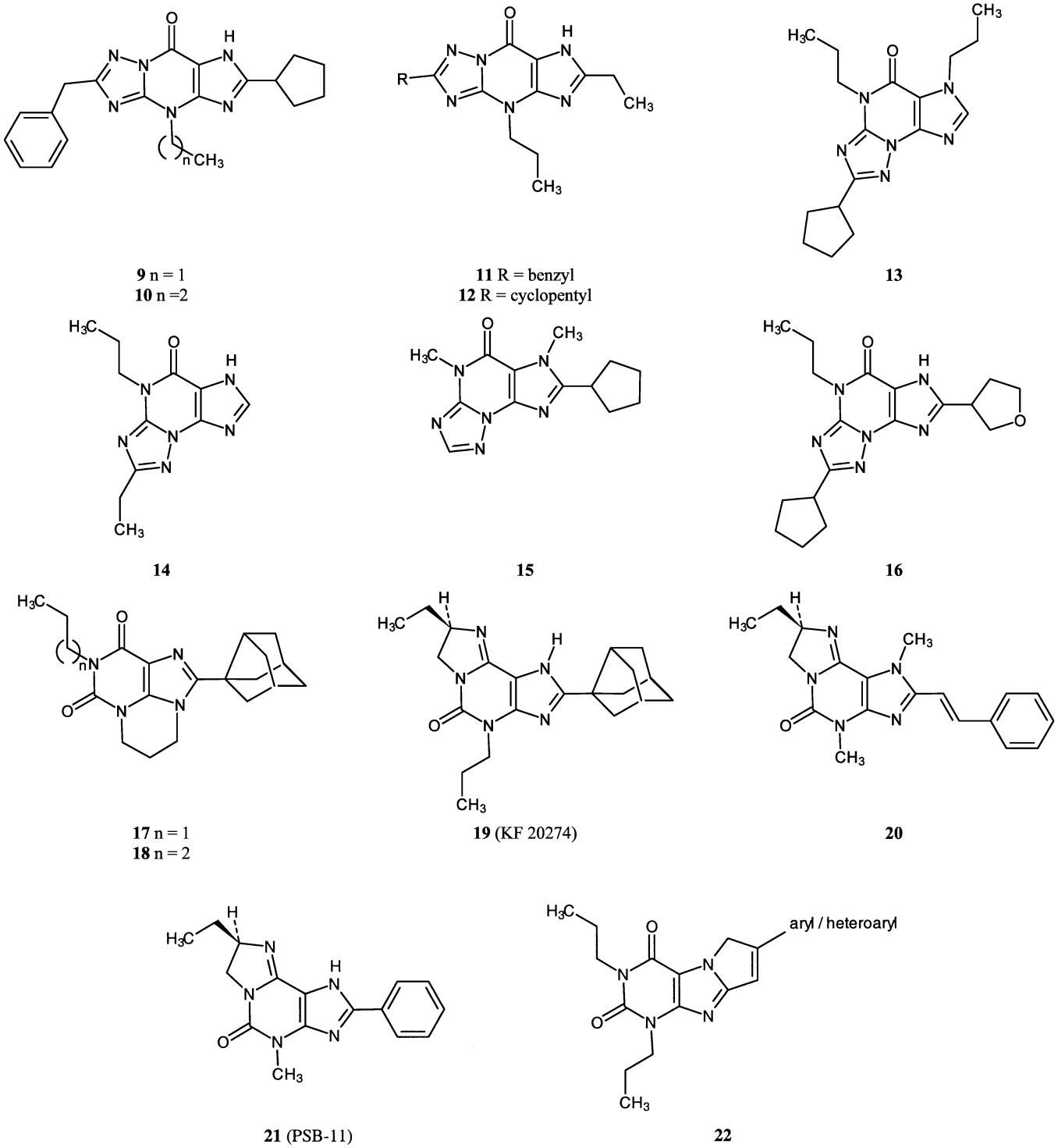


Fig. 3. Structures of annelated xanthine AR antagonists.

form was developed as a novel antagonist radioligand for the human A_3 ARs [37]. Type E annelation is represented by hetero/aryl pyrrolinopurinones with general structure **22** [38]. Unfortunately such compounds have shown negligible affinity for A_1- and A_{2A} ARs and very low selectivity.

In our efforts to develop new ligands for A_{2A} AR the structure of MSX-2 (**4**) and other 8-styryl xanthines were used as a template (Fig. 4). An 8-styrylxanthines are active in their (*E*)-configuration. *Z*-isomers show a great decrease both in potency and selectivity to A_{2A} ARs. However they are highly flexible molecules and

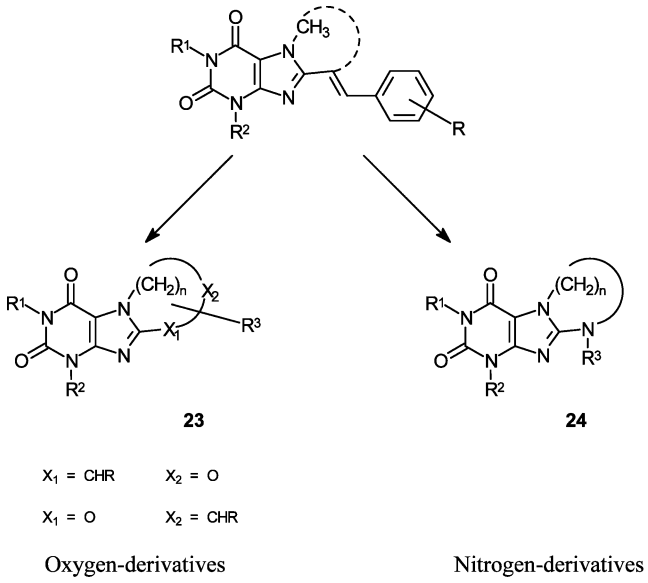


Fig. 4. Oxygen or nitrogen containing annelated theophylline analogs.

their structures observed in solution depend strongly on the environment. Moreover dissolved in organic solvents these compounds at low concentration (0.1 mM) undergo rapid photoisomerisation from *E*-isomer to an equilibrium mixture of *E*/*Z*-isomers when exposed to visible light. In our research program new compounds envisaged as constrained bioisosteric analogs of 8-styrylxanthines were designed: the group of oxygen- or nitrogen-containing annelated theophylline derivatives **23** and **24**. Obtained were oxazolo-, oxazino- and oxazepino-purindiones as oxygen containing fused derivatives of theophylline as well as imidazo-, pyrimido- and diazepino-purindiones as nitrogen containing tricyclic compounds [39–41]. The aim of the present work was to synthesise target compounds with an (un)substituted hetero/aryl group connected by different spacers with the pyrimido[2,1-*f*]purindione system. The designed distance from the tricyclic structure equalled 0–6 carbon (or their isosteric) atoms. Additionally R^1 and R^2 substituents were varied. Dimethyl substituted derivatives **25a**–**25l** (Fig. 5) were obtained starting from theophylline. For di-*n*-propyl derivatives **25m** and **25n** of tricyclic purinones the appropriate xanthine had to be constructed and subsequently converted to the tricyclic structures. Some steps of the syntheses involved reactions performed under microwave irradiation according to Refs. [42,43].

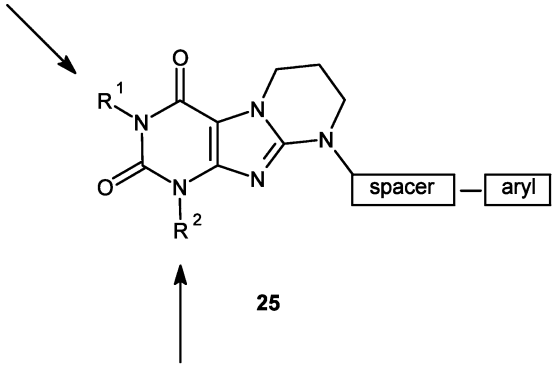


Fig. 5. Pyrimido[2,1-*f*]purindiones (**25**) investigated as AR antagonists.

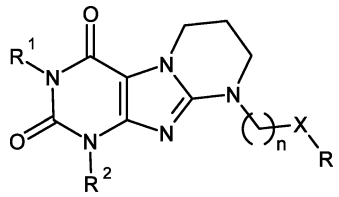
Spatial properties of the obtained compounds were examined by X-ray structure determination and by molecular modeling. Physicochemical properties of the obtained compounds were examined on the basis of theoretical (pK_a, log *P*, Log *D*) calculations and experimental data (TLC, HPLC).

The synthesised compounds were evaluated in vitro for their affinities to A_1 and A_{2A} ARs in assays at rat brain membrane preparations using [^{3}H]CCPA for rA_1 and [^{3}H]MSX-2 for A_{2A} ARs (data for some compounds are presented in Table 1).

Selected compounds (**25h**, **25j** and **25n**) were additionally investigated in binding assays at human recombinant A_{2B} and A_3 ARs (with [^{3}H]ZM 241 385 used for hA_{2B} and [^{3}H]PSB-11 for hA_3 ARs). Dipropyl derivative **25n** exhibited a K_i value of 0.59 μM at human A_{2B} and of 3.66 μM at human A_3 ARs, while for dimethyl derivatives **25h** and **25j** K_i values were higher than 10 μM. The most potent ligands were characterised as antagonists at A_{2A} AR in Na^+ shift assays [44]. Generally, the investigated compounds from the dimethyl series **25a**–**25l** have mainly shown selective adenosine A_{2A} receptors antagonistic properties depending on the distance of the aromatic ring from the tricyclic structure and the substitution pattern of the aromatic ring. Introduction of a heteroatom into the spacer allowed to obtain the selective A_1 AR antagonist **25j**. A larger spacer was advantageous for A_{2A} AR affinity and phenethyl derivatives (**25h** and **25i**) were the most potent compounds in this series. However with a further increase in chain length, the selectivity toward adenosine A_{2A} receptor dropped again. Similar effects had the introduction of *n*-propyl substituents (**25m**, **25n**). Compounds with higher activity were obtained but the selectivity toward all investigated ARs was decreased. Non-apparent QSAR correlation was observed between physicochemical properties descriptors and the affinity values at A_{2A} ARs. It is supposed that mainly spatial placement of the aromatic substituent toward the tricyclic structure is responsible for the differences in the activity. The investigated compounds were somewhat less potent at human as compared to rat A_{2A} ARs.

The compounds were tested in vivo according to the National Institutes of Health's Antiepileptic Drug Development Program in Bethesda [45,46] Table 1 (activity expressed in Anticonvulsant Screening Project—ASP classes 1–3). Activity as anticonvulsants, mainly in chemically induced seizures, have been shown by the compounds without or with a short spacer mainly

Table 1
Pharmacological characterisation of compounds of general structure **25**



25

No	R^1	R^2	*n*	X	R	In vitro		In vivo[a]	
						K_i [μM]		ASP class[b]	Positive tests[c]
						A_1	A_{2A}		
a	methyl	methyl	0	–	phenyl	> 25	3.52	3	
b	methyl	methyl	0	–	4-Cl phenyl	> 25	1.12	1	MES, ScMet
c	methyl	methyl	0	–	3-Cl phenyl	> 25	> 25	3	
d	methyl	methyl	0	–	2-Cl phenyl	> 25	2.35	3	
e	methyl	methyl	1	–	phenyl	3.58	1.09	1	ScMet
f	methyl	methyl	1	–	4-Cl phenyl	> 25	4.28	1	ScMet
g	methyl	methyl	1	–	4-F phenyl	> 25	2.33	1	MES, ScMet
h	methyl	methyl	2	–	phenyl	> 25	0.32	3	
i	methyl	methyl	2	–	4-Cl phenyl	6.47	0.48	3	
j	methyl	methyl	2	O	4-Cl phenyl	4.22	> 25	3	
k	methyl	methyl	2	O	benzyl	3.16	3.69	–	
l	methyl	methyl	2	O	4-Cl benzyl	> 25	1.33	–	
m	*n*-propyl	*n*-propyl	0	–	4-Cl phenyl	0.36	0.38	–	
n	*n*-propyl	*n*-propyl	2	–	phenyl	0.62	0.86	–	

[a] Administered into the mice in doses of 30, 100 and 300 mg/kg as a suspension in 0.5% methylcellulose.
[b] Classification is as follows: 1—anticonvulsant activity at 100 mg/kg or less; 2—anticonvulsant activity at doses greater than 100 mg/kg; 3—compound inactive at 300 mg/kg.
[c] Used tests: electrically induced seizures—maximal electroshock (MES) and chemically induced seizures—subcutaneous pentylenetetrazole (ScMet) tests.

in the group of benzyl derivatives (**25e**, **25f**, **25g**). Compounds with longer spacer (**25e**, **25g**, **25h**, **25i** and **25j**) exhibited neurotoxic effects (rotorod test) according to ASP criteria [45,46]. The most potent 4-fluorophenyl derivative **25g** was advanced for further investigations. There was no apparent correlation between anticonvulsant activity and AR affinity.

Acknowledgements

This study was supported in part by the Polish State Committee for Scientific Research (Grant No 6 P05F 024 21), and by the Deutsche Forschungsgemeinschaft (GRK-677). The authors are grateful to Professor James P. Stables for providing anticonvulsant test data through the Antiepileptic Drug Development Program, National Institutes of Health, Bethesda, Maryland, USA.

References

[1] B.B. Fredholm, A.P. IJzerman, K.A. Jacobson, K.-N. Klotz, J. Linden, Pharmacol. Rev., International Union of Pharmacology XXV Nomenclature and Classification of Adenosine Receptors 53 (2001) 527–552.
[2] J.A. Ribeiro, D. Cunha-Reis, L.V. Lopes, J.E. Coelho, A.R. Costenla, P. Correia-de-Sá, R.A. Cunha, A. de Mendonça, A.M. Sebastião, Drug Dev. Res. 52 (2001) 337–345.
[3] F. Ciruela, M. Escriche, M.M. Soloviev, E.I. Canela, J. Burgeño, J. Mallol, W.-Y. Chan, C. Lluis, R.A.J. McIlhinney, R. Franco, Drug Dev. Res. 52 (2001) 316–322.
[4] J.A. Ribeiro, Eur. J. Pharmacol. 375 (1999) 101–113.
[5] S.M. Kaiser, R.J. Quinn, Drug Discov. Today 4 (1999) 542–551.
[6] S.-A. Poulsen, R.J. Quinn, Bioorg. Med. Chem. 6 (1998) 619–641.
[7] M.P. DeNinno, Annu. Rep. Med. Chem. 33 (1998) 111–120.
[8] A.H. Dickenson, R. Suzuki, A.J. Reeve, CNS Drugs 13 (2000) 77–85.
[9] V. Shneyvays, N. Safran, I. Halili-Rutman, A. Shainberg, Drug Dev. Res. 50 (2000) 324–337.
[10] A.P. Kourounakis, P.A.M. Van der Klein, A.P. IJzerman, Drug Dev. Res. 49 (2000) 227–237.
[11] C.E. Müller, B. Stein, Curr. Pharm. Des. 2 (1996) 501–530.

[12] S. Hess, Expert Opin. Ther. Patents 11 (2001) 1533–1561.
[13] K.-N. Klotz, J. Hessling, C. Owman, B. Kull, B.B. Fredholm, M.J. Lohse, Naunyn-Schmiedeberg's Arch. Pharmacol. 357 (1998) 1–9.
[14] K.-N. Klotz, Naunyn-Schmiedeberg's Arch. Pharmacol. 362 (2000) 382–391.
[15] C.E. Müller, Curr. Med. Chem. 7 (2000) 1269–1288.
[16] R.F. Bruns, J.H. Fergus, J. Pharm. Pharmacol. 41 (1989) 590–594.
[17] J.C. Shryock, M.J. Ozeck, L. Belardinelli, Mol. Pharmacol. 53 (1998) 886–893.
[18] M. El Yacoubi, C. Ledent, M. Parmentier, J. Costentin, J.-M. Vaugeois, Eur. J. Pharmacol. 401 (2000) 63–77.
[19] M.D. Chordia, L.J. Murphree, T.L. Macdonald, J. Linden, R.A. Olsson, Bioorg. Med. Chem. Lett. 12 (2002) 1563–1566.
[20] P.G. Baraldi, A.N. Zaid, I. Lampronti, F. Fruttarolo, M.G. Pavani, M.A. Tabrizi, J.C. Shryock, E. Leung, R. Romagnoli, Bioorg. Med. Chem. Lett. 10 (2000) 1953–1957.
[21] C.E. Müller, Il Farmaco 56 (2001) 77–80.
[22] E. Ongini, A. Monopoli, B. Cacciari, P.G. Baraldi, Il Farmaco 56 (2001) 87–90.
[23] R. Sauer, J. Maurinsh, U. Reith, F. Fülle, K.-N. Klotz, C.E. Müller, J. Med. Chem. 43 (2000) 440–448.
[24] K.A. Jacobson, A.P. IJzerman, J.M. Linden, Drug Dev. Res. 47 (1999) 45–53.
[25] Linden J.M., US6117878 (2000).
[26] Y.-C. Kim, X.-D. Ji, N. Melman, J. Linden, K.A. Jacobson, J. Med. Chem. 43 (2000) 1165–1172.
[27] A.M. Hayallah, J. Sandoval-Ramirez, U. Reith, U. Schobert, B. Preiss, B. Schumacher, J.W. Daly, C.E. Müller, J. Med. Chem. 45 (2002) 1500–1510.
[28] C.A. Salvatore, M.A. Jacobson, H.E. Taylor, J. Linden, R.G. Johnson, Proc. Natl. Acad. Sci. USA 90 (1993) 10365–10369.
[29] Y.-C. Kim, Y. Karton, X.-D. Ji, N. Melman, J. Linden, K.A. Jacobson, Drug Dev. Res. 47 (1999) 178–188.
[30] Boehringer Ingelheim: WO9803511 (1998).
[31] Boehringer Ingelheim Pharm: EP0978517 (2000).
[32] Boehringer Ingelheim Pharm: WO9965912 (1999).
[33] Boehringer Ingelheim Pharm: WO0012511 (2000).
[34] S. Weyler, C.E. Müller, Arch. Pharm. Pharm. Med. Chem. 334 (Suppl. 2) (2001) C80 52.
[35] F. Suzuki, J. Shimada, H. Nonake, A. Ishii, S. Shiozaki, S. Ichikawa, E. Ono, J. Med. Chem. 35 (1992) 3578–3581.
[36] C.E. Müller, M. Thorand, R. Qurishi, M. Diekmann, K.A. Jacobson, W.L. Padgett, J.W. Daly, J. Med. Chem. 45 (2002) 3440–3450.
[37] C.E. Müller, M. Diekmann, M. Thorand, V. Ozola, Bioorg. Med. Chem. Lett. 12 (2002) 501–503.
[38] P.G. Baraldi, A.Z. Nasser, F. Fruttarolo, M.A. Tabrizi, M.del.C Nunez, G. Spalluto, R. Romagnoli, Synthesis (2001) 773–775.
[39] S. Rockitt, H. Duddeck, A. Drabczyńska, K. Kieć-Kononowicz, Eur. J. Org. Chem. (2000) 3489–3496.
[40] S. Rockitt, R. Wartchow, H. Duddeck, A. Drabczyńska, K. Kieć-Kononowicz, Z. Naturforsch. 56b (2001) 319–324.
[41] K. Kieć-Kononowicz, A. Drabczyńska, E. Pękala, B. Michalak, C.E. Müller, B. Schumacher, J. Karolak-Wojciechowska, H. Duddeck, S. Rockitt, R. Wartchow, Pure Appl. Chem. 73 (2001) 1411–1420.
[42] D. Bogdał, Wiadomości Chemiczne 53 (1999) 66–99.
[43] D. Bogdał, J. Pielichowski, K. Jaskot, Org. Prep. Proc. Int. 30 (1998) 427–432.
[44] Z.-G. Gao, A.P. IJzerman, Biochem. Pharmacol. 60 (2000) 669–676.
[45] R.L. Krall, J.K. Penry, B.G. White, H.J. Kupferberg, E.A. Swinyard, Epilepsia 19 (1978) 400–428.
[46] R.A. Porter, J.J. Cereghino, G.D. Gladding, H.J. Kupferberg, B. Scoville, B.G. White, Cleveland Clin. Q. 51 (1984) 293–305.