Novel antagonists for the human adenosine A2A and A3 receptor via purine nitration: synthesis and biological evaluation of C2-substituted 6-trifluoromethylpurines and 1-deazapurines

Koch, M.

Citation for published version (APA):
Biological evaluation of substituted 6-trifluoromethylpurines and 1-deazapurines on adenosine receptors

ABSTRACT
The purine and 1-deazapurine analogues that were synthesized and discussed in chapters 2, 3, 4 and 5 were evaluated for their affinity for the human adenosine receptor family. The 6-trifluoromethyl substituted analogues I – III show significant affinity for the adenosine A₃ receptor; functional tests revealed strong antagonistic activity. The 2-substituted 1-deazapurines IV showed moderate affinity and antagonistic properties for the adenosine A₂A receptor.
6.1 INTRODUCTION

Adenosine receptors are distributed in variable levels of expression in many different tissue types in the human body. This has led to intensive research towards the therapeutic potential of controlling the activity of the adenosine receptors. Purine derivatives have been widely explored as adenosine receptor ago- and antagonists. However, work on purine ligands has mainly focused on straightforward analogues of adenine. The N-6 amino function has usually been preserved and substituted with a group to improve affinity for the adenosine receptor. In our purine series, we introduced the trifluoromethyl group at C-6 as a replacement of the 6-amino group. As described in the previous chapters, because of the synthetic difficulties, only a very limited set of 6-trifluoromethyl analogues has been published before. A limited set of small substituents (NH₂, S-Et, Cl) has been introduced at C-2. N-9 is usually substituted with ribose, THP or a small alkyl group. The introduction of substituents at C-8 has not been described in combination with 6-trifluoromethyl substitution in purines. Two C8-trifluoromethyl substituted purine analogs (2-Cl, 2-NH₂) were described. No adenosine receptor interaction data were reported for these 8-trifluoromethyl compounds.

For the 6-perfluoroalkyl purines described by Hocek and Hockova, cytostatic activity was found for only one adenosine analogue: 6-trifluoromethyl-9-β-D-ribofuranosyl purine. Even close structural analogs were all inactive, and no mention was made of possible interaction with adenosine receptors. Abbott Japan describes 9H-purine derivatives as possible TNFα (tumor necrosis factor) antagonists. Two of the examples described in this patent application have a 6-trifluoromethyl group, but there is no information as to the interactions with adenosine receptors. Nagano et al. describe yet another group of trifluoromethylpurines as potential anti-tumor agents. None of these compounds showed the desired activity, and the paper does not mention interactions with adenosine receptors. The closest analogs with published adenosine receptor affinity data are two C2-CF3 substituted adenosines published by Ohno et al.

Also for 1-deazapurines, only limited information is available about affinity as ligands for adenosine receptors. 1-Deaza adenosines are known from a patent from Ciba Geigy AG, and were also described by J. E. Francis et al., The 1-deaza adenosines described in the
documents all contain the 9-β-D-ribofuranosyl substituent, characteristic for the natural ligand adenosine. These compounds were shown to have affinity for adenosine receptors, notably for the adenosine A$_2$ receptor subtype, for which they are agonists. Since the A$_{2B}$ receptor was not known at that time, no information about A$_{2A}$ or A$_{2B}$ specificity is described. Also our laboratory published data on adenosine receptors for 1-deazaadenosines, showing selective affinity for A$_1$ subtype receptors. The compounds described in Chapter 5 of this thesis are novel and do not contain the ribose moiety. Especially the 1-deazapurine bases substituted at C-2 are completely different from previously reported analogues. Therefore the action of the the new classes of compounds obtained was tested on adenosine receptors. The studies will give insight in the structural requirements for receptor interaction and may have potential in the search for new selective therapeutics.

### 6.2 Adenosine receptor screening

The compounds were screened for activity at Solvay Pharmaceuticals, partly outsourced to Cerep, France, on adenosine receptors in the following ways.

**Affinity tests**

Affinity of the compounds for the different human adenosine receptors was determined using the following methods:

- Human adenosine-A$_1$ receptors: receptor binding assay described by Townsend, using human recombinant receptors expressed in CHO cells, and [3H]DPCPX as radioligand.
- Human adenosine-A$_{2A}$ receptors: receptor binding assay described by D. R. Luthin et al., using human recombinant receptors expressed in HEK-293 cells, and [3H]CGS 21680 as radioligand.
- Human adenosine-A$_{2B}$ receptors: receptor binding assay described by J. H. Stehle et al., using human recombinant receptors expressed in HEK-293 cells, and [3H]MRS 1754 as radioligand.
- Human adenosine-A$_3$ receptors: receptor binding assay described by C. A. Salvatore et al. expressed in HEK-293 cells, and [125I]-AB-MECA as radioligand.

The $K_i$ values of the compounds were calculated by the formula: $K_i = EC_{50}/[1 + \text{(concentration radioligand}/K_m\text{ radioligand})]$, Where $K_i$ is the binding affinity of the inhibitor, the $EC_{50}$ is the concentration of the ligand under investigation required to
displace 50% of specific binding of the radioligand. The $pK_i$ is defined as the negative logarithm of the $K_i$.

**Functional tests**

Functional tests for the $A_3$ receptor subtype are performed with a dual mode Aequorin screen, which measures both agonism and antagonism. A screen is used where the G protein-coupled receptor (GPCR) is expressed in an apoaequorine containing cell line. Aequorin is an alternative assay methodology for luminescent measurement of calcium and can be adapted to high throughput screens. The advantage of this screen is the fast screening of antagonists and agonists with the same technical setup. Aequorin is a calcium-sensitive photoprotein isolated from the jellyfish *Aequorea victoria* that has been used as a calcium indicator for more than three decades. Its low affinity for Ca makes aequorin a good sensor in the range of biological calcium ion concentrations, with a large dynamic range. The active protein is formed from apoaequorin and its cofactor, coelenterazine. In the presence of molecular oxygen, the binding of calcium ions to aequorin induce a conformational change resulting in the oxidation of coelenterazine and subsequent emission of a blue light flash. Upon addition of an agonist, aequorin and calcium are released resulting in light emission during 20 -30 seconds. By luminometry fast and secure detection can be obtained. For subsequent antagonist testing a reference agonist is injected at fixed concentrations after pretreatment with test compound and light emission is recorded. The antagonistic efficacy of the test compound will be proportional to the inhibition of intensity of emitted light.

Both $pEC_{50}$ and $pA_2$ values have been determined for selected test compounds. The $pEC_{50}$ describes the agonist properties of the compound. The term $EC_{50}$ relates to the half maximal effective concentration of a drug which induces a response halfway between baseline and maximum. It is commonly used as a measure of drug agonistic potency. The $pEC_{50}$ is defined as the negative logarithm of the $EC_{50}$.

The value $pA_2$ refers to the antagonistic action of the compounds and this value can be derived from a Schild plot. The Schild plot is a pharmacological method to study ligand-receptor interaction. To construct a Schild plot, the dose-response curve for a reference
agonist is determined in the presence of various concentrations of a competitive antagonist. From this experiment the $pA_2$ is determined which reflects the efficacy with which the agonistic interaction with the receptor is antagonized by the test compound (i.e., the equilibrium dissociation constant). The x-intercept of the fitted regression line is an estimate of $pA_2$ which is the estimated equilibrium dissociation constant for the antagonist ($pA_2$ corresponds to the dose of antagonist that requires a 2-fold increase in agonist concentration.\textsuperscript{18})

6.2.1 EVALUATION OF SUBSTITUTED 6-TRIFLUOROMETHYLPURINE DERIVATIVES

The compounds that were described in this thesis were selected for affinity studies on the adenosine receptors. Affinity tests were performed on human adenosine receptors. A selection of active compounds was further tested for functional activity. The in vitro screening results of the 6-trifluoromethylpurine analogues are given in Table 6.1.
Table 6.1: Affinity of 6-trifluoromethyl substituted purines

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>pK_i</th>
<th>pK_i</th>
<th>pK_i</th>
<th>pK_i</th>
<th>pEC_50</th>
<th>pA_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NO₂</td>
<td>CH₃</td>
<td>H</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>4.8</td>
<td>&lt;5</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td>&lt;5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>5.4</td>
<td>4.7</td>
<td>&lt;5.0</td>
<td>7.7</td>
<td>&lt;5.0</td>
<td>6.8</td>
</tr>
<tr>
<td>7</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.5</td>
<td>&lt;5.0</td>
<td>5.8</td>
</tr>
<tr>
<td>8</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>5.1</td>
<td>4.9</td>
<td>&lt;5.0</td>
<td>6.5</td>
<td>&lt;5.0</td>
<td>7.4</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>6.0</td>
<td>4.9</td>
<td>&lt;5.0</td>
<td>7.9</td>
<td>&lt;5.0</td>
<td>8.7</td>
</tr>
<tr>
<td>10</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>5.2</td>
<td>4.8</td>
<td>4.9</td>
<td>5.8</td>
<td>&lt;5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>11</td>
<td>CH₃</td>
<td>Br</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>CH₃</td>
<td>I</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.6</td>
<td>&lt;5.0</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>CH₃</td>
<td>O</td>
<td></td>
<td>&lt;5.0</td>
<td>5.1</td>
<td></td>
<td>5.9</td>
<td>&lt;5.0</td>
<td>6.8</td>
</tr>
<tr>
<td>14</td>
<td>CH₃</td>
<td>S</td>
<td></td>
<td>5.2</td>
<td>&lt;5.0</td>
<td>5.8</td>
<td>&lt;5.0</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>CH₃</td>
<td>N</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>CH₃</td>
<td>N</td>
<td></td>
<td>&lt;5.0</td>
<td>4.8</td>
<td></td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>CH₃</td>
<td>O</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>CH₃</td>
<td>HN</td>
<td></td>
<td>5.5</td>
<td>5.9</td>
<td></td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Biological evaluation of substituted 1-deazapurines and 6-trifluoromethylpurines

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>hA1</th>
<th>hA2A</th>
<th>hA2B</th>
<th>hA3</th>
<th>pK_i</th>
<th>pK_i</th>
<th>pK_i</th>
<th>pEC_{50}</th>
<th>pA_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>CH₃</td>
<td></td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>5.1</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>CH₃</td>
<td></td>
<td></td>
<td>5.1</td>
<td>&lt;5.0</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>CH₃</td>
<td></td>
<td>F</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>CH₃</td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.7</td>
<td>&lt;5.0</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>CH₃</td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>CH₃</td>
<td></td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>CH₃</td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>7.5</td>
<td>&lt;5.0</td>
<td>9.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Ribose¹</td>
<td>H</td>
<td></td>
<td>5.3</td>
<td>5.5</td>
<td></td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Bocom</td>
<td>H</td>
<td></td>
<td>5.6</td>
<td>&lt;5.0</td>
<td></td>
<td>7.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Bocom</td>
<td>H</td>
<td></td>
<td>5.6</td>
<td>4.7</td>
<td>7.1</td>
<td>&lt;5.0</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>ribose</td>
<td>H</td>
<td></td>
<td>5.8</td>
<td>5.5</td>
<td></td>
<td>-.-</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Ribose = β-D-ribofuranosyl

n= 1-3

hA₁ Receptor Binding on Human Adenosine A₁ Receptor; CEREP 801-1h
Receptor Binding Assay

hA₂A Receptor Binding on Human Adenosine A₂A Receptor; CEREP 801-2ah
Receptor Binding Assay

hA₂b Receptor Binding on Human Adenosine A₂b Receptor; CEREP 801-2bh
Receptor Binding Assay
Stehle et al. (1992) Mol. Endocrinol. 6: 384-393

hA₃ Receptor Binding on Human Adenosine A₃ Receptor; CEREP 801-3h
Receptor Binding Assay
Salvatore et al. (1993) Proc Natl Acad Sci USA 90: 10365-10369

hA₃ Functional Screen Human Adenosine A₃ Receptor, Aequorin dual mode (Solvay Pharmaceuticals Weesp)
Chapter 1 describes why it is important to search for new ligands for the A$_{2A}$ receptor and which synthetic strategies were viable. However, as shown in Table 6.1 these ligands show a complete lack of affinity for the adenosine A$_{2A}$ receptor, which was not expected from the models described in Chapter 1. On the other hand, some compounds show high affinity for the adenosine A$_3$ receptor. Especially compounds 6, 9, 26, 27 and 28 show high affinity with pK$_i$ values around 8 (K$_i$ around 10 nM). The selectivity for the A$_3$ receptor over the A$_1$ and A$_{2A}$ receptor is excellent. For many ligands described in literature a higher overlap is seen with A$_1$ receptor affinity, which is apparently less in these compounds.

**Influence of C-2 substituents**

Entries 2 - 5 show that the introduction of cycloalkyl amines at C-2 does not result in good affinity for any of the adenosine receptors. The introduction, however, of substituents containing aromatic residues at C-2 immediately raises affinity for the adenosine A$_3$ receptor as shown for the benzyl and phenethyl amino substituted entries. The introduction of a 4-hydroxyl moiety at the phenethyl group (conversion of compound 26 to compound 27) leads to a further increase of affinity with a pK$_i$ of 8.0 (K$_i$ = 10 nM). Combining this optimal R$_1$ substitution with the best observed R$_2$ (H) and R$_3$(H) may lead to even better affinity.

**Influence of N-9 substituents: H, Methyl, Ribose and Bocom**

Interestingly, substituents at N-9 have strong influence on affinity for the A$_3$ receptor as seen for the phenethyl compounds 9, 10 and 26: pK$_i$, methyl 5.8; H 7.9 and ribose 7.5 respectively. This change in activity is very interesting. The results show that changing the ribose group for hydrogen (the unprotected base N9) is not detrimental for activity at all. This may have consequences for pharmacokinetics. A big problem in drug research is targeting drugs to the central nervous system. For many compounds the blood brain barrier is often difficult to pass. With the benzylamino substitution at C-2 (compounds 6, 7, 8) we observe the same trend for the N-9-methyl (pK$_i$ 5.5) the N9-H (pK$_i$ 7.7) and the N-9-ribose system (pK$_i$ 6.5). Methyl substituents at N-9 lower the affinity for the A$_3$ receptor while the free purine base is equally or more active than the corresponding riboside. It may be that N-9 H leads to a stronger complex via H-bond formation in the active site of the receptor.
It is also quite interesting to observe entries 28 and 29 where N-9 is protected with the newly developed Bocom group. Both the 2-phenethyl and the 2-benzyl substituted systems show high affinity with very good selectivity for the A3 receptor, pK_i being 7.6 and 7.1, respectively.

Compound 29, with Bocom protection at N-9, does not show strong agonistic activity, or antagonistic activity. In view of the low affinity of the N-9 alkylated compounds, it is not likely to be a spatial effect. It is more likely to be the result of an electronic effect of H-bonding with N-H or interaction with the carbonyl group of the Bocom protecting group.

**Results of functional tests pA_2** and **pEC_50**

A selection of the compounds was tested for functional agonistic or antagonistic activity on the A3 receptor after observing affinity for this receptor subtypes (Table 6.1). From the functional tests it can be seen that all compounds act as moderate to strong antagonists on the A3 receptor (pA_2’s ranging from 5 – 9.8). No agonistic activity was observed. The relation between affinity and antagonistic potency seems to be highly dependent on substitution pattern. On the one hand the nucleoside analogues containing a ribose moiety were demonstrated to be potent. This is quite remarkable for adenosine nucleoside analogs, which are usually known to act as agonists. Recently Volpini et al. presented a series of 8-substituted alkynylpurines which were the first examples of adenosine receptor antagonists with an intact ribose moiety.\(^\text{19}\) On the other hand the Bocom-protected compound 29 shows hardly any antagonistic activity (nor agonistic activity). This might possibly reflect a different (allosteric) interaction mode.

**Literature comparison of selected compounds: influence of C6 substituents: CF_3, NH_2**

The laboratory of Cristalli described a series of 9-alkyl (ethyl) purines as adenosine receptor ligands.\(^\text{20}\) All compounds disclosed, show adenosine A_{2A} receptor antagonism, with less A_3 affinity. Our synthesized C6 amino variant 31 (with a methyl group at N-9 instead of the ethyl group) behaves according to the findings of Cristalli and shows A_{2A} affinity. With the conversion of 6-amino to CF_3 however (compounds 9, 10 and 26), the selectivity is strongly shifted to the A_3 receptor. IJzerman and Cristalli published the results of a functional screening for the A_{2B} receptor where also compound 33 and the 6-amino variant of 8 are
published with a reduced potency\textsuperscript{21}. 1-deazapurine is published as the only deazapurine with a low functional activity. In our study no functional tests have been performed for the A\textsubscript{2B} receptor, but affinity for this receptor is low.

| Table 6.2: Comparison of 6-trifluoromethylpurines with 6-amino variants |
|-----------------------------|----------------|----------------|----------------|----------------|
| Entry | R\textsubscript{1} | R\textsubscript{2} | hA\textsubscript{1} | hA\textsubscript{2A} | hA\textsubscript{2B} | hA\textsubscript{3} |
| 9 | CF\textsubscript{3} | H | 6.0 | 4.9 | <5 | 7.9 |
| 10 | CF\textsubscript{3} | CH\textsubscript{3} | 5.2 | 4.8 | 4.9 | 5.8 |
| 26 | CF\textsubscript{3} | ribose | <5.0 | <5.0 | <5.0 | 7.5 |
| 31 | NH\textsubscript{2} | CH\textsubscript{3} | 6.3 | 6.8 | 5.7 |
| 32\textsuperscript{a} | NH\textsubscript{2} | -CH\textsubscript{2}-CH\textsubscript{3} | 6.5 | 6.8 | 5.5 | 5.6 |
| 33\textsuperscript{a} | NH\textsubscript{2} | ribose | 6 | 7.2 |

\textsuperscript{a} literature values Cristalli

**Literature comparison: C2- Alkoxy substituents**

When the conversion of the amino group to trifluoromethyl was combined with a 2-alkoxy moiety, the same effect on selectivity was observed as for the 2-phenethylamino analogs. Compound 30 showed moderately selective A\textsubscript{3} affinity. All 2-alkoxyadenosines described for example by Ueeda et al.\textsuperscript{22} are adenosine A\textsubscript{2} selective. It has to be noted that the A\textsubscript{3} receptor was not known at that time. Adenosine analogs disclosed in WO 2004/069185 are all presented as selective adenosine A\textsubscript{1} (partial) agonists.\textsuperscript{23}

**Effect of trifluoromethyl group on adenosine receptor affinity**

The effect of the trifluoromethyl group at the C-6 position on the affinity and selectivity is dramatic. Apparently the trifluoromethyl function has the interesting capacity of changing the binding mode of the purines in such a way that they do not fit in the A\textsubscript{2A} receptor, but become effective ligands for the A\textsubscript{3} receptor. We speculate that H bonding differences via fluorine may be at the basis of these findings.
Effect of 8-substitution

The introduction of substituents at C-8, which proved to be useful in enhancing $A_{2A}$ activity in adenosine analogues in literature, does not give the anticipated effect on $A_{2A}$ affinity. On the other hand, also the $A_3$ affinity becomes much less prominent after C8 substitution and values are all in the same ball park. The highest affinity is found for the 8-substituted phenyl acetylene derivative 23 with a $pK_i$ of 6.3. Remarkably, also the 8-cyclopentyl amine substituted derivative shows a $pK_i$ of 6.2 and a moderate affinity for the $A_{2A}$ receptor with a $pK_i$ of 5.9. Morelli et al. published data for 8-substituted 9-ethyl adenines. They report that 8-bromo-9-ethyladenine, 8-ethoxy-9-ethyladenine and 8-furyl-9-ethyladenine have been characterized in vitro as efficient adenosine receptor antagonists with $A_{2A}$ affinity.

Derivatives with aromatic systems at the 8-position seem to be well tolerated and the heteroaryl substituted systems like 2-furyl compound 13 and 2-thienyl compound 14 are equipotent.

6.2.2 LIPOPHILICITY

Most of the current potent and selective non nucleoside $A_3$ adenosine receptor antagonists, including substituted pyridines, dihydropyridines, triazoloquinazolines, and pyrazolotriazolopyrimidines, have a relatively high lipophilicity ($logP > 3.7$). This may contribute to their observed very low degree of water-solubility. Better water-soluble $A_3$ adenosine receptor antagonists are considered to be more effective pharmacological tools for in vitro and in vivo studies. For bioavailability and distribution through the body, lipophilicity of the compounds may be important. Usually, therapeutic drugs acting on the central nervous system have an optimal $logP$ between 2 and 4. For selected compounds the $logP$ was measured with a specially designated HPLC system which gives fast results and are generally in line with results of the more laborious octanol-water distribution measurements. This system is used as a standard in pharmaceutical industries to test lipophilicity. Table 6.3 shows that the furyl compound has a rather high $logP$. In a pharmaco-dynamic system higher $logP$’s should not be problematic: The in vitro $logP$ value is not a predictor for the in vivo efficacy of the compound, since in this process many other factors and transport mechanism of drugs through the body will play a role.
Table 6.3: lipophilicity of selected compounds at pH 11

<table>
<thead>
<tr>
<th>Entry</th>
<th>HPLC-logP</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>4.3</td>
</tr>
<tr>
<td>31</td>
<td>2.4</td>
</tr>
<tr>
<td>13</td>
<td>6.1</td>
</tr>
</tbody>
</table>

6.2.3 MODELING STUDIES

As mentioned before, the results show that replacement of the amino group of the adenine by the trifluoromethyl group shifts the affinity for the adenosine receptors towards the A3 receptor. In first instance, in analogy with reports from the literature, we expected to generate A2A receptor ligands. One of the major changes from previously reported analogs was the introduction of – CF₃ at the C6 which normally is substituted with -NH₂. So, the question is: why is the affinity towards the A2A receptor lost?

To look further into that, a more specific pharmacophore modeling study was re-initiated at Solvay Pharmaceuticals. Pharmacophore modeling was performed with the GASP (Genetic Algorithm Similarity Program) module as implemented in Sybyl 7.0, running on a Silicon Graphics Octane2. GASP automatically assigns features to atoms and moieties of the antagonists (e.g. donor and acceptor atoms, hydrophobic centers and normals on aromatic planes). For donor and acceptor atoms corresponding acceptor and donor sites are additionally assigned. These site-points represent interaction points on the receptor and are placed in the direction of the lone pair or hydrogen atom of the acceptor and donor atom, respectively. During the derivation of a pharmacophoric model, all possible combinations of common features are superimposed. The solution possessing the lowest rmsd value (root mean square deviation), combined with good volume overlap and low energy conformers of the ligands, constitute the resulting pharmacophore.

In Scheme 6.1 the very strong antagonists SCH-63390 and the xanthine derivative MSX-2 were selected for direct comparison with our designed candidate for the A2A receptor 13.
Scheme 6.1 Target compound 13 and two selective A$_{2A}$ antagonists were selected for a pharmacophore modelling study.

Figure 6.1 shows that GASP produced a 4-point pharmacophore with good spatial overlap of the compounds. The pharmacophore consists of three acceptor atoms, which lie in one plane, and a hydrophobic area, which is represented by the centroid of the fitted aromatic phenyl rings of MSX-2 (shown in green) and SCH63390 (shown in purple). The donor sites interacting with the ligand acceptor atoms are also shown and are colored purple. Although GASP attempts to fit low energy conformers, the conformations of MSX-2 and SCH-63390 were somewhat strained. A minimization was therefore carried out, after which the compounds were re-aligned. This did not have a significant effect on the pharmacophore model.

Figure 6.1 A$_{2A}$ Pharmacophore model analysis show inverted position for compound 13. SCH-63390 (purple color), the xanthine derivative MSX-2 (green color) and designed compound 13 (orange color)
It is observed that the A$_{2A}$ antagonists SCH-63390 (purple color) and the xanthine derivative MSX-2 (green color) overlap very well. However, when compound 13 was modeled in this study, a surprising fact was observed: Compared with its putative bioisosteric NH$_2$ group our substrate 13 fits in the pocket exactly in the opposite inverted position with its trifluoromethyl group. The 6-amino group of SCH-63390 is rather in the same direction as the N-methyl group of 13, while the CF$_3$ group of 13 points in exactly the opposite way. In addition, the overlap in the aromatic part with the phenethyl group at C-2 is somewhat distorted, while the phenyl moieties of the MSX-2 and SCH-63390 fit very well in the hydrophobic area.

It was then attempted to force superposition of 13 with the A$_{2A}$ ligands to explore new possibilities for manipulation. If the furyl moieties of SCH-63390 and 13 are superimposed, it is clear that the phenethyl part does not fit well and is not stabilised via pi-pi interactions. Possibly, when benzylamine would have been chosen at C2 or some substituents were added to the aromatic ring, the fit in the A$_{2A}$ receptor pocket might be better. Likewise the N-9 methyl group does not fit well in the model (Figure 6.2). As discussed in chapter 2, the trifluoromethyl group can be considered sterically as large as an isopropyl group. An isosteric replacement of the NH$_2$ with a perfluoroalkyl group (-CH$_2$-F, or –CF$_2$-H), introducing the electronic properties of fluorine and at the same time preserving a hydrogen donor/acceptor moiety, may possibly an option for future research.
Some information can be found in the literature regarding the observed adenosine A₃ receptor affinity of the compounds synthesized by us.

Recently, Volpini and coworkers reported a receptor homology modelling/receptor modelling study for the A₃ receptor and found interesting structural relations for the A₃ agonists. The agonist 2-phenylethynyladenosine was docked in its anti conformation. The alkynyl chain is accommodated in a hydrophobic region between helices 3, 4 and 5, see Figure 6.3. The ribose moiety, which is crucial for agonist activity, is in close contact with helices 3 and 7. In particular the 5'-hydroxyl group is within hydrogen-bond distance to Asn274 (TM7), while the 3'-hydroxy group, which has been widely demonstrated to be essential for full agonistic activity, is closely linked to the crucial His272 (TM7). The adenine region is found between helices 3 and 6, with Asn250 (TM6) hydrogen-bonded to the amine in 6 position of the purine ring and Thr94 very close to N-3. The receptor ligand complex is more stable than its individual separated molecules by about 60 kcal/mol.
In contrast, 8-phenylethynyladenosine cannot bind to the A₃ receptor in the same way because the alkynyl chain in C-8 position would overlap with helix 6. So the binding mode of this molecule should be different. Volpini et al. propose that the purine moieties of 2-phenylethynyladenosine and 8-phenylethynyladenosine can be superimposed in such a way that the C-2 of a molecule matches the C8 of the other one as shown in Figure 6.4.

This leads to a very good steric and electrostatic correspondence at the level of the purine moieties and the phenylethynyl chains, but there is no overlap of the ribose moieties. As a consequence, 8-phenylethynyladenosine would not be able to stimulate the receptor response anymore. Recently, the A₃ receptor morphology was further studied using molecular modelling via agonist screening.²⁶
Biological evaluation of substituted 1-deazapurines and 6-trifluoromethylpurines

Figure 6.4 Superimposition of 2- and 8-phenylethynyladenosine (left) with the C-2 of a molecule matching the C-8 of the other one and vice versa, and relative docking of 8-phenylethynyladenosine into the seven trans-membrane domain of the human A<sub>3</sub> receptor (right). Picture adapted from Volpini et al.

Latest information on the crystal structure of the adenosine A<sub>2A</sub> receptor indicates structural changes towards originally used ligand models. Our model database set-up using A<sub>2A</sub> adenosine receptor antagonists, did not describe the actual (dynamic) modes of the receptor. Very recently, the structural basis of ligand subtype selectivity within the family of adenosine receptors was checked and confirmed by generating 3D models of all four subtypes using the recently determined crystal structure of the adenosine A<sub>2</sub> receptor as a template, and employing the methodology of ligand-guided receptor optimization for refinement. This approach produced 3D conformational models of adenosine receptor subtypes that effectively explain binding modes and subtype selectivity for a diverse set of known adenosine receptor antagonists.27

Earlier research of the group of Baraldi indicated that there may be a structural relation between A<sub>2A</sub> antagonists and ligands with A<sub>3</sub> receptor affinity.28 Via a rational approach they were able to convert strong A<sub>2A</sub> antagonists to molecules with A<sub>3</sub> receptor affinity via simple
exchange of structural moieties. Over 100 pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c] pyrimidine derivatives that are potent A2A antagonists and a set of N-6-substituted phenyl carbamoyl adenosine-5’-uronamides with strong A3 agonistic activity were selected. They linked and exchanged the amino group of the receptor antagonists with the phenyl-carbamoyl moiety that is typical of A3 receptor agonists in an attempt to modulate the affinity and selectivity of these compounds at human A3 receptor subtypes. A series of new synthesized hybrid molecules (5-N-(substitutedphenylcarbamoyl)amino-8-substituted-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidines) was prepared with superior antagonistic activity on the human A3 receptor.

In the series of compounds we have studied the introduction of the trifluoromethyl group possibly interacts in such a way that structurally the compounds fit in the A3 receptor, but, the ribose moieties are unable to stimulate the receptor, leading to antagonistic activity. In our latest modelling studies of the A2A pharmacophores in Figure 6.1 we observe that our target molecules fit upside down in the model. In addition, with the findings of Volpini and co-workers this may explain the observed A3 antagonistic activity. An option for future research would be introduction of -CH2F or CHF2 functionality at C6 as previously described for some analogs of Hocek, favouring H-bond donor acceptor functions, described in chapter 2.

Our work on the adenosine receptors was described in a review article describing recent activities in the research and patent field on adenosine agonists and antagonists. The authors compared a large set of publications and shared their hope that the large volume of new adenosine compounds would lead to a new wave of compounds entering the market for new unmet medical needs.29

Combining these separate pieces of information might lead to the following considerations on our series:

- Introduction of the trifluoromethyl group changes (and obviously decreases) the interaction with the A2A receptor.
- As is the case in the reported series, this results apparently in a concomitant increase in A3 receptor affinity.
In analogy with a reported example, the ribose moiety, when present, might be in a position unable to stimulate the receptor. Although generally linked to agonistic effects, in our case clear A₃ antagonism is observed.

6.2.4 PHARMACOLOGICAL FUNCTION OF A₃ RECEPTOR ANTAGONISTS

The ligands synthesised by us that have surprising A₃ antagonist activity have many potential therapeutic applications. There are many reports that the A₃ adenosine receptor plays a role in a diverse range of diseases by regulating the cell cycle and apoptotic cell death via activating mast cells, eosinophils, neutrophils, and natural killer cells.³⁷,³⁹,³⁸ These reports suggest that A₃ adenosine receptor-selective agents might be therapeutically useful for treatment of human diseases. Indeed, recent studies using mast cell lines and A₃ adenosine receptor-deficient mice have shown that the A₃ adenosine receptor antagonists have potential as anti-asthmatic and anti-inflammatory drugs.³³,³⁴ Highly potent and selective antagonists for the human A₃ adenosine receptor have been screened and identified (Kᵢ values in the 0.1 nM range). However, due to low interspecies homology between human and rodent A₃ adenosine receptor, 1000 fold lower affinities for the rodent A₃ adenosine receptor (Kᵢ values in the 0.1 mM range) was observed than for the human A₃ adenosine receptor. This discrepancy in activity makes it difficult to relate animal studies to future therapy in human. Recently, A₃ adenosine-humanized mice were reported, in which the mouse A₃ adenosine gene was replaced by its human counterpart. This may open new options for pharmacological testing.³⁶,³¹ Antagonists for A₃ receptor promise to be useful for the treatment of inflammation and in regulation of cell growth.

THERAPEUTIC POTENTIAL OF A₃ ADENOSINE RECEPTOR ANTAGONISTS

GLAUCOMA

Glaucoma, characterized by elevated intraocular pressure, is a leading cause of irreversible blindness in the world. Patients with glaucoma may require long-term administration of intraocular pressure-lowering medications. These medications belong to several classes of molecules including beta-adrenergic blockers, cholinesterase inhibitors, alpha-adrenergic agonists, carbonic anhydrase inhibitors, and ocular hypotensive lipids. Most intraocular pressure-lowering medications are associated with mild adverse effects, however several of
them are associated with systemic risks as well as serious ocular effects, especially following chronic use. It was found that A3 adenosine receptors regulate Cl- channels of non pigmented ciliary epithelial cells. In addition, selective A3 antagonists lowered intraocular pressure in mouse and monkeys. These results suggest that reducing Cl-channel activity with A3 antagonists may provide a novel approach for treating glaucoma. A recent study across several animal species concludes that antagonist derivatives derived from A3 agonist Cl-IB-MECA (2-Chloro-N6-(3-iodobenzyl)adenosine-5’-N-methylcarboxamide) lower intraocular pressure and is further investigated.32

**ASTHMA AND COPD**

Asthma and chronic obstructive pulmonary disease (COPD) are lung diseases that afflict many people. It has been shown that adenosine is generated at sites of tissue damage and hypoxia. Accordingly, adenosine levels are elevated in the bronchoalveolar lavage fluid and in exhaled breath condensate of asthmatic patients where significant lung inflammation and hypoxia exist.33 Recent studies demonstrate that elevations in adenosine evoke signalling pathways that lead to chronic lung disease. Additional evidence linking adenosine to asthma and COPD include the observations that exposing patients to exogenous adenosine can elicit bronchoconstriction, whereas adenosine has little effect on control subjects. In addition in mice research, the A3 receptor was found to be expressed in eosinophils and mucus-producing cells in the airways of adenosine deaminase-deficient mice. Treatment of adenosine deaminase-deficient mice with MRS 1523, a selective A3 receptor antagonist, prevented airway eosinophilia and mucus production.34 The A3 adenosine receptors may serve important regulatory roles in the inflamed lung and selective A3 receptor antagonists may be therapeutically useful.35 A recent review describes the options for use of adenosine modulating therapeutics, including the A3 receptor.36

**Cancer and cell growth inhibition**

Human A3 adenosine receptors play an essential role in several physiopathological processes and selective ligands (agonists and antagonists) have been evaluated as anticancer therapeutic agents. Among these ligands, derived from agonist Cl-IB-MECA, truncated D-4’-
Biological evaluation of substituted 1-deazapurines and 6-trifluoromethylpurines

thioadenosine derivatives lacking the 4'-hydroxymethylene moiety were reported, changing the functionality for the A₃ receptor to antagonists.⁷ This truncated thio-Cl-IB-MECA was reported for use in leukaemia, bladder cancer³⁸ and thyroid cancer.³⁹

6.3 BIOLOGICAL EVALUATION OF SUBSTITUTED 1-DEAZAPURINES

In Table 6.4 the results of the receptor study for the 1-deazapurine series from Chapter 5 are presented. In general, it can be seen that there is moderate activity on adenosine receptors. It is interesting to see that introducing a methyl group at N-9 in compound 32 results in slight affinity for the A₃ receptor (entry 33). When the nitro group of compound 34 is converted into a nitroso moiety (entry 35) slightly enhanced affinity for the A₁ (pKi <5 to 5.5) and A₃ (pKi <5 to 5.9) receptor is observed.

The introduction of a cyclic alkyl structure at C-2 does not enhance affinity for one of the receptors, see entry 37. When aromatic substituents are introduced as in compound 39, enhanced affinity is observed for the A₂A receptor. The introduction of one or two methoxy groups (entries 40 and 41 respectively), does not change selectivity for the A₂A receptor dramatically.

Surprisingly, compounds 42 and 43 exhibit some affinity for the A₂A receptor. In addition these are the only examples that show higher affinity for the A₁ receptor too. The cyclohexyl substituent in being more flexible than the phenyl group in compound 39, is apparently capable of accommodating the binding pocket of the A₁ receptor somewhat better.
Table 6.4: Biological evaluation of substituted 1-deazapurines

<table>
<thead>
<tr>
<th>Entry</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>hA_1</th>
<th>hA_2A</th>
<th>hA_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>-NO_2</td>
<td>-N_3</td>
<td>H</td>
<td>&lt;5.0</td>
<td>5.1</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>33</td>
<td>-NO_2</td>
<td>-N_3</td>
<td>-CH_3</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>5.7</td>
</tr>
<tr>
<td>34</td>
<td>-NO_2</td>
<td>-NH_2</td>
<td>-CH_3</td>
<td>&lt;5.0</td>
<td>5.4</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>35</td>
<td>-N=O</td>
<td>-NH_2</td>
<td>-CH_3</td>
<td>5.5</td>
<td>5.1</td>
<td>5.9</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>4.8</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>5.1</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td>5.6</td>
<td>5.5</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td>5.4</td>
<td>5.9</td>
<td>5.6</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td>&lt;5.0</td>
<td>6.0</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td>5.2</td>
<td>5.7</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td>6.0</td>
<td>6.1</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td>5.5</td>
<td>5.7</td>
<td>&lt;5.0</td>
</tr>
</tbody>
</table>
In previous work on 1-deazapurine ribosides, high affinity of the 6-N-iodobenzyl-2-nitro-1-deazapurine riboside for the A3 receptor (9.8 nM) was observed. The unsubstituted 6-amino variant also showed some affinity for the A3 receptor (216 nM = pKi 6.6). The 1-deazapurine N9-H and N-9-Me synthesized in this thesis (compounds 32 - 35) are much less potent than the corresponding ribosides. It is noted that the affinity is decreased when a nitrogen atom is missing in the purine system. The introduction of substituents on C-2 directs affinity towards the A2A receptor (Ki around 790 nM), which was not seen before for these 1-deazapurines. As mentioned in chapter 5, Pharmacopeia Drug Discovery a biopharmaceutical company, also recently targeted the A2A adenosine receptor with functionalized 1-deazapurines. Their 2- and 8-substituted compounds showed affinity of around Ki 10µM (10,000 nM) for the A2A adenosine receptor, a more than tenfold lower activity compared to our substrates. No affinity data on the other adenosine receptors were given.

6.4 CONCLUDING REMARKS

The in vitro screening of the synthesised purine compounds, identified structures with (very) high affinity and selectivity for A3 adenosine receptors. We have shown that a simple conversion from an amino to a trifluoromethyl group may lead to new types of selective adenosine receptor antagonists. The resulting 2,6,8-trisubstituted trifluoromethylpurines were shown to be both potent and selective human A3 receptor antagonists. Such compounds may have potential therapeutic applications in glaucoma or treatment of asthma.

Nitrated 1-deazapurine bases have moderate affinity for adenosine receptors. The introduction of substituents at C-2 of 1-deazapurine systems results in somewhat higher affinity for the A2A receptor.

6.5 ACKNOWLEDGEMENTS

We wish to thank Jennifer McCormack and Mia Pras-Raves from Solvay Pharmaceuticals for their contribution to the modelling studies.
6.6 EXPERIMENTAL

General information.

In vitro affinity for human adenosine A3 receptors

Briefly, membrane preparations were obtained from human recombinant (HEK 293) cells in which the human adenosine-A3 receptor was stably expressed. Membranes were incubated at 22°C for 90 minutes with $[^{125}\text{I}]-\text{AB-MECA}$ in the absence or presence of test compounds in a concentration range from 10 µM down to 0.1 nM, diluted in a suitable buffer. Separation of bound radioactivity from free was done by filtration through Packard GF/B glass fiber filters with several washings with ice-cold buffer using a Packard cell harvester. Bound radioactivity was measured with a scintillation counter (Topcount, Packard) using a liquid scintillation cocktail (Microscint 0, Packard). Measured radioactivity was plotted against the concentration of the displacing test compound and displacement curves were calculated by four-parameter logistic regression, resulting in IC$_{50}$ values, i.e. that concentration of displacing compound by which 50% of the radioligand is displaced. Affinity pK$_i$ values were calculated by correcting the IC$_{50}$ values for radioligand concentration and its affinity for the human adenosine-A3 receptor according to the Cheng-Prusoff equation:

$$pK_i = - \log(\text{IC}_{50} / (1+S/K_d))$$

in which the IC$_{50}$ is as described above, S is the concentration $[^{125}\text{I}]-\text{AB-MECA}$ used in the assay expressed in mol/l (typically 0.1 nM), and K$_d$ is the equilibrium dissociation constant of $[^{125}\text{I}]-\text{AB-MECA}$ for human adenosine-A3 receptors (0.22 nM).

Determination of In Vitro Functional Activity on Human Adenosine A3 Receptors Using an Aequorin-Based Assay

A stable monoclonal hA3-Aequorin cell line was provided by Euroscreen. The coding region encoding the human Adenosine A3 receptor was amplified by polymerase chain reaction (PCR), using human lung cDNA as template. The PCR product was ligated in the expression vector pEFIN3 (Invitrogen), and the complete sequence of the insert was then established. Sequencing revealed a complete identity with the sequence published by
Biological evaluation of substituted 1-deazapurines and 6-trifluoromethylpurines

Salvatore, C. A. et al. (Acc. Number GenBank: L22607). The expression plasmid containing the coding sequence of the human Adenosine A₃ receptor was transfected in CHO-K1 cells stably expressing mitochondrially targeted Aequorin and Gα₁₆. Resistant clones were selected in the presence of 400 micro g/ml G418 and isolated by limiting dilution. The clone with the best response to 2-CI-IB-MECA was selected for further work. The hA₃-Aequorin cells express mitochondrially targeted apo-Aequorin. Cells have to be loaded with coelenterazine, in order to reconstitute active Aequorin. After binding of agonists to the hA₃ receptor, the intracellular calcium concentration increases, this leads to a luminescent response. Binding of calcium to the apo-Aequorin/coelenterazine complex leads to an oxidation reaction of coelenterazine, which results in the production of apo-Aequorin, coelenteramide, CO₂ and light (λₘₐₓ 469 nm). This luminescent response is dependent on the agonist concentration.

Assay-protocol:
- The loaded cells were diluted 18 times in Dulbecco's medium without PhenolRed (Gibco BRL), pre-heated to 37°C, resulting in a concentration of 2.8*10⁵ cells/ml and stirred for 1 hour at room temperature. Per well 10 µl compound of control in white 96-well plates and 90 µl of diluted cells (2.5*10⁴ cells/well) was added. After that, chemoluminescence was measured immediately for 20 seconds. In antagonist mode 50 µl 10⁻⁶ N6-benzyl-NECA is added as the agonist, followed by measuring chemoluminescence immediately for 20 seconds using the MicroBetaJet (PerkinElmer).
6.7 REFERENCES

   (h) Mitsui Chemicals WO 02/031176
4 Sakaguchi J.; Takeshita M.; Izumi T, Abbott Japan, JP 2004 217582
7 Fujimoto, R.A.; Francis J.E., Hutchison, A.J., Ciba Geigy AG 2003, EP03/54180
   1995 47 307 - 313
12 Stehle J. H.; Rivkees, S.A.; Lee, J.J.; Weaver, D.R.; Deeds, J.D.; Reppert, S.M. Mol.
   Endocrinol. 1992 6 384 - 393
   Sci. 1993 90 10365 - 10369
14 Le Poule, E.; Hisada, Z.; Deltheux, M. J. Biomol. Screen. 2002 7 57 - 65
16 Brini, M.; Marsault, R.; Bastianutto, C. J. Biol. Chem. 1995 270 9896 - 9903
17 Shimomura, O.; Johnson, F.H. Nature 1975 256 236 - 238
18 Arunkakshana, O.; Schild, H.O. Br. J. Pharmacol. 1959 65 48 - 58
19 Volpini R.; Costanzi, S.; Lambertucci, C.; Vittori, S.; Lorenzen, A.; Klotz, K.-N.; Cristalli,
   17 969 - 985
22 Ueeda, M.; Thompson, R.D.; Arroyo, L.H.; Olsson, R.A J. Med. Chem. 1991 34 1334 -
   1339
23 CV Therapeutics Inc. PCT Int. Appl. WO 2004/069185
   2003 193 - 204
27 Katritch,V.; Abagyan, R. Neuropharmac. 2011 1 108 - 115
Biological evaluation of substituted 1-deazapurines and 6-trifluoromethylpurines

29 Neil J.; Gessi, S.; Borea, P.A.; Polosa, R. Exp. Opin. Ther. Patents 2007 17 979 - 991
30 Yamano, K; Inoue, M.; Satoh, M. Biochem. Pharm. 2006 3 294 - 306
31 Yamano, K; Inoue, M.; Satoh, M. J. Pharmacol. Sci. 2006 100 89
35 van den Berge, M.; Hylkema, M. N.; Versluis, M.; Postma, D. S. Drugs in R&D 2007 8 1 13 - 23
38 Kim, H.; Kang, J.W.; Do, Y. Anticanc. Res. 2010 30 2823 - 2830