

# N9-Benzyl-substituted 1,3-dimethyl- and 1,3-dipropyl-pyrimido[2,1-f]purinediones: Synthesis and structure–activity relationships at adenosine A<sub>1</sub> and A<sub>2A</sub> receptors

Anna Drabczyńska,<sup>a</sup> Christa E. Müller,<sup>b</sup> Janina Karolak-Wojciechowska,<sup>c</sup>  
Britta Schumacher,<sup>b</sup> Anke Schiedel,<sup>b</sup> Olga Yuzlenko<sup>a</sup> and Katarzyna Kieć-Kononowicz<sup>a,\*</sup>

<sup>a</sup>Jagiellonian University, Medical College, Faculty of Pharmacy, Department of Technology and Biotechnology of Drugs, Medyczna 9, Pl 30-688 Kraków, Poland

<sup>b</sup>Pharmaceutical Institute, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

<sup>c</sup>Institute of General and Ecological Chemistry, Technical University of Łódź, Żwirki 36, Pl 90-924 Łódź, Poland

Received 14 July 2006; revised 30 March 2007; accepted 13 April 2007

Available online 19 April 2007

**Abstract**—Synthesis and physicochemical properties of *N*-benzyl pyrimido[2,1-*f*]purinediones are described. These derivatives were synthesized by the cyclization of 7-chloropropyl-8-bromo-1,3-dimethyl- or 1,3-dipropyl xanthine derivatives with corresponding (un)substituted benzylamines. Dipropyl derivatives were obtained under microwave irradiation conditions either. The obtained compounds (**1–20**) were evaluated for their affinity to adenosine A<sub>1</sub> and A<sub>2A</sub> receptors, selected compounds were additionally investigated for affinity to the A<sub>3</sub> receptor subtype. The results of the radioligand binding assays to A<sub>1</sub> and A<sub>2A</sub> adenosine receptors showed that most of the 1,3-dimethyl-9-benzylpyrimidopurinediones exhibited selective affinity to A<sub>2A</sub> receptors at micromolar or submicromolar concentrations (for example, derivative **9** with *o*-methoxy substituent displayed a *K<sub>i</sub>* value of 0.699 μM at rat A<sub>2A</sub> receptor with more than 36-fold selectivity). Contrary to previously described arylpyrimido[2,1-*f*]purinediones dipropyl derivatives (compounds **15–20**) showed affinity to both kinds of receptors increased, however A<sub>1</sub> affinity increased to a larger extent, with the result that A<sub>2A</sub> selectivity was abolished. The best adenosine A<sub>1</sub> receptor ligand was *m*-chlorobenzyl derivative **18** (*K<sub>i</sub>* = 0.089 μM and 5-fold A<sub>1</sub> selectivity). Structure–activity relationships were discussed with the analysis of lipophilic and spatial properties of the investigated compounds. Pharmacophore model of adenosine A<sub>1</sub> receptor antagonist was adopted for this purpose.

© 2007 Elsevier Ltd. All rights reserved.

## 1. Introduction

The physiological effects of extracellular adenosine are mediated by four G-protein coupled receptors: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> adenosine receptors (ARs).<sup>1,2</sup> The ARs may either inhibit (A<sub>1</sub>, A<sub>3</sub>) or stimulate (A<sub>2A</sub>, A<sub>2B</sub>) adenylate cyclase activity. Coupling to other second messenger systems has been described, including phospholipase C stimulation (A<sub>1</sub>, A<sub>2B</sub>, A<sub>3</sub>).<sup>3</sup> Selective A<sub>1</sub> AR antagonists have demonstrated promising therapeutic potential for the treatment of cognitive diseases, renal failure, Alzheimer's disease and cardiac failure.<sup>4</sup> In the central nervous system the A<sub>2A</sub> ARs are present in high density in basal ganglia and are able to cross-talk

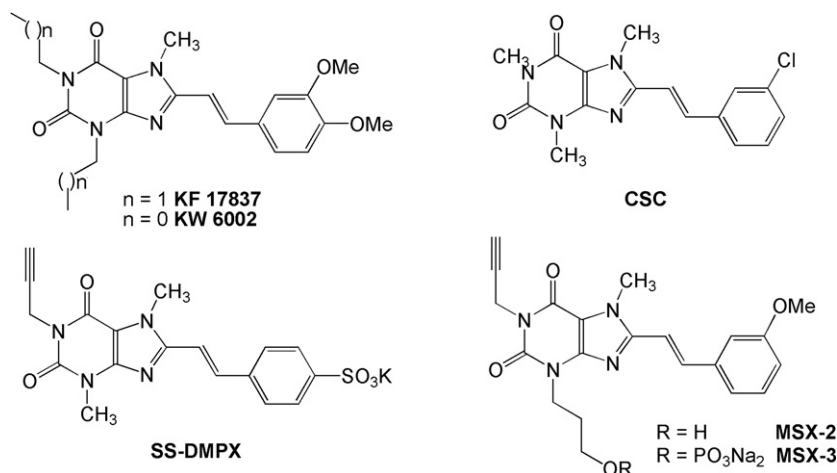
with dopamine receptors.<sup>5,6</sup> Adenosine A<sub>2A</sub> receptor antagonists may be useful for the treatment of acute and chronic neurodegenerative disorders such as cerebral ischemia, Parkinson's and Huntington's diseases, as drugs controlling motor functions and exhibiting neuroprotective properties.<sup>7–11</sup>

The involvement of AR interaction in seizure disorders has been discussed.<sup>7,10–14</sup> Adenosine and specific AR agonists exert potent anticonvulsant effects in a variety of seizure models, primarily via activation of the A<sub>1</sub> AR subtype. AR antagonists were described to produce proconvulsant effects.<sup>10,12–14</sup>

Since ARs offer an attractive target for drug development several studies were carried out in order to find subtype-selective AR antagonists.<sup>15–22</sup> Styryl xanthines have been recognized as potent and selective adenosine A<sub>2A</sub> receptor antagonists (Fig. 1). The first very potent

**Keywords:** Adenosine A<sub>1</sub>; A<sub>2A</sub> receptor antagonists; Pyrimido[2,1-*f*]purinediones; Tricyclic xanthine derivatives.

\*Corresponding author. Tel./fax: +48 12 657 04 88; e-mail: [mfonono@cyf-kr.edu.pl](mailto:mfonono@cyf-kr.edu.pl)



**Figure 1.** Structures of styryl xanthines with adenosine  $A_{2A}$  receptor antagonistic properties.

and selective  $A_{2A}$  adenosine receptor antagonists were KF 17837<sup>23,24</sup> and CSC.<sup>25</sup> KF 17837, being more potent  $A_{2A}$  adenosine receptor antagonist than CSC, was less selective vs  $A_1$  adenosine receptor subtype. Two major problems are connected with this group of compounds: low water solubility<sup>26</sup> and rapid photoisomerisation.<sup>27,28</sup> To enhance water solubility of styryl xanthines two main approaches have been used by Müller and coworkers: introduction of polar groups on the phenyl ring (SS-DMPX)<sup>29</sup> or preparation of phosphate pro-drugs (MSX-2, MSX-3).<sup>30</sup> Compound KW-6002 is being evaluated in phase II clinical trials as potential antiparkinsonian and antidepressant drug.<sup>31,32</sup>

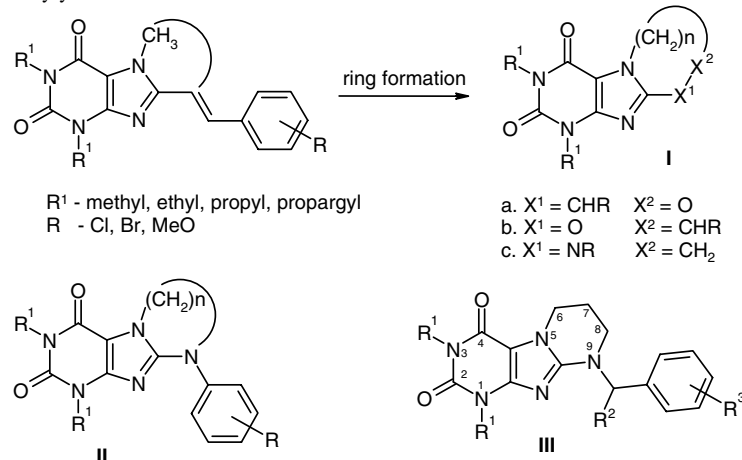
In our studies we focused on the search for  $A_1/A_{2A}$  AR ligands among tricyclic xanthine derivatives.<sup>16,33–35</sup> Compounds (I) with oxygen or nitrogen containing fused rings were obtained<sup>16,33,34</sup> as constrained analogues of 8-styrylxanthines (Fig. 2).<sup>18,36</sup> Thus imidazo-, pyrimido- and 1,3-diazepino[2,1-*f*]purinediones were synthesized (I). One series of compounds (II) possessed variously substituted aryl moiety placed directly at the nitrogen atom of the fused ring. In vitro studies have shown their  $A_{2A}$  AR

affinity and selectivity, in vivo some of the compounds displayed anticonvulsant activity. The pyrimidine annelated ring (II) was beneficial for both receptor affinity and anticonvulsant activity. Continuing our research in the group of tricyclic derivatives of purinediones<sup>16,33,34</sup> pyrimido[2,1-*f*]purinediones with (un)substituted benzyl moieties at the annelated ring were synthesized. Such compounds (III), possessing aryl substituent placed in the greater (two bonds) distance from the nitrogen atom of the annelated ring, were designed as better mimicking the structure of styryl xanthines.

To gain insight into the structure affinity relationships of this class of compounds, the lead structure benzylpyrimidotheophylline (I) was modified by:

- elongation of  $R^1$  substituents (replacement of the methyl groups in theophylline by propyl groups as in the standard xanthine  $A_1$  antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX)),
- introducing  $R^2$  substituent to the benzyl methylene,
- introducing  $R^3$  substituent in position *o*-, *m*- or *p*- of the aromatic ring,

8-styrylxanthine derivatives



**Figure 2.** Oxygen or nitrogen containing annelated xanthine derivatives as constrained analogues of 8-styryl xanthines.

- replacement of the phenyl ring by the potentially bioisosteric heterocycles furane or pyridine.

The synthesized compounds were evaluated *in vitro* for their affinity to ARs. Physicochemical properties of tested compounds were estimated by means of calculations. In order to analyze three-dimensional properties of this group of compounds structure examination was performed by means of molecular modelling and X-ray analysis for two selected compounds.

## 2. Chemistry

The synthesis of tricyclic 1,3-dimethylpyrimidopurinedione with benzyl substituents in the 9-position of the annelated ring was accomplished as shown in Figure 3. As starting material 7-(3-chloropropyl)-8-bromotheophylline was used which was obtained as previously described.<sup>37</sup> The compound was reacted with the substituted in *o*-, *m*- or *p*-position benzylamines under various reaction conditions with regard to the amount of amine, solvent, and reaction time. The data are summarized in Table 1.

The synthesis of compounds **1** and **12** had been described previously,<sup>38</sup> but their structures had only been determined by UV spectra and no biological activity has been reported.

For the preparation of 1,3-dipropylxanthine as starting material for cyclization to the corresponding dipropyl-substituted pyrimidopurinediones standard procedures were applied.<sup>39,40</sup> Subsequent bromination, introduction of the chloropropyl chain in position 7 by two phase alkylation with 1-chloro-3-bromopropane followed by cyclization with benzylamines yielded the target tricyclic 9-benzyl-substituted compounds (Fig. 3).

Alternatively, dipropyl derivatives **15–20** could also be prepared by microwave flash-heating method. Thus, 8-bromo-7-(3-chloropropyl)-1,3-dipropylxanthine was

melted with the appropriate benzylamine in a microwave oven (power 300–750 W) for 4–7 min.

Although the yields of the traditional and microwave method were comparable, the advantages of the last one were a dramatically reduced reaction time and a better quality (higher purity) of the products.

The structures of the synthesized compounds were confirmed by UV, IR and <sup>1</sup>H NMR spectra. The UV spectra showed a bathochromic shift of  $\lambda_{\text{max}}$  from ca. 276 nm to about 300 nm, typical for 8-aminoxanthine derivatives.<sup>41</sup> The IR showed absorption bands typical for xanthine derivatives.<sup>42</sup>

## 3. X-ray structure analysis of compounds **8** and **14**

Monocrystals suitable for X-ray structural studies were obtained only for 2 of 20 N9-benzyl-substituted pyrimido[2,1-*f*]purinediones. Then the structures of **8** and **14** were determined. In the studied molecules the basic tricyclic pyrimido[2,1-*f*]purinedione is exactly the same (Fig. 4). The purinedione skeleton is coplanar. In the annelated pyrimidine ring three carbon atoms (C6, C7 and C8) possess sp<sup>3</sup> hybridization and this ring in both molecules adopts sofa conformation with the C7 atom in flap position. The C11 atom from substituent at N9 resides in an equatorial position. It is important that C7 and C11 atoms are located on opposite sides of tricycle (*trans* with respect to main skeleton). In the structure of **8** the phenyl attached to C11 is inclined to the pyrimido[2,1-*f*]purinedione at 75.1(1)°. For the furane ring, placed at C11 in molecule **14**, a respective angle equal to 80.89(7)° was found.

The structures of **8** and **14** constituted starting points for subsequent molecular modelling of the molecules described in this study. All molecules contain rotational one atomic-linker (sp<sup>3</sup>-hybridized C11), which joins together pyrimido[2,1-*f*]purinedione and the aromatic substituent. Conformational analysis of that molecule

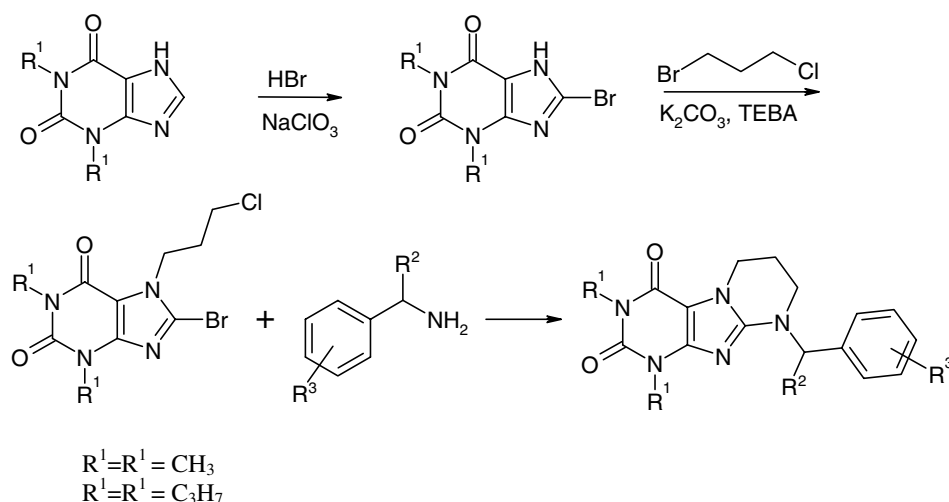
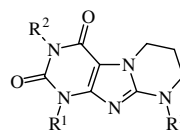


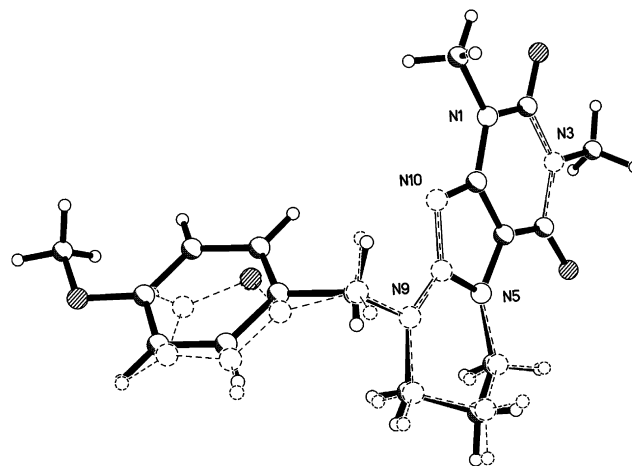
Figure 3. Synthesis of 1,3-dialkyl-9-benzylpyrimido[2,1-*f*]purinediones.

**Table 1.** Physical data and reaction conditions of 9-benzylpyrimido[2,1-f]-purinediones

| Compound | R <sup>1</sup>  | R <sup>2</sup>  | R <sup>3</sup> | Mol. form. m.w.  | Mp [°C]              | Yield [%] | Reaction medium    | Reaction time [h] | Crystal. solvent   | TLC R <sub>f</sub> |
|----------|-----------------|-----------------|----------------|--|----------------------|-----------|--------------------|-------------------|--------------------|--------------------|
| 1        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>17</sub> H <sub>19</sub> O <sub>2</sub> N <sub>5</sub> , 325.40                 | 197–198 <sup>a</sup> | 70        | —                  | 3                 | Ethanol            | 0.56A              |
| 2        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>18</sub> H <sub>21</sub> O <sub>2</sub> N <sub>5</sub> , 339.39                 | 223–224              | 58        | —                  | 4                 | Ethanol            | 0.72A              |
| 3        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>17</sub> H <sub>18</sub> O <sub>2</sub> N <sub>5</sub> Cl, 359.81               | 230–232              | 70        | Me-Digol           | 10                | Methoxy-ethanol    | 0.65A              |
| 4        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>17</sub> H <sub>18</sub> O <sub>2</sub> N <sub>5</sub> Cl, 359.81               | 233–235              | 63        | Me-Digol           | 8                 | Methoxy-ethanol    | 0.65A              |
| 5        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>17</sub> H <sub>18</sub> O <sub>2</sub> N <sub>5</sub> Cl, 359.81               | 168–169              | 50        | Me-Digol           | 10                | Methoxy-ethanol    | 0.58A              |
| 6        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>17</sub> H <sub>17</sub> O <sub>2</sub> N <sub>4</sub> Cl <sub>2</sub> , 394.26 | 195–196              | 63        | Me-Digol           | 9                 | <i>n</i> -Propanol | 0.94A              |
| 7        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>17</sub> H <sub>18</sub> O <sub>2</sub> N <sub>5</sub> F, 343.35                | 210–212              | 40        | Me-Digol           | 8                 | Methoxy-ethanol    | 0.58A              |
| 8        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>18</sub> H <sub>21</sub> O <sub>3</sub> N <sub>5</sub> , 355.39                 | 185–187              | 59        | Propanol           | 3                 | Propanol           | 0.63A              |
| 9        | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>18</sub> H <sub>21</sub> O <sub>3</sub> N <sub>5</sub> , 355.39                 | 217–218              | 72        | Me-Digol           | 10                | <i>n</i> -Butanol  | 0.68A              |
| 10       | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>18</sub> H <sub>21</sub> O <sub>3</sub> N <sub>5</sub> , 355.39                 | 166–167              | 55        | Me-Digol           | 10                | Ethanol            | 0.64A              |
| 11       | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>23</sub> H <sub>23</sub> O <sub>2</sub> N <sub>5</sub> , 401.45                 | 247–249              | 70        | Methoxy-ethanol    | 10                | Propanol           | 0.74A              |
| 12       | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>18</sub> H <sub>21</sub> O <sub>2</sub> N <sub>5</sub> , 339.40                 | 137 <sup>a</sup>     | 80        | <i>n</i> -Propanol | 6                 | 50% Ethanol        | 0.56A              |
| 13       | CH <sub>3</sub> | CH <sub>3</sub> |                | C <sub>16</sub> H <sub>18</sub> O <sub>2</sub> N <sub>6</sub> , 326.35                 | 188–190              | 49        | Butanol            | 6                 | Isopropanol        | 0.56B              |

|           |  |                 |                 |  |         |    |                    |    |                          |       |
|-----------|--|-----------------|-----------------|--|---------|----|--------------------|----|--------------------------|-------|
| <b>14</b> |  | CH <sub>3</sub> | CH <sub>3</sub> | C <sub>15</sub> H <sub>17</sub> O <sub>3</sub> N <sub>5</sub> , 315.33   | 188–189 | 76 | <i>n</i> -Propanol | 8  | Ethanol                  | 0.67A |
| <b>15</b> |  |                 |                 | C <sub>22</sub> H <sub>29</sub> O <sub>2</sub> N <sub>5</sub> , 395.50   | 126–128 | 86 | DMF                | 5  | Ethanol/H <sub>2</sub> O | 0.72C |
| <b>16</b> |  |                 |                 | C <sub>21</sub> H <sub>26</sub> O <sub>2</sub> N <sub>5</sub> Cl, 415.92 | 128–130 | 83 | DMF                | 10 | Ethanol/H <sub>2</sub> O | 0.66C |
| <b>17</b> |  |                 |                 | C <sub>21</sub> H <sub>26</sub> O <sub>2</sub> N <sub>5</sub> Cl, 415.92 | 129–132 | 70 | DMF                | 10 | Ethanol/H <sub>2</sub> O | 0.59C |
| <b>18</b> |  |                 |                 | C <sub>21</sub> H <sub>26</sub> O <sub>2</sub> N <sub>5</sub> Cl, 415.92 | 121–124 | 66 | DMF                | 10 | Ethanol/H <sub>2</sub> O | 0.43C |
| <b>19</b> |  |                 |                 | C <sub>22</sub> H <sub>29</sub> O <sub>3</sub> N <sub>5</sub> , 422.50   | 108–110 | 88 | DMF                | 10 | Ethanol/H <sub>2</sub> O | 0.52C |
| <b>20</b> |  |                 |                 | C <sub>21</sub> H <sub>26</sub> O <sub>2</sub> N <sub>5</sub> F, 399.47  | 119–121 | 70 | DMF                | 10 | Ethanol/H <sub>2</sub> O | 0.49C |

TLC A, benzene/acetone/methanol 1:1:1; C, toluene/acetone 7:3.

<sup>a</sup> Ref. 38 for compound **1** mp 198.5–200 °C, for compound **12** mp 137 °C.

**Figure 4.** Superimposition of molecules **8** and **14** with respect to pyrimidopurinedione moiety atom.

fragment was performed with  $\Phi = \text{C8–N9–C11–C12}$  torsion angle rotation.<sup>43</sup> Two energy minima (at  $\Phi = \pm 70^\circ$  and  $\pm 50^\circ$  for **8** and **14**, respectively) correspond to *trans* and *cis* location of C7 and C11 atoms with respect to the main molecule skeleton. Conformations present in both studied structures are close to slightly deeper energy minimum describing *trans* form.

In both structures main supramolecular motives are formed by weak intermolecular interactions of O2...H–C8(sp<sup>3</sup>) (Table 2b) and (Fig. 5). It should be pointed out that the carbon atom C8 is located as an exact neighbour of the electron-withdrawing non-planar nitrogen N9 (see Table 2a) and that the partial charge on respective hydrogens equals about 0.11e.<sup>44</sup> In the crystals of **8**, due to the 4-OCH<sub>3</sub> substitution of the phenyl ring, an additional weak H-bond of C14–H14...O18 is also observed.

#### 4. Pharmacology

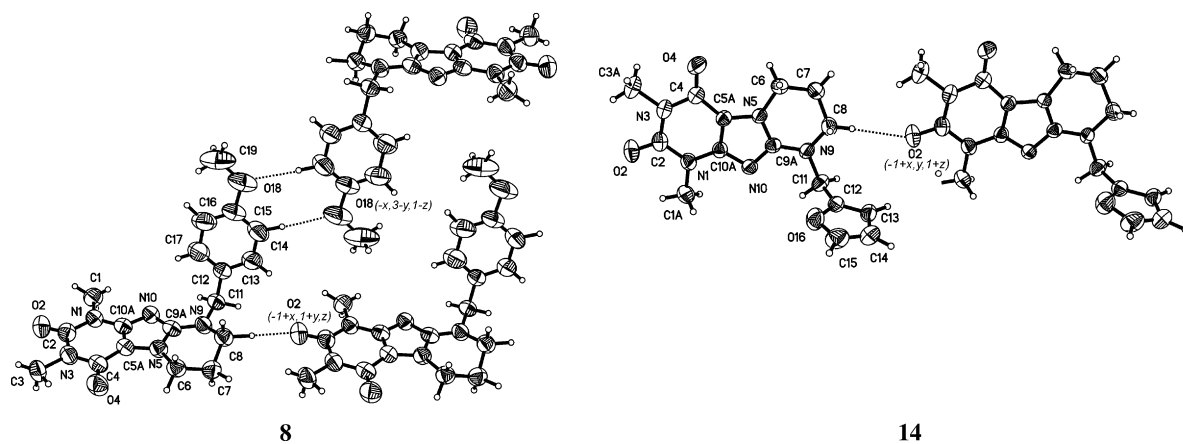
All compounds were tested in vitro in radioligand binding assays for affinity to A<sub>1</sub> and A<sub>2A</sub> adenosine receptors in rat cortical membrane and rat striatal membrane preparations, respectively. As A<sub>1</sub> AR radioligand [<sup>3</sup>H]2-chloro-*N*<sup>6</sup>-cyclopentyladenosine ([<sup>3</sup>H]CCPA) and as A<sub>2A</sub> receptor ligand [<sup>3</sup>H]1-propargyl-3-hydroxypropyl-7-methyl-8-(3-methoxystyryl)-xanthine ([<sup>3</sup>H]MSX-2) were used.<sup>45,46</sup> Selected compounds were additionally investigated at human A<sub>1</sub>, A<sub>2A</sub> and A<sub>3</sub> ARs in mammalian cells recombinantly expressing the respective receptor subtype. As A<sub>3</sub> radioligand we used [<sup>3</sup>H]2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purine-5-one ([<sup>3</sup>H]PSB-11<sup>22</sup>). The results are presented in Table 3. The standard antagonists caffeine (non-selective) and KW-6002 (A<sub>2A</sub>-selective) were included for comparison.

#### 5. Biological results and discussion

The results of the radioligand binding assays to A<sub>1</sub> and A<sub>2A</sub> adenosine receptors showed that most of the 1,3-dimethyl-9-benzylpyrimidopurinediones exhibited affinity

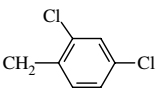
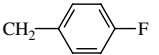
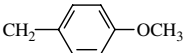
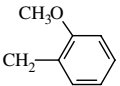
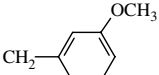
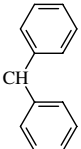
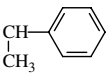
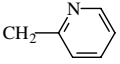
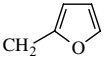

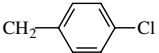
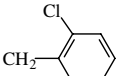
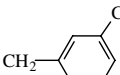
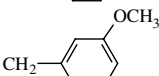
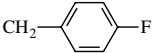
**Table 2.** Selected geometrical data from **8** and **14** molecules

| (a) Molecule geometry                   |                 |                  |          |             |             |               |
|---|-----------------|------------------|----------|-------------|-------------|---------------|
|   |                 |                  | <b>8</b> |             |             | <b>14</b>     |
| Dihedral angle skeleton/ring at C11 [°] |                 |                  | 75.1(1)  |             |             | 80.89(7)      |
| Σ angles at N9 [°]                      |                 |                  | 354.9    |             |             | 358.5         |
| C8–N9–C11–C12 [°]                       |                 |                  | 68.1(2)  |             |             | 56.8(2)       |
| (b) Weak H-bonds in the crystals:       |                 |                  |          |             |             |               |
|   | D–H ... A       | Sym. code        | D–H (Å)  | H ... A (Å) | D ... A (Å) | D–H ... A (°) |
| <b>8</b>                                | C8–H8A ... O2   | −1 + x, 1 + y, z | 1.23     | 2.17        | 3.344(3)    | 158           |
|   | C17–H17 ... O18 | −x, 3 − y, 1 − z | 0.96     | 2.60        | 3.354(5)    | 164           |
| <b>14</b>                               | C8–H8B ... O2   | −1 + x, y, 1 + z | 0.95     | 2.53        | 3.458(2)    | 166           |

**Figure 5.** H-bonds net in the crystals of compounds **8** and **14**.**Table 3.** Affinities of 9-benzylpyrimido[2,1-f]purinediones at A<sub>1</sub> and A<sub>2A</sub> adenosine receptors

| Compound  | R <sup>3</sup> | A <sub>1</sub> <sup>a</sup> versus [ <sup>3</sup> H] CCPA |                      | A <sub>2A</sub> selectivity A <sub>1</sub> /A <sub>2A</sub> | A <sub>2A</sub> <sup>b</sup> versus [ <sup>3</sup> H] MSX-2 |                      |
|---|----------------|---|----------------------|---|---|----------------------|
|   |                | % inhibition at 25 μM (or 2.5 μM)                         | K <sub>i</sub> ± SEM |   | % inhibition at 25 μM (or 2.5 μM)                           | K <sub>i</sub> ± SEM |
| Caffeine  | —              | n.d. <sup>c</sup>   | 18.8 ± 5.6           | —   | n.d. <sup>c</sup>   | 32.5 ± 8.0           |
| KW-6002   | Figure 1       | n.d. <sup>c</sup>   | 0.23 ± 0.03          | —   | n.d. <sup>c</sup>   | 0.00515 ± 0.00025    |
| R <sup>1</sup> =R <sup>2</sup> =CH <sub>3</sub> |                |   |                      |   |   |                      |
| <b>1</b>  |                | 66 ± 3  | 3.58 ± 0.17          | 3.28  | 96 ± 2  | 1.09 ± 0.30          |
| <b>2</b>  |                | (0)   | ≥2.5                 |   | (26 ± 26)   | >2.5                 |
| <b>3</b>  |                | 27 ± 5  | ≥25                  |   | 70 ± 9  | 4.28 ± 0.44          |
| <b>4</b>  |                | (10 ± 10)   | >2.5                 |   | (75 ± 4)  | 1.24 ± 0.20          |
| <b>5</b>  |                | 67 ± 1  | 1.42 ± 0.08          |   | 93 ± 4  | 1.12 ± 0.14          |

Table 3 (continued)

| Compound  | R <sup>3</sup>  | A <sub>1</sub> <sup>a</sup> versus [ <sup>3</sup> H] CCPA |                        | A <sub>2A</sub> selectivity A <sub>1</sub> /A <sub>2A</sub> | A <sub>2A</sub> <sup>b</sup> versus [ <sup>3</sup> H] MSX-2 |   |
|---|---|---|------------------------|---|---|---|
|   |   | % inhibition at 25 μM (or 2.5 μM)                         | K <sub>i</sub> ± SEM   |   | % inhibition at 25 μM (or 2.5 μM)                           | K <sub>i</sub> ± SEM                    |
| 6   |    | 34 ± 11   | ≥25                    |   | 75 ± 7  | 2.48 ± 0.07                             |
| 7   |    | 17 ± 10   | >25                    |   | 86 ± 3  | 2.33 ± 0.21                             |
| 8   |    | 28 ± 2  | >25                    |   | 83 ± 1  | 5.49 ± 0.54                             |
| 9   |    | 18 ± 6 (11 ± 1) <sup>d</sup>                              | >25 (>25) <sup>d</sup> |   | 99 ± 1  | 0.699 ± 0.016 (11.3 ± 2.3) <sup>d</sup> |
| 10  |    | 60 ± 7  | 2.50 ± 0.12            | 1.9   | 84 ± 7  | 1.31 ± 0.13                             |
| 11  |    | (7 ± 2))  | >2.5                   |   | (5 ± 5)   | >2.5                                    |
| 12  |   | 35 ± 8  | ≥25                    | 8.8   | 92 ± 1  | 2.84 ± 0.17                             |
| 13 <sup>c</sup>   |  | 23 ± 4  | 17.8 ± 0.51            | 7   | 79 ± 7  | 2.53 ± 0.21                             |
| 14  |  | 56 ± 1  | 3.43 ± 0.46            |   | 74 ± 4  | 3.31 ± 0.64                             |
| A <sub>1</sub> selectivity A <sub>2A</sub> /A <sub>1</sub>    |   |   |                        |   |   |   |
| R <sup>1</sup> =R <sup>2</sup> =C <sub>3</sub> H <sub>7</sub> |   |   |                        |   |   |   |
| 15  |  | 91 ± 1  | 1.00 ± 0.15            | 0.73  | 60 ± 5(at 3 μM)   | 0.73 ± 0.09                             |
| 16 <sup>f</sup>   |  | 28 ± 1  | 0.23 ± 0.15            | 3.6   | 72. ± 8   | 0.87 ± 0.41                             |
| 17  |  | 89 ± 3  | 0.48 ± 0.08            | 3.6   | 88 ± 1  | 1.73 ± 0.35                             |
| 18 <sup>g</sup>   |  | 87 ± 31.86 (at 1 μM)                                      | 0.089 ± 0.029          | 14.5  | 92 ± 2  | 0.478 ± 0.022                           |
| 19  |  | 94 ± 1  | 0.18 ± 0.04            | 6.28  | 72 ± 10   | 1.13 ± 0.14                             |
| 20  |  | 89 ± 1  | 0.71 ± 0.13            | 4.8   | 82 ± 10   | 3.43 ± 0.71                             |

<sup>a</sup> Rat brain cortical membranes.<sup>b</sup> Rat brain striatal membranes.<sup>c</sup> n.d., not determined.<sup>d</sup> Data from human recombinant receptors permanently expressed in CHO cells.<sup>e</sup> K<sub>i</sub> value of **13** at human A<sub>3</sub> ARs >> 10 μM (4% inhibition of radioligand binding at 10 μM).<sup>f</sup> K<sub>i</sub> value of **16** at human A<sub>3</sub> ARs: 1.60 ± 0.30 μM.<sup>g</sup> K<sub>i</sub> value of **18** at human A<sub>3</sub> ARs: 1.29 ± 0.31 μM.



to A<sub>2A</sub> receptors but poor A<sub>1</sub> affinity (Table 3). The lead structure benzylpyrimidopurinedione (**1**) showed affinity for A<sub>1</sub> and A<sub>2A</sub> receptors in the low micromolar concentration range with 3-fold A<sub>2A</sub> selectivity ( $K_i A_{2A} = 1.09 \mu\text{M}$ , ( $K_i A_1 = 3.58 \mu\text{M}$ ). Introduction of substituents R<sup>3</sup> (CH<sub>3</sub>, OCH<sub>3</sub>, Cl, F) in the *p*-position of the benzyl residue was disadvantageous for A<sub>2A</sub> affinity (e.g., **2**, **3**, **7**, **8**). Better tolerated were substituents in the *m*- or *o*-position. The *o*-methoxy derivative **9** was the best A<sub>2A</sub> ligand ( $K_i = 0.699 \mu\text{M}$ ) of the presented series.

Introduction of a substituent R<sup>2</sup> at the methylene linker of the benzyl residue (methyl in **12**, phenyl in **11**) was disadvantageous too. The phenyl substituent (**11**) decreased affinity to both kinds of receptors practically to zero (5% and 7% inhibition of radioligand binding at 2.5  $\mu\text{M}$ ). The isosteric exchange of the phenyl ring (in the 9-benzyl substituent) by a heterocyclic ring (furyl in **14**, pyridyl in **13**) had the similar effect. Taking into account previously described results<sup>33</sup> on the arylpyrimidopurinediones (**II**), examined for the profile at A<sub>2A</sub> activity in sodium chloride shift experiments, it might be suggested that recently investigated compounds also exhibit A<sub>2A</sub> antagonistic activity.<sup>47</sup>

The most interesting effects were observed in compounds showing an elongation of the substituents in the 1- and 3-positions from methyl to propyl (compounds **15**–**20**). Affinity to both kinds of receptors increased, however A<sub>1</sub> affinity increased to a larger extent, with the result that A<sub>2A</sub> selectivity was abolished (compound **15**). All investigated dipropyl-substituted compounds showed submicromolar A<sub>1</sub> affinity (with the exception of **15**,  $K_i = 1.00 \mu\text{M}$ ). The best A<sub>1</sub> ligand was compound **18** ( $K_i = 0.089 \mu\text{M}$  and 5-fold A<sub>1</sub> selectivity).

The most potent and at the same time most selective compound **9** was additionally tested at human A<sub>1</sub> and

A<sub>2A</sub> receptors to investigate possible species differences. In fact, **9** exhibited lower affinity for the human than for the rat receptors: its  $K_i$  value at the human A<sub>2A</sub> receptor ( $K_i = 11.3 \mu\text{M}$ ) was 16-fold lower than that at the rat receptor ( $K_i = 0.699 \mu\text{M}$ ). The same tendency, lower affinity for human than for rat A<sub>2A</sub> receptors, has been observed for other members of the pyrimido[2,1-*f*]purinedione family.<sup>35</sup> Three selected compounds were investigated at human A<sub>3</sub> ARs, one dimethyl derivative (**13**) and two dipropyl derivatives (**16**, **18**). While the dimethyl derivative was inactive at A<sub>3</sub> receptors, the dipropyl derivatives exhibited  $K_i$  values of 1–2  $\mu\text{M}$ . This result is in accordance with data from bicyclic xanthine derivatives, in which a dipropyl substitution is very favourable for A<sub>3</sub> affinity, in contrast to a dimethyl substitution.<sup>21,48</sup> It can be concluded that the *N,N*-dipropyl substitution of the xanthine part is not only unfavourable for A<sub>2A</sub>-selectivity versus A<sub>1</sub>, but also versus A<sub>3</sub> ARs.

## 6. Structure–activity relationships

Starting with structure–activity relationship analysis, the  $K_i$  values of pyrimido[2,1-*f*]purinedione of both studied receptors (A<sub>1</sub> and A<sub>2A</sub>) were examined as a function of the calculated lipophilicity descriptor, log *P* (and log *D*).<sup>49</sup> All indispensable numerical data are collected in Table 4 while results in graphical forms are presented in Figures 6 and 7. After primary analysis two series of derivatives were selected: one with R<sup>1</sup>=CH<sub>3</sub> (compounds **1**–**14**) and the other one with R<sup>1</sup>=C<sub>3</sub>H<sub>7</sub> (compounds **15**–**20**). This is in accordance with two clusters observed on the diagrams of  $K_i$  value versus log *P* (Figs. 6 and 7). Two linear relationships—involving log *P* for both series of compounds and affinity to A<sub>1</sub> receptor ( $K_i$  A<sub>1</sub> values on Fig. 7)—were shown. Linear equations make it impossible to estimate the optimal values of

**Table 4.** Physicochemical, geometrical and electronic parameters of studied pyrimido[2,1-*f*]purinediones considered in QSAR studies

|           | Pharmacological data |                       | Lipophilicity |              | Geometrical data        |            | QSAR   |       |         | Electronic parameters |       |
|-----------|----------------------|-----------------------|---------------|--------------|-------------------------|------------|--------|-------|---------|-----------------------|-------|
|           | $K_i$ A <sub>1</sub> | $K_i$ A <sub>2A</sub> | log <i>P</i>  | log <i>D</i> | <i>V</i> A <sub>3</sub> | d <i>V</i> | MR     | MP    | Hyd. en | HOMO                  | DP    |
| <b>1</b>  | 3.58                 | 1.09                  | 1.50          | 1.12         | 920.06                  | 202.28     | 89.06  | 37.17 | −0.84   | −8.44                 | 5.328 |
| <b>2</b>  |                      |                       | 1.93          | 1.53         | 972.03                  | 254.25     | 94.10  | 39.00 | 0.32    | −8.42                 | 5.502 |
| <b>3</b>  |                      | 4.28                  | 2.22          | 1.96         | 963.04                  | 245.26     | 93.86  | 39.10 | −0.51   | −8.51                 | 4.881 |
| <b>4</b>  |                      | 1.24                  | 2.21          | 1.95         | 951.57                  | 233.79     | 93.86  | 39.10 | −0.64   | −8.46                 | 4.576 |
| <b>5</b>  | 1.42                 | 1.12                  | 2.28          | 2.02         | 958.87                  |            | 93.86  | 39.10 | −0.39   | −8.49                 | 4.227 |
| <b>6</b>  |                      | 2.48                  | 3.00          | 2.83         | 994.44                  | 276.62     | 98.67  | 41.02 | −0.33   | −8.53                 | 4.037 |
| <b>7</b>  |                      | 2.33                  | 1.65          | 1.38         | 929.30                  | 211.52     | 89.27  | 37.08 | −0.55   | −8.51                 | 4.894 |
| <b>8</b>  |                      | 5.49                  | 1.45          | 1.11         | 997.83                  | 280.05     | 95.52  | 39.64 | −2.52   | −8.41                 | 4.420 |
| <b>9</b>  |                      | 0.70                  | 1.46          | 1.12         | 992.26                  | 274.48     | 95.52  | 39.64 | 1.09    | −8.34                 | 6.775 |
| <b>10</b> | 2.5                  | 1.31                  | 1.56          | 1.22         | 996.26                  | 279.15     | 95.52  | 39.64 | −2.35   | −8.42                 | 5.448 |
| <b>11</b> |                      |                       | 3.18          | 2.90         | 1103.95                 | 386.17     | 113.34 | 46.83 | −1.46   | −8.39                 | 5.266 |
| <b>12</b> |                      | 2.84                  | 2.03          | 1.76         | 960.72                  | 291.31     | 93.48  | 39.00 | −0.38   | −8.40                 | 5.449 |
| <b>13</b> | 17.8                 | 2.53                  | 0.40          | 0.32         | 962.10                  | 244.32     | 86.50  | 37.13 | −1.03   | −8.31                 | 6.876 |
| <b>14</b> | 3.43                 | 3.31                  | 0.90          | 0.71         | 879.32                  | 161.54     | 81.45  | 31.90 | −3.33   | −8.43                 | 5.777 |
| <b>15</b> | 1.00                 | 0.73                  | 2.86          | 2.42         | 1168.71                 |            | 112.64 | 46.34 | −1.54   | −8.36                 | 5.090 |
| <b>16</b> | 0.23                 | 0.87                  | 4.17          | 3.93         | 1159.92                 | 235.30     | 112.41 | 46.44 | 0.73    | −8.44                 | 4.730 |
| <b>17</b> | 0.48                 | 1.73                  | 4.16          | 3.92         | 1147.89                 | 223.45     | 112.41 | 46.44 | 0.57    | −8.39                 | 4.099 |
| <b>18</b> | 0.089                | 1.29                  | 4.22          | 3.99         | 1159.02                 |            | 112.41 | 46.44 | 0.68    | −8.45                 | 3.820 |
| <b>19</b> | 0.18                 | 1.13                  | 3.40          | 3.08         | 1193.65                 | 274.20     | 114.06 | 46.98 | −1.30   | −8.35                 | 6.153 |
| <b>20</b> | 0.71                 | 3.43                  | 3.59          | 3.35         | 1125.25                 | 201.94     | 107.82 | 44.42 | 0.65    | −8.45                 | 4.777 |



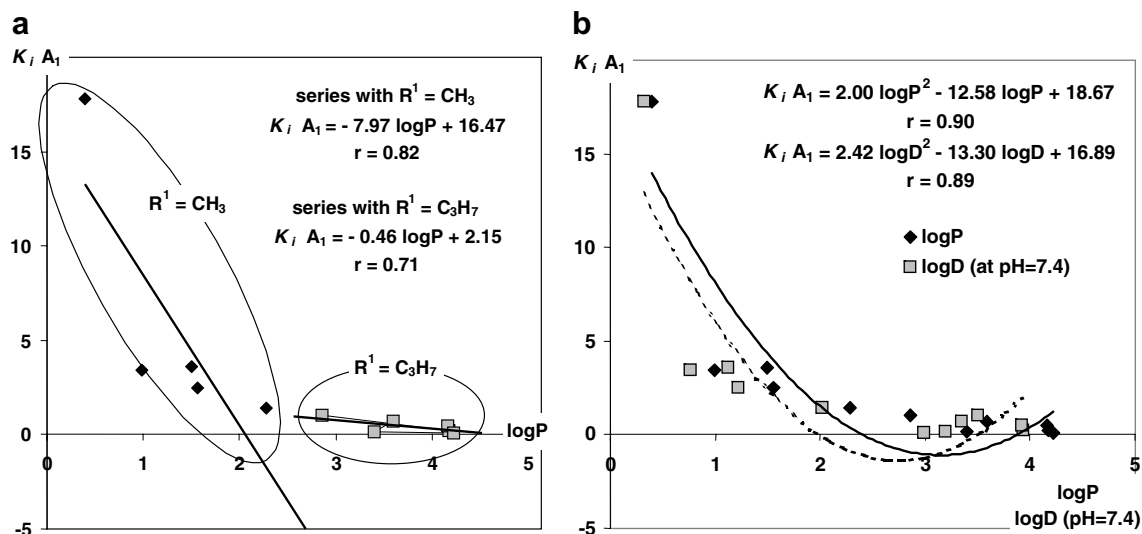


Figure 6. Relationships of  $K_i$  to  $A_1$  receptor in the function of  $\log P$  for two series of derivatives with identical  $R^1$  substituents.

$\log P$ . Considering both series as one group, the second degree polynomial equation between  $\log P$  and  $K_i$  for  $A_1$  enables to evaluate the optimal value of lipophilicity for pyrimido[2,1-*f*]purinediones. This optimum was found at about 3 for  $\log P$  and  $\log D$  (at pH 7.4) (Fig. 6b). Contrary to the above, the relationships between  $\log P$  and the activity towards adenosine  $A_{2A}$  receptor ( $K_i$  for  $A_{2A}$  values in Fig. 7) are not obvious. It means that lipophilicity for the activity for adenosine  $A_{2A}$  receptor is less significant than that to  $A_1$  receptor. In Table 4 also some other similarity descriptors—geometrical and electronic ones—are collected for studied pyrimido[2,1-*f*]purinediones. Unfortunately, no explicit conclusions could have been drawn based on one or multilinear relationships.<sup>50</sup>

### 6.1. Benzylpyrimido[2,1-*f*]purinediones and $A_1$ receptor interactions

The relationships of  $K_i = f(\log P \text{ and/or } \log D)$  pointed out an importance of lipophilicity for activity of the ben-

zylpyrimido[2,1-*f*]purinediones to  $A_1$  receptor (Fig. 6) but not to  $A_{2A}$  one (Fig. 7). The impact of lipophilicity for  $A_1$  receptor ligands is manifested also in the appropriate pharmacophore model which consists of as many as three lipophilic pockets essential for ligand–receptor interactions.<sup>51,52</sup> Besides these, two H-bond acceptors and one H-bond donor are needed for binding. Keeping this in mind, that model has been adopted to benzylpyrimido[2,1-*f*]purinedione molecules in the manner depicted on Figure 8.

Two  $R^1$  substituents from the studied benzylpyrimido[2,1-*f*]purinediones are located in the lipophilic pockets P2 and P3. In accordance with the data from Tables 3 and 4, replacement of both  $R^1=CH_3$  by  $C_3H_7$  increases  $\log P$  value as well as molecule activity for adenosine  $A_1$  receptor. Thus, two three-atomic chains more efficiently fix the molecule at the respective binding site of the receptor. Furthermore, purine oxygen—located between  $R^1$  substituents—is shielded by spaces occupied by the propyl chains. In consequence, as it was observed in

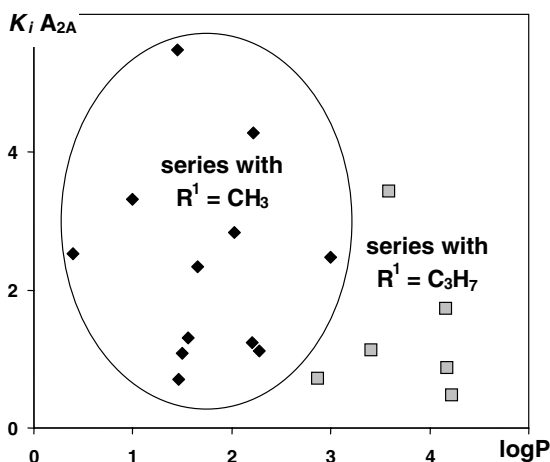


Figure 7. Relationships of  $K_i$  to  $A_{2A}$  receptor with  $\log P$  values.

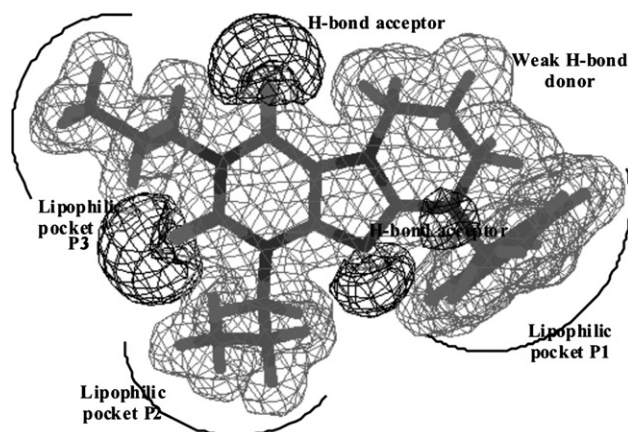


Figure 8. Model of interaction of benzylpyrimido[2,1-*f*]purinediones with  $A_1$  receptor.

**Table 5.** Spectroscopic data of compounds **1–20**

| Compound  | UV [nm]           | IR [cm <sup>-1</sup> ]                             | <sup>1</sup> H NMR  |
|-----------|-------------------|--|---|
| <b>1</b>  | 299 <sup>38</sup> | 1704 CO (pos. 2)<br>1654 CO (pos. 4)               | 2.11–2.18 (m, 2H, CH <sub>2</sub> CH <sub>2</sub> –CH <sub>2</sub> ), 3.18 (t, 2H, <i>J</i> = 5.54 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.41 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.56 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.26 (t, 2H, <i>J</i> = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.78 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.30–7.41 (m, 5H, phenyl)  |
| <b>2</b>  | 301               | 1704 CO (pos. 2)<br>1657 CO (pos. 4)               | 2.07–2.13 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> CH <sub>2</sub> ), 2.34 (s, 3H, CH <sub>3</sub> ), 3.26 (t, 2H, <i>J</i> = 5.78 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.36 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.52 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.21 (t, 2H, <i>J</i> = 6.02 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.70 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.12–7.23 (m, 4H, phenyl)                                    |
| <b>3</b>  | 301               | 1697 CO (pos. 2)<br>1655 CO (pos. 4)               | 2.08–2.17 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> CH <sub>2</sub> ), 3.24–3.27 (m, 2H, CH <sub>2</sub> N <sub>9</sub> ), 3.37 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.52 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.23 (t, 2H, <i>J</i> = 6.00 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.71 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.24–7.34 (m, 4H, phenyl)   |
| <b>4</b>  | 302               | 2705 CO (pos. 2)<br>1651 CO (pos. 4)               | 2.16–2.23 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> CH <sub>2</sub> ), 3.36 (t, 2H, <i>J</i> = 5.64 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.41 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.54 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.29 (t, 2H, <i>J</i> = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.92 (s, 2H, CH <sub>2</sub> N <sub>9</sub> ), 7.26–7.32 (m, 2H, H4,5-phenyl), 7.39–7.45 (m, 2H, H3,6-phenyl)                               |
| <b>5</b>  | 301               | 1691 CO (pos. 2)<br>1660 CO (pos. 4)               | 2.14–2.22 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> CH <sub>2</sub> ), 3.30 (t, 2H, <i>J</i> = 5.64 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.41 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.56 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.27 (t, 2H, <i>J</i> = 5.91 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.75 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.21–7.25 (m, 1H, H2-phenyl), 7.31–7.34 (m, 3H, H4,5,6-phenyl)                               |
| <b>6</b>  | 301               | 1702 CO (pos. 2)<br>1654 CO (pos. 4)               | 2.12–2.21 (m, 2H, CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 3.31–3.37 (m, 5H, N <sub>3</sub> CH <sub>3</sub> + CH <sub>2</sub> N <sub>9</sub> ), 3.50 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.25 (s, 2H, <i>J</i> = 6.23 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.83 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.21–7.43 (m, 3H, phenyl)  |
| <b>7</b>  | 304               | 1702 CO (pos. 2)<br>1662 CO (pos. 4)               | 2.07–2.17 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> CH <sub>2</sub> ), 3.24 (t, 2H, <i>J</i> = 5.00 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.38 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.53 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.22 (t, 2H, <i>J</i> = 6.25 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.70 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.00–7.07 (m, 2H, H2,6-phenyl), 7.27–7.32 (m, 2H, H3,5-phenyl)                               |
| <b>8</b>  | 301               | 1697 CO (pos. 2)<br>1656 CO (pos. 4)               | 2.05–2.15 (m, 2H, CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 3.23 (t, 2H, <i>J</i> = 5.38 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.38 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.53 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 3.80 (s, 3H, OCH <sub>3</sub> ), 4.21 (t, 2H, N <sub>5</sub> CH <sub>2</sub> ), 4.67 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 6.86–6.90 (m, 2H, H3,5-phenyl), 7.23–7.27 (m, 2H, H2,6-phenyl)                   |
| <b>9</b>  | 300               | 1702 CO (pos. 2)<br>1651 CO (pos. 4)               | 2.07–2.17 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> –CH <sub>2</sub> ), 3.05–3.37 (m, 5H, N <sub>3</sub> CH <sub>3</sub> , CH <sub>2</sub> N <sub>9</sub> ), 3.52 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 3.84 (s, 3H, OCH <sub>3</sub> ), 4.22 (t, 2H, <i>J</i> = 6.25 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.76 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 6.88–6.96 (m, 2H, H4,5-phenyl), 7.25–7.31 (m, 2H, H3,6-phenyl)                          |
| <b>10</b> | 302               | 1698 CO (pos. 2)<br>1653 CO (pos. 4)               | 2.07–2.10 (m, CH <sub>2</sub> –CH <sub>2</sub> –CH <sub>2</sub> ), 3.27 (t, 2H, <i>J</i> = 5.65 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.68 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.51 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 3.79 (s, 3H, OCH <sub>3</sub> ), 4.32 (t, 2H, <i>J</i> = 6.02 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.71 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 6.91–6.97 (m, 2H, H4,5-phenyl), 7.22–7.30 (m, 2H, H2,6-phenyl) |
| <b>11</b> | 299               | 1700 CO (pos. 2)<br>1648 CO (pos. 4)               | 2.10–2.19 (m, 2H, CH <sub>2</sub> –CH <sub>2</sub> –CH <sub>2</sub> ), 3.15 (t, 2H, <i>J</i> = 5.88 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.35 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.51 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.21 (t, 2H, <i>J</i> = 6.01 Hz, N <sub>5</sub> CH <sub>2</sub> ), 7.06 (s, 1H, CH), 7.25–7.38 (m, 10H, phenyl)   |
| <b>12</b> | 299 <sup>15</sup> | 1690 CO (pos. 2)<br>1659 CO (pos. 4)               | 1.63 (t, 3H, <i>J</i> = 6.25 Hz), 1.94–2.07 (m, 2H, CH <sub>2</sub> N <sub>9</sub> ), 2.91–3.02 (m, 1H, CH <sub>2</sub> N <sub>9</sub> ), 3.17–3.26 (m, 1H, CH <sub>2</sub> N <sub>9</sub> ), 3.38 (s, 3H, N <sub>3</sub> CH <sub>3</sub> ), 3.54 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.13–4.27 (m, 2H, N <sub>5</sub> CH <sub>2</sub> ), 5.83–5.91 (q, <i>J</i> = 7.5 Hz, 1H, N <sub>9</sub> –CH), 7.26–7.37 (m, 5H, phenyl)                   |
| <b>13</b> | 299               | 1703 CO (pos. 2)<br>1654 CO (pos. 4)               | 2.11–2.21 CH <sub>2</sub> –CH <sub>2</sub> –CH <sub>2</sub> ), 3.37–3.45 (m, 5H, N <sub>3</sub> CH <sub>3</sub> + CH <sub>2</sub> N <sub>9</sub> ), 3.50 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.25 (t, 2H, <i>J</i> = 6.00 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.86 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.25–7.36 (m, 2H, H3,5-pyridyl), 7.65–7.71 (td, 1H, H4-pyridyl), 8.56–8.58 (m, 1H, H6-pyridyl)                                   |
| <b>14</b> | 302               | 3100 furan<br>1700 CO (pos. 2)<br>1652 CO (pos. 4) | 2.09–2.18 (m, 2H, CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 3.31–3.36 (m, 5H, N <sub>3</sub> CH <sub>3</sub> , CH <sub>2</sub> N <sub>9</sub> ), 3.53 (s, 3H, N <sub>1</sub> CH <sub>3</sub> ), 4.24 (t, 2H, <i>J</i> = 6.00 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.71 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 6.30–6.35 (m, 2H, H3,4-furyl), 7.38 (s, 1H, H5-furyl)  |

|           |     |                  |   |   |
|-----------|-----|------------------|---|---|
| <b>15</b> | 300 | 1687 CO (pos. 2) | 0.92–0.98 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ), 1.57–1.82 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> + H <sub>2</sub> O), 2.06–2.13 (m, 2H, N <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>9</sub> ), 2.34 (s, 3H, CH <sub>3</sub> phenyl), 1654 CO (pos. 4)                                     | 3.23 (t, 2H, J = 5.77 Hz, CH <sub>2</sub> N <sub>9</sub> ), 3.91–4.03 (m, 4H, N <sub>1</sub> , N <sub>3</sub> , CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ), 4.20 (t, 2H, J = 6.05 Hz, N <sub>9</sub> CH <sub>2</sub> ), 7.13–7.23 (m, 4H, phenyl)  |
| <b>16</b> | 302 | 1686 CO (pos. 2) | 0.92–0.98 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ), 1.55–1.83 (m, 6H, 2 CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> + H <sub>2</sub> O), 2.08–2.16 (m, 2H, N <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>9</sub> ), 3.24 (t, 2H, J = 5.64 Hz, 1653 CO (pos. 4)                                 | CH <sub>2</sub> N <sub>9</sub> ), 3.94–4.02 (m, 4H, N <sub>1</sub> , N <sub>3</sub> , CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ), 4.22 (t, 2H, J = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.68 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.24–7.32 (m, 4H, phenyl)   |
| <b>17</b> | 300 | 1694 CO (pos. 2) | 0.91–0.97 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ), 1.56–1.81 (m, 4H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 2.11–2.19 (m, 2H, N <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>9</sub> ), 3.32–3.57 (m, 2H, CH <sub>2</sub> N <sub>9</sub> ), 1654 CO (pos. 4)                         | 3.91–4.01 (m, 4H, N <sub>1</sub> , N <sub>3</sub> , CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ), 4.24 (t, 2H, J = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.86 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.22–7.26 (m, 2H, H <sub>4,5</sub> -phenyl), 7.38–7.41 (m, 2H, H <sub>3,6</sub> -phenyl)  |
| <b>18</b> | 302 | 1695 CO (pos. 2) | 0.92–0.98 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ), 1.59–1.84 (m, 4H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 2.10–2.17 (m, 2H, N <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>9</sub> ), 3.25–3.50 (m, 2H, CH <sub>2</sub> N <sub>9</sub> ), 1653 CO (pos. 4)                         | 3.91–4.03 (m, 4H, N <sub>1</sub> , N <sub>3</sub> , CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ), 4.42 (t, 2H, J = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.68 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 7.18–7.34 (m, 4H, phenyl)   |
| <b>19</b> | 300 | 1698 CO (pos. 2) | 0.92–0.97 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ), 1.60–1.81 (m, 4H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 2.07–2.15 (m, 2H, N <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>9</sub> ), 3.25 (t, 2H, J = 5.78 Hz, 1653 CO (pos. 4)   | CH <sub>2</sub> N <sub>9</sub> ), 3.79 (s, 3H, OCH <sub>3</sub> ), 3.91–4.02 (m, 4H, N <sub>1</sub> , N <sub>3</sub> , CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ), 4.21 (t, 2H, J = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.68 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 6.81–6.90 (m, 3H, H <sub>4,5,6</sub> -phenyl), 7.23–7.28 (m, 1H, H <sub>2</sub> -phenyl) |
| <b>20</b> | 302 | 1686 CO (pos. 2) | 0.92–0.98 (m, 6H, 2 CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ), 1.60–1.84 (m, 6H, 2 CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> + H <sub>2</sub> O), 2.07–2.15 (m, 2H, N <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N <sub>9</sub> ), 3.24 (t, 2H, J = 5.64 Hz, 1654 CO (pos. 4) | CH <sub>2</sub> N <sub>9</sub> ), 3.91–4.03 (m, 4H, N <sub>1</sub> , N <sub>3</sub> , CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ), 4.21 (t, 2H, J = 6.05 Hz, N <sub>5</sub> CH <sub>2</sub> ), 4.68 (s, 2H, N <sub>9</sub> CH <sub>2</sub> ), 6.98–7.06 (m, 2H, H <sub>2,6</sub> -phenyl), 7.26–7.32 (m, 2H, H <sub>3,5</sub> -phenyl)                                  |

the structure of 1,3-dipropyl-arylpyrimido[2,1-*f*]purinedione, that oxygen is going to be not effective as H-bond acceptor.<sup>33</sup> Aromatic substituent located at N<sub>9</sub>-nitrogen is filling the third lipophilic area, marked as P1 in Figure 8. It is essential that replacement of phenyl by heterocycles (pyridyl or furane in compounds **13** and **14**, respectively) decreases the activity of the compound. We suppose that the properties and capacity of P1 pocket should be studied more intensively on the base of pyrimido[2,1-*f*]purinediones with a variety of spacers joining aromatic group with the main skeleton.

Beside lipophilic pockets, the model in Figure 8 consists of two H-bond acceptors: one at the not protected purine oxygen and the second one at the imidazole nitrogen. Reference pharmacophore model for ligands of A<sub>1</sub> receptor comprises also H-bond donor, it should be located in the proximity of tetrahydropyrimidine ring. H-Bond donor in this area of the molecule was detected in the crystal structure of **8** and **14** (see above). It involves endocyclic tetrahydropyrimidine carbon atom (C8) with hydrogens of higher acidity to form C–H...O weak interaction (Fig. 5).

## 7. Conclusions

A series of 20 new benzyl derivatives of pyrimido[2,1-*f*]purinediones was obtained. The new compounds were tested for their adenosine receptor affinity. 1,3-Dimethyl derivatives exhibited adenosine A<sub>2A</sub> receptor selectivity and affinity at micromolar and submicromolar concentrations. 1,3-Dipropylbenzylpyrimidopurinediones have shown submicromolar affinity and selectivity towards A<sub>1</sub> AR. *p*-Substituents of the benzyl residues, substituent R<sup>2</sup> at the methyl linker and isosteric exchange of the phenyl moiety were disadvantageous for A<sub>1</sub> and A<sub>2A</sub> adenosine receptor activity. SAR studies have revealed dependence between A<sub>1</sub> AR affinity and log *P* value. Pharmacophore model for A<sub>1</sub> R ligands has been created.

## 8. Experimental

### 8.1. Chemistry

Melting points were determined on a MEL-TEMP II apparatus and are uncorrected. IR spectra were recorded as KBr discs on a FT Jasco IR 410 spectrometer. <sup>1</sup>H NMR spectra were recorded on a Varian-Mercury 300 MHz (compounds **1**, **4**, **5**, **15–20**) or Bruker VM 250 (compounds **3**, **6–9**, **11–14**) or Bruker Ac 200F (compounds **2**, **10**) spectrometer in CDCl<sub>3</sub>, using TMS as an internal standard. UV spectra were recorded on a Jasco UV/VIS V-530 apparatus in concentration 10<sup>−5</sup> mol/L in methanol (Table 9). Elemental analyses (C, H, N) were within ±0.4% of theoretical values and were performed on an Elemental-Vario-EL III apparatus.

TLC data were obtained with Merck-Silica gel 60F<sub>254</sub> aluminium sheets, using benzene/acetone 7:3 (A), benzene/acetone/methanol 3:3:3 (B) and toluene/acetone

**Table 6.** Reaction conditions and yields of 1,3-dipropyl-9-benzylpyrimido[2,1-*f*]-purinediones obtained by microwave method

| Compound  | Reaction time [min] | Microwave power [W] | Yield[%] |
|-----------|---------------------|---------------------|----------|
| <b>15</b> | 5                   | 450                 | 85       |
| <b>16</b> | 10                  | 750                 | 82       |
| <b>17</b> | 7                   | 450                 | 85       |
| <b>18</b> | 10                  | 450                 | 90       |
| <b>19</b> | 4                   | 300                 | 87       |
| <b>20</b> | 10                  | 450                 | 98       |

7:3 (C) as developing systems. The plates were visualized with UV light.

**8.1.1. General procedure for the synthesis of 9-benzyl 1,3-dimethyl-6,7,8,9-tetrahydro pyrimido[2,1-*f*]purine-2,4 (1H,3H)-dione (1–14).** A mixture of 0.66 g (2 mmol) of 7-(3-chloropropyl)-8-bromotheophylline<sup>37</sup> and corresponding amine (4–30 mmol) was refluxed in appropriate solvent (propanol, butanol, methoxyethanol, Me-Digol) or without solvent for 4–10 h (see Table 1). After cooling the precipitate was separated, washed with ethanol and water. All compounds were purified by crystallization (see Table 1).

**8.1.2. General procedure for the synthesis of 9-benzyl 1,3-dipropyl-6,7,8,9-tetrahydro pyrimido[2,1-*f*]purine-(1H,3H)-dione (15–20).** Starting materials.

**8.1.2.1. 8-bromo-1,3-dipropylxanthine.** A mixture of 12.15 g (50 mmol) of 1,3-dipropylxanthine,<sup>39,40</sup> 39 ml acetic acid and 6.8 ml (75 mmol) of 40% HBr was stirred at 50 °C until dissolution was completed. The temperature was raised to 58 °C and the solution of 2.04 g

(18 mmol) NaClO<sub>3</sub> in 14.4 ml of water was added dropwise during 1 h.

Heating and stirring was continued lasting for 2 h. The white solid precipitated, which was filtered off on the next day, washed with cold water, hot water and ethanol.

The yield 78%, mp 179–182 °C.

Spectral data:

UV:  $\lambda_{\max}$  = 279 nm.

IR [cm<sup>-1</sup>]: 3063 (NH), 2965–2874 (–CH<sub>2</sub>), 1705 [–CO (pos. 2)], 1660 [–CO (pos. 4)], 760 (–CH<sub>2</sub>).

<sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 0.95–1.01 (m, 6H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); 1.68–1.88 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); 4.04–4.10 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); 13.55 (s, 1H, NH).

**8.1.2.2. 1,3-dipropyl-7-(3-chloropropyl)-8-bromoxanthine.** A mixture of 15.3 g (24 mmol) of 1,3-dipropyl-8-bromoxanthine, 5.92 g (43 mmol) of anhydrous K<sub>2</sub>CO<sub>3</sub>, 7.88 ml (80 mmol) of 1-bromo-3-chloropropane, 0.49 g (2 mmol) of TEBA and 150 ml of acetone was refluxed for 10 h with stirring. The unreacted 1,3-dipropyl-8-bromoxanthine and inorganic salts were filtered off from the hot mixture. This precipitate contained also a small amount of the main product. The acetone filtrate was cooled overnight. The precipitated product was separated. To remove unreacted 1,3-dipropyl-8-bromoxanthine these two crops of product were stirred at room temperature with 5% NaOH, filtered off and washed with water.

**Table 7.** Crystal data and structure refinement details for **8** and **14**

|   | <b>8</b>  | <b>14</b>  |
|---|---|--|
| Empirical formula                           | C <sub>18</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>   | C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>3</sub>                                      |
| Formula weight                              | 355.40  | 315.33   |
| Temperature                                 | 293(2) K  | 293(2) K   |
| Wavelength                                  | 1.54178 Å   | 0.71073 Å  |
| Crystal system                              | Triclinic   | Monoclinic   |
| Space group                                 | P-1   | P2 <sub>1</sub> /n   |
| Unit cell dimensions                        | <i>a</i> = 5.0020(10) Å<br><i>b</i> = 9.653(2) Å<br><i>c</i> = 17.745(4) Å<br>$\alpha$ = 91.99(3)°<br>$\beta$ = 92.18(3)°<br>$\gamma$ = 91.52(3)° | <i>a</i> = 5.1665(8) Å<br><i>b</i> = 30.497(4) Å<br><i>c</i> = 9.6592(13) Å<br>$\beta$ = 94.56(1)° |
| Volume                                      | 855.3(3) Å <sup>3</sup>   | 1503.0(4)  |
| Z, Calculated density                       | 2, 1.380 Mg/m <sup>3</sup>  | 4, 1.394 Mg/m <sup>3</sup>   |
| Absorption coefficient                      | 0.797 mm <sup>-1</sup>  | 0.101 mm <sup>-1</sup>   |
| <i>F</i> (000)                              | 376   | 664  |
| Crystal size                                | 0.1 × 0.1 × 0.4 mm  | 0.25 × 0.3 × 0.5 mm  |
| Theta range for data index                  | 2.5–80.0°   | 2.52–25°   |
| Ranges                                      | –6:h:6, –12:k:12, 0:l:22  | –5:h:6, –36:k:36, –11:l:11   |
| Diffractometer                              | KM-4  | KM-4 with CCD detector   |
| Refl. collected/unique                      | 7098/3549 [ <i>R</i> (int) = 0.107]   | 16,079/2647 [ <i>R</i> (int) = 0.028]  |
| Refinement method                           | Full-matrix-block least-squares on <i>F</i> <sup>2</sup>  | Full-matrix-block least-squares on <i>F</i> <sup>2</sup>   |
| Data/parameters                             | 3549/242  | 2647/212   |
| Goodness-of-fit on <i>F</i> <sup>2</sup>    | 0.91  | 1.06   |
| Final <i>R</i> [ <i>I</i> > 2σ( <i>I</i> )] | <i>R</i> 1 = 0.0643, <i>wR</i> 2 = 0.1291   | <i>R</i> 1 = 0.0361, <i>wR</i> 2 = 0.1078  |
| Largest diff. peak and hole                 | 0.30 and –0.32 e Å <sup>-3</sup>  | 0.17 and –0.16 e Å <sup>-3</sup>   |

**Table 8.** Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **8** and **14**

| <b>8</b>  |            |             |             |            |
|-----------|------------|-------------|-------------|------------|
| O2        | 0.4882(5)  | 0.4127(2)   | 0.19775(13) | 0.0769(9)  |
| O4        | 0.6224(5)  | 0.7831(3)   | 0.04895(13) | 0.0830(9)  |
| O18       | 0.2069(7)  | 1.3238(3)   | 0.53674(15) | 0.1190(2)  |
| N1        | 0.2545(5)  | 0.6036(2)   | 0.22333(13) | 0.0563(8)  |
| N3        | 0.5524(5)  | 0.5976(3)   | 0.12334(14) | 0.0631(9)  |
| N5        | 0.2028(5)  | 0.9300(2)   | 0.14438(12) | 0.0555(8)  |
| N9        | −0.1235(5) | 1.0453(2)   | 0.21194(12) | 0.0562(8)  |
| N10       | 0.0137(5)  | 0.8153(2)   | 0.23758(13) | 0.0558(8)  |
| C1A       | 0.1162(7)  | 0.5372(3)   | 0.28392(17) | 0.0692(9)  |
| C2        | 0.4339(6)  | 0.5308(3)   | 0.18332(18) | 0.0618(9)  |
| C3A       | 0.7419(7)  | 0.5185(4)   | 0.08060(18) | 0.0778(9)  |
| C4        | 0.5031(6)  | 0.7341(3)   | 0.10225(16) | 0.0602(9)  |
| C5A       | 0.3139(6)  | 0.7985(3)   | 0.14664(15) | 0.0558(9)  |
| C6        | 0.2644(6)  | 1.0440(3)   | 0.09553(16) | 0.0617(9)  |
| <b>14</b> |            |             |             |            |
| O2        | 0.9957(5)  | 0.11487(10) | 0.3774(2)   | 0.0634(10) |
| O4        | 1.1365(5)  | 0.02913(9)  | 0.7744(3)   | 0.0647(10) |
| O16       | 0.5151(5)  | 0.22594(9)  | 1.0728(3)   | 0.0674(10) |
| N1        | 0.7593(5)  | 0.13134(9)  | 0.5629(3)   | 0.0455(10) |
| N3        | 1.0592(5)  | 0.07217(9)  | 0.5757(3)   | 0.0464(10) |
| N5        | 0.7185(5)  | 0.08714(8)  | 0.9026(3)   | 0.0371(8)  |
| N9        | 0.3916(5)  | 0.12808(9)  | 1.0033(3)   | 0.0451(9)  |
| N10       | 0.5225(5)  | 0.14169(8)  | 0.7710(3)   | 0.0412(9)  |
| C1A       | 0.6218(8)  | 0.16754(12) | 0.4864(4)   | 0.0582(14) |
| C2        | 0.9410(6)  | 0.10686(12) | 0.4981(3)   | 0.0466(12) |
| C3A       | 1.2505(7)  | 0.04603(14) | 0.5039(4)   | 0.0633(16) |
| C4        | 1.0168(6)  | 0.06021(11) | 0.7166(3)   | 0.0449(12) |
| C5A       | 0.8277(6)  | 0.08712(10) | 0.7717(3)   | 0.0384(10) |
| C6        | 0.7669(7)  | 0.05672(11) | 1.0214(4)   | 0.0468(12) |
| C7        | 0.5336(7)  | 0.05825(12) | 1.1085(4)   | 0.0538(12) |
| C8        | 0.4559(7)  | 0.10537(12) | 1.1365(3)   | 0.0497(11) |
| C9A       | 0.5381(6)  | 0.11997(10) | 0.8947(3)   | 0.0378(10) |
| C10A      | 0.7035(6)  | 0.12081(10) | 0.6979(3)   | 0.0389(11) |
| C11       | 0.2136(7)  | 0.16588(11) | 0.9989(4)   | 0.0488(12) |
| C12       | 0.2984(6)  | 0.20180(10) | 1.0982(3)   | 0.0420(11) |
| C13       | 0.2031(7)  | 0.21669(11) | 1.2151(4)   | 0.0482(12) |
| C14       | 0.3641(8)  | 0.25200(13) | 1.2678(4)   | 0.0602(14) |
| C15       | 0.5477(8)  | 0.25645(13) | 1.1803(5)   | 0.0697(16) |

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

### Spectral data:

UV:  $\lambda_{\text{max}} = 279.5 \text{ nm}$ .

IR [ $\text{cm}^{-1}$ ]: 2965–2873 (–CH<sub>2</sub>), 1702 [–CO (pos. 2)], 1659 [–CO (pos. 4)], 750 (CH<sub>2</sub>).

<sup>1</sup>H NMR  $\delta = 0.92$ – $0.99$  (m, 6H, 2CH<sub>3</sub>–CH<sub>2</sub>–CH<sub>2</sub>), 1.58–1.84 (m, 4H, 2CH<sub>3</sub>–CH<sub>2</sub>–CH<sub>2</sub>), 2.28–2.37 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>2</sub>Cl), 3.43–3.63 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>2</sub>Cl), 3.92–4.04 (m, 2H, 2CH<sub>3</sub>–CH<sub>2</sub>–CH<sub>2</sub>), 4.49–4.51 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>2</sub>Cl).

**8.1.2.3. Cyclization.** *Method a.* A mixture of 1 mmol of 1,3-dimethyl- or 1,3-dipropyl-7-(3-chloropropyl)-8-bromoxanthine with 4 mol of corresponding benzylamine and 2 ml of DMF was refluxed for 5–10 h. The products were isolated from reaction mixture by adding water and cooling overnight in refrigerator. The crystalline products were filtered off and purified by dissolving in hot ethanol and precipitated with water.

**Table 9.** Elemental analysis

| Compound  |        | Analyses [%] |      |       |
|-----------|--------|--------------|------|-------|
|           |        | C            | H    | N     |
| <b>1</b>  | Ref. 6 | 62.76        | 5.89 | 21.53 |
|           |        | 62.65        | 6.15 | 21.52 |
| <b>2</b>  |        | 63.71        | 6.24 | 20.64 |
|           |        | 63.44        | 6.15 | 20.96 |
| <b>3</b>  |        | 56.75        | 5.05 | 19.46 |
|           |        | 56.59        | 4.87 | 19.28 |
| <b>4</b>  |        | 56.75        | 5.05 | 19.46 |
|           |        | 56.83        | 5.06 | 19.26 |
| <b>5</b>  |        | 56.75        | 5.05 | 19.46 |
|           |        | 56.52        | 5.23 | 19.61 |
| <b>6</b>  |        | 51.79        | 4.35 | 17.77 |
|           |        | 51.66        | 4.08 | 17.53 |
| <b>7</b>  |        | 59.46        | 5.29 | 20.40 |
|           |        | 59.35        | 5.05 | 20.39 |
| <b>8</b>  |        | 60.83        | 5.96 | 19.71 |
|           |        | 60.64        | 5.85 | 19.48 |
| <b>9</b>  |        | 60.83        | 5.96 | 19.71 |
|           |        | 60.46        | 5.84 | 19.90 |
| <b>10</b> |        | 60.83        | 5.96 | 19.71 |
|           |        | 61.09        | 5.64 | 19.46 |
| <b>11</b> |        | 68.80        | 5.78 | 17.44 |
|           |        | 68.80        | 5.50 | 17.21 |
| <b>12</b> | Ref. 6 | 63.69        | 6.24 | 20.64 |
|           |        | 63.76        | 6.62 | 20.72 |
| <b>13</b> |        | 58.90        | 5.57 | 25.75 |
|           |        | 58.93        | 5.74 | 26.10 |
| <b>14</b> |        | 57.14        | 5.44 | 22.21 |
|           |        | 57.20        | 5.33 | 22.22 |
| <b>15</b> |        | 66.83        | 7.40 | 17.71 |
|           |        | 66.63        | 7.44 | 17.40 |
| <b>16</b> |        | 60.64        | 6.31 | 16.83 |
|           |        | 60.53        | 6.32 | 16.53 |
| <b>17</b> |        | 60.64        | 6.31 | 16.83 |
|           |        | 60.83        | 6.29 | 16.93 |
| <b>18</b> |        | 60.64        | 6.31 | 16.83 |
|           |        | 60.74        | 6.31 | 16.52 |
| <b>19</b> |        | 64.22        | 7.11 | 17.02 |
|           |        | 64.10        | 7.19 | 17.00 |
| <b>20</b> |        | 63.14        | 6.57 | 17.53 |
|           |        | 62.85        | 6.55 | 17.50 |

The spectral data are summarized in Table 5.

*Method b.* A mixture of 0.39 g (1 mmol) of 1,3-dipropyl-7-(3-chloropropyl)-8-bromoxanthine and 4 mmol of benzylamines was melted in microwave oven during 4–10 min (power 300–750 W). After cooling acetonitrile was added and precipitate of the unreacted benzylamine salts was filtered off, the filtrate was evaporated under reduced pressure. To the residue water was added and the precipitated product was collected (compounds **15**, **16**, **18**, **20**). Compound **19** was separated by dissolving the reaction mixture in ethanol and precipitated with water. Compound **17** was isolated from the reaction mixture by adding acetonitrile. The precipitate was washed with water to remove benzylamine salts.

The reaction conditions and the yields are presented in Table 6.



## 8.2. Adenosine receptor binding assays

Adenosine receptor binding assays were performed as previously described using rat brain cortical membrane preparations for A<sub>1</sub> AR assays and rat brain striatal membrane preparations for A<sub>2A</sub> assays.<sup>48,53,54</sup> Frozen rat brains (unstripped) were obtained from Pel-Freez<sup>®</sup>, Rogers, Arkansas, USA. For assays at human A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> ARs, CHO cell membranes containing the human receptors were used as described.<sup>53,55</sup> [<sup>3</sup>H]2-chloro-*N*<sup>6</sup>-cyclopentyladenosine ([<sup>3</sup>H]CCPA) was used as the A<sub>1</sub> radioligand, [<sup>3</sup>H]3-(3-hydroxypropyl)-7-methyl-8-(*m*-methoxystyryl)-1-propargylxanthine ([<sup>3</sup>H]-MSX-2) as the A<sub>2A</sub> radioligand,<sup>56</sup> [<sup>3</sup>H]4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo[2,3-*a*]<sup>1,3,5</sup>-triazin-5-4-(amino)-ethyl)phenol ([<sup>3</sup>H]ZM241385) as the A<sub>2B</sub> receptor radioligand<sup>57</sup> and [<sup>3</sup>H]-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]-purine-5-one ([<sup>3</sup>H]PSB-11) as the A<sub>3</sub> AR radioligand.<sup>22</sup> Initially, a single high concentration of compound (25 μM at A<sub>1</sub> and A<sub>2A</sub>, 10 μM at A<sub>2B</sub> and A<sub>3</sub> receptors) was tested in three (A<sub>1</sub>, A<sub>2A</sub>) or two (A<sub>2B</sub>, A<sub>3</sub>) independent experiments. For potent compounds, curves were determined using 6–7 different concentrations of test compounds spanning three orders of magnitude. Data were analyzed using the PRISM program version 3.0 (Graph Pad, San Diego, CA, USA).

## 8.3. X-ray structure analysis of **8** and **14**

*Crystal data for 8*: C<sub>18</sub>H<sub>21</sub>N<sub>5</sub>O<sub>3</sub>; mol. mass 355.40; triclinic; space group: *P*-1; *a* = 5.0020(10) Å; *b* = 9.653(2) Å; *c* = 17.745(4) Å; α = 91.99(3)°, β = 92.18(3)°, γ = 91.52(3)°, *V* = 855.3(3) Å<sup>3</sup>; *z* = 2; *dx* = 1.380 Mg/m<sup>3</sup>; μ = 0.797 mm<sup>−1</sup>; *F*(000) = 376; final *R* = 0.064 for 3549 reflections [*I* > 4σ(*I*)].

*Crystal data for 14*: C<sub>15</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>; mol. mass 315.33; monoclinic; space group: *P*2<sub>1</sub>/*n*; *a* = 5.1665(8) Å; *b* = 30.497(4) Å; *c* = 9.6592(13) Å; β = 94.56(1)°; *V* = 1503.0(4) Å<sup>3</sup>; *z* = 4; *dx* = 1.394 Mg/m<sup>3</sup>; μ = 0.101 mm<sup>−1</sup>; *F*(000) = 664; final *R* = 0.036 for 2647 reflections [*I* > 4σ(*I*)].

The crystals of **8** and **14** were obtained by slow evaporation of ethanol solution. The measurement of the crystal **8** was performed on a Kuma4CCD κ-axis diffractometer with graphite-monochromated MoKα radiation at room temperature. The measurement of the crystal **14** was performed on a KM-4 four-cyclic diffractometer with graphite-monochromated CuKα 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 collected in Table 7. The structures were solved by direct methods<sup>58</sup> and refined using SHELXL.<sup>58</sup> The full-matrix least-squares refinement was based on *F*<sup>2</sup>. The positions of all H-atoms were found from electron density Δρ map and refined in riding model with the isotropic displacement parameters of 1.5 times the respective *U*<sub>eq</sub> values for the parent-atoms. Atomic scattering factors were those as in SHELXL.<sup>59</sup> Atomic coordinates are gathered in Table 8. Crystallographic data (excluding structural factors) for the structure reported

in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition numbers: CCDC 623700 for **8** and CCDC 623701 for **14**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EW, UK (Fax: Int code + (1223)336-033; E-mail: deposit@ccdc.cam.ac.uk).

## 8.4. Computational procedures

The starting models of molecules were based on crystallographic data for **8** and **14** using the PCMOD.6 program.<sup>43</sup> The geometry of the molecules was optimised with MOPAC 6.0 using AM1 Hamiltonians in an aqueous environment (dielectric constants equal 78.4).<sup>44</sup>

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.<sup>49</sup> Multiple Linear Regression equations were computed by means of the QSAR-PC:PAR program written by R.C. Coburn.<sup>50</sup>

## Acknowledgments

This work was supported by Polish State Committee for Scientific Research (Grant No. 6 P05F 024 21) and by the Deutsche Forschungsgemeinschaft (GRK 677). C.E.M. is grateful for support by the Fonds der Chemischen Industrie and the Bundesministerium für Bildung und Forschung (BMBF), Germany.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.04.018.

## References and notes

1. Fredholm, B. B.; Arslan, G.; Haldner, L.; Kull, B.; Schulte, G.; Wasserman, W. *Naumyn-Schmiedeberg's Arch. Pharmacol.* **2000**, 362, 364.
2. Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K.-N.; Linden, J. *Pharm. Rev.* **2001**, 53, 527.
3. Ralevic, V.; Burnstock, G. *Pharmacol. Rev.* **1998**, 50, 413.
4. Poulsen, S.-A.; Quinn, R. J. *Bioorg. Med. Chem.* **1998**, 6, 619.
5. Ferrè, S.; Fredholm, B. B.; Morelli, M.; Popoli, P.; Fuxe, K. *Trends Neurosci.* **1997**, 20, 482.
6. Ferrè, S.; Popoli, P.; Giménez-Llort, L.; Rimondini, R.; Müller, C. E.; Strömberg, I.; Ögren, S. O.; Fuxe, K. *Parkinsonism Relat. Disord.* **2001**, 7, 235.
7. Moreau, J.-L.; Huber, G. *Brain Res. Rev.* **1999**, 31, 65.
8. Müller, C. E. *Drugs Future* **2000**, 25, 1043.
9. Ongini, E.; Schubert, P. *Drug Dev. Res.* **1998**, 45, 387.
10. Impagnatiello, F.; Bastia, E.; Ongini, E.; Monopoli, A. *Emerg. Ther. Targets* **2000**, 4, 635.
11. Ribeiro, J. A.; Sebastião, A. M.; deMendonça, A. *Prog. Neurobiol.* **2003**, 68, 377.
12. De Sarro, G.; De Sarro, A.; Di Paola, E. D.; Bertorelli, R. *Eur. J. Pharmacol.* **1999**, 371, 137.

13. Dunwiddie, T. V. *Adv. Neurol.* **1999**, 79, 1001.
14. McGaraughty, S.; Cowart, M.; Jarvis, M. F.; Berman, R. F. *Curr. Top. Med. Chem.* **2005**, 5, 43.
15. Hess, S. *Expert. Opin. Ther. Patents* **2001**, 11, 1.
16. Kieć-Kononowicz, K.; Drabczyńska, A.; Pękala, E.; Michalak, B.; Müller, C. E.; Schumacher, B.; Karolak-Wojciechowska, J.; Duddeck, H.; Rocktt, S.; Wartchow, R. *Pure Appl. Chem.* **2001**, 73, 1411.
17. Da Settimo, F.; Primofiore, G.; Taliani, S.; Marini, A. M.; La Motta, C.; Novellino, E.; Greco, G.; Lavecchia, A.; Trincavelli, L.; Martini, C. *J. Med. Chem.* **2001**, 44, 316.
18. Baraldi, P. G.; Tabrizi, M. A.; Bovero, A.; Avitabile, B.; Preti, D.; Fruttarolo, F.; Romagnoli, R.; Varani, K.; Borea, P. A. *Eur. J. Med. Chem.* **2003**, 38, 367.
19. Ferrarini, P. L.; Betti, L.; Cavallini, T.; Giannaccini, G.; Lucacchini, A.; Manera, C.; Martinelli, A.; Ortore, G.; Saccomanni, G.; Tuccinaroli, T. *J. Med. Chem.* **2004**, 47, 3019.
20. Yan, L.; Müller, C. E. *J. Med. Chem.* **2004**, 47, 1031.
21. Müller, C. E. *Curr. Top. Med. Chem.* **2003**, 3, 445.
22. Müller, C. E.; Diekmann, M.; Thorand, M.; Ozola, V. *Bioorg. Med. Chem. Lett.* **2002**, 12, 501.
23. Shimada, J.; Suzuki, F.; Nokada, H.; Ishii, S.; Ichikawa, S. *J. Med. Chem.* **1992**, 36, 2342.
24. Nonaka, H.; Ichimura, M.; Takeda, M.; Nonaka, Y.; Shimada, J.; Suzuki, F.; Yamaguchi, K.; Kase, H. *Eur. J. Pharmacol., Mol. Pharm. Sect.* **1994**, 267, 335.
25. Jacobson, K. A.; Gallo-Rodriguez, C.; Melman, N.; Fischer, B.; Maillard, M.; van Bergen, A.; van Galen, P. J. M.; Karton, Y. J. *J. Med. Chem.* **1993**, 36, 1333–1342.
26. Jackson, E. K.; Herzer, W. A.; Suzuki, F. *J. Pharmacol. Exp. Ther.* **1993**, 267, 1304.
27. Nonaka, Y.; Shimada, J.; Nonaka, H.; Koike, N.; Aoki, N.; Kobayashi, H.; Kase, H.; Yamaguchi, K.; Suzuki, F. *J. Med. Chem.* **1993**, 36, 3731.
28. Hockemeyer, J.; Burbiel, J. C.; Müller, C. E. *J. Org. Chem.* **2004**, 69, 3308.
29. Müller, C. E.; Sandoval-Ramirez, J.; Schobert, U.; Geis, U.; Frobenius, W.; Klotz, K. N. *Bioorg. Med. Chem.* **1998**, 6, 707.
30. Sauer, R.; Maurinsh, J.; Reith, U.; Fülle, F.; Klotz, K. N.; Müller, C. E. *J. Med. Chem.* **2000**, 43, 440.
31. Knutsen, L. J. S.; Weiss, S. M. *Curr. Opin. Invest. Drugs* **2001**, 2, 668.
32. Rabasseda, X.; Sorbera, L. A.; Martin, L.; Leeson, P. A.; Castañer, J. *Drugs Future* **2001**, 26, 20.
33. Drabczyńska, A.; Müller, C. E.; Lacher, S. K.; Schumacher, B.; Karolak-Wojciechowska, J.; Nasal, A.; Kawczak, P.; Yuzlenko, O.; Pękala, E.; Kieć-Kononowicz, K. *Bioorg. Med. Chem.* **2006**, 14, 7258.
34. Sauer, R.; Maurinsh, J.; Reith, U.; Fülle, F.; Klotz, K.-N.; Müller, C. E. *J. Med. Chem.* **2000**, 43, 440.
35. Drabczyńska, A.; Schumacher, B.; Müller, C. E.; Karolak-Wojciechowska, J.; Michalak, B.; Pękala, E.; Kieć-Kononowicz, K. *Eur. J. Med. Chem.* **2003**, 38, 397.
36. Drabczyńska, A.; Müller, C. E.; Schumacher, B.; Hinz, S.; Karolak-Wojciechowska, J.; Michalak, B.; Pękala, E.; Kieć-Kononowicz, K. *Bioorg. Med. Chem.* **2004**, 12, 4895.
37. Rockitt, S.; Wartchow, R.; Duddeck, H.; Drabczyńska, A.; Kieć-Kononowicz, K. *Z. Naturforsch.* **2001**, 56B, 319.
38. Eckstein, M.; Łosoń, W. *Dissert. Pharm. Pharmaceut.* **1968**, 20, 35.
39. Papesch, V.; Grove, M.; Schroeder, E. F.; Serle, G. D. United States Patent 1952, 2, 602.795; *Chem. Abstr.* **1953**, 47, 4920f.
40. Kramer, G. L.; Garst, J. E.; Mitchel, S. S.; Wells, J. N. *Biochemistry* **1997**, 16, 3316–3321.
41. Bergmann, F.; Dickstein, S. *J. Am. Chem. Soc.* **1955**, 77, 691.
42. Rybar, A.; Antoš, K. *Coll. Czech. Chem. Commun.* **1970**, 35, 1415.
43. PCMODEL 6 Molecular Modeling Software for the IBM PC, Serena Software, Bloomington, 1996.
44. MOPAC program version 6, QCPE No. 455; Department of Chemistry, Indiana University, Bloomington, IN 47405, 1990.
45. Müller, C. E.; Maurinsh, J.; Sauer, R. *Eur. J. Pharm. Sci.* **2000**, 10, 259.
46. Klotz, K.-N.; Lohse, M. J.; Schwalbe, U.; Cristalli, G.; Vittori, S.; Grifantini, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1989**, 340, 679.
47. Gao, Z. G.; IJzerman, A. P. *Biochem. Pharmacol.* **2000**, 60, 669.
48. Hayallach, A. M.; Sandoval-Ramirez, J.; Reith, U.; Preiss, B.; Schumacher, B.; Daly, J. W.; Müller, C. E. *J. Med. Chem.* **2002**, 45, 1500.
49. PALLAS for Windows 1.2, CompuDrug Chemistry LTD, 1995.
50. QSAR-PC program, R. A. Coburn; BIOSOFT, Cambridge, UK.
51. Da Settimo, F.; Primofiore, G.; Taliani, S.; Marini, A. M.; La Motta, C.; Novellino, E.; Greco, G.; Lavecchia, A.; Trincavelli, L.; Martini, C. *J. Med. Chem.* **2001**, 44, 316.
52. Bondavalli, F.; Botta, M.; Bruno, O.; Ciacci, A.; Corelli, F.; Fossa, P.; Lucacchini, A.; Manetti, F.; Martini, C.; Menozzi, G.; Mosti, L.; Ranise, A.; Schenone, S.; Tafi, A.; Trincavelli, M. A. *J. Med. Chem.* **2002**, 45, 4875.
53. Müller, C. E.; Thorand, M.; Quirshi, R.; Dieckmann, M.; Jacobson, K. A.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **2002**, 45, 3440–3450.
54. Hess, S.; Müller, C. E.; Frobenius, W.; Reith, U.; Klotz, K.-N.; Eger, K. *J. Med. Chem.* **2000**, 43, 4636–4646.
55. Klotz, K.-N.; Hessling, J.; Hegler, J.; Owman, C.; Kull, B.; Fedholm, B. B.; Lohse, M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, 357, 1–9.
56. Müller, C. E.; Maurinsh, J.; Sauer, R. *Eur. J. Pharm. Sci.* **2000**, 10, 259–265.
57. Ji, X.-D.; Jacobson, K. A. Use of the triazolotriazine [<sup>3</sup>H]ZM 241385 as a radioligand at recombinant human A<sub>2B</sub> adenosine receptors. *Drug Des. Discov.* **1999**, 16, 217–226.
58. Sheldrick, G. M. *SHELXL-93. A FORTRAN-77 Program for the Refinement of Crystal Structures from Diffraction Data*; University of Göttingen: Germany, 1993.
59. Sheldrick, G. M. *SHELXTL PC<sup>MT</sup>*; Siemens Analytical X-Ray Instruments Inc.: Madison, Wisconsin, USA, 1990.