Molecular Recognition at the Active Site of Catechol-O-methyltransferase (COMT): Adenine Replacements in Bisubstrate Inhibitors


Abstract: L-Dopa, the standard therapeutic for Parkinson’s disease, is inactivated by the enzyme catechol-O-methyltransferase (COMT). COMT catalyzes the transfer of an activated methyl group from S-adenosylmethionine (SAM) to its catechol substrates, such as l-dopa, in the presence of magnesium ions. The molecular recognition properties of the SAM-binding site of COMT have been investigated only sparsely. Here, we explore this site by structural alterations of the adenine moiety of bisubstrate inhibitors. The molecular recognition of adenine is of special interest due to the great abundance and importance of this nucleobase in biological systems. Novel bisubstrate inhibitors with adenine replacements were developed by structure-based design and synthesized using a nucleosidation protocol introduced by Vorbrüggen and co-workers. Key interactions of the adenine moiety with COMT were measured with a radiochemical assay. Several bisubstrate inhibitors, most notably the adenine replacements thiopyridine, purine, N-methyladenine, and 6-methylpyrurine, displayed nanomolar IC₅₀ values (median inhibitory concentration) for COMT down to 6 nM. A series of six cocrystal structures of the bisubstrate inhibitors in ternary complexes with COMT and Mg²⁺ confirm our predicted binding mode of the adenine replacements. The cocrystal structure of an inhibitor bearing no nucleobase can be regarded as an intermediate along the reaction coordinate of bisubstrate inhibitor binding to COMT. Our studies show that solvation varies with the type of adenine replacement, whereas among the adenine derivatives, the nitrogen atom at position 1 is essential for high affinity, while the exocyclic amino group is most efficiently substituted by a methyl group.

Introduction

Low dopamine levels in the human brain are a characteristic of Parkinson’s disease.[1] The gold standard to treat the disease is oral administration of l-dopa, a prodrug that is converted to dopamine in the brain.[2–6] However, before it reaches its target, l-dopa is rapidly inactivated to a large extent by the enzyme catechol-O-methyltransferase (COMT: EC number: 2.1.1.6). COMT catalyzes the transfer of a methyl group from S-adenosylmethionine (SAM) to catechol substrates in the presence of Mg²⁺ ions. Consequently, the bioavailability of l-dopa is greatly enhanced by COMT inhibition, resulting in a concomitant increase of dopamine levels in the brain.[10,11]

Potent bisubstrate inhibitors of COMT, which are competitive for both the catechol and the SAM binding sites, were introduced by our group.[12] The X-ray crystal structure of the ternary complex of ligand 1 (median inhibitory concentration IC₅₀ = 9 nM, inhibitory constant Kᵢ = 2 nM,[13] see Figure 1) with COMT and a Mg²⁺ ion (PDB code: 1JR4) was used as the basis for a detailed exploration of the molecular recognition properties of the entire active site of COMT.[15,16] Previously, we were able to demonstrate that 5-nitrocatechol anchors are not required for high-affinity bisubstrate inhibition and can be replaced with lipophilic substituents, such as a 4-fluorophenyl ring, without loss in activity against COMT.[17,18] The predicted orientation of the 4-fluorophenyl ring in the active site was recently confirmed by a series of X-ray cocrystal structure analyses.[19] We also showed that the adenine-bound water molecule imported with the ligand (Figure 1) can be expelled by N(6)-alkyl substituents and that the energetic gain of this water replacement amounts down to ΔG ≡ −1.8 kcal mol⁻¹.[20] Here, we carry the investigation of the adenine binding site one step further. Using bisubstrate inhibitors as a tool, we perform a thorough structural investigation of the molecular properties that ligands must exhibit for tight binding into the adenine

Supported information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201003648.

Keywords: adenine substitutes · bisubstrate inhibitors · catechol-O-methyltransferase · molecular recognition · structure-based design
site. Adenine nucleotides bind to a plethora of metabolic and regulatory proteins including polymerases, transferases, kinases, oxidoreductases, and ATPases.[20–25] Our analysis of adenine replacements in COMT inhibitors is of general interest not only with respect to SAM-binding proteins but also to avoid potential specificity issues with other enzyme classes.

Results and Discussion

Modeling: All bisubstrate inhibitors were evaluated by computational modeling using the software MOLOC.[26] The in silico design was based on the X-ray co-crystal structure of 1 in complex with COMT (Figure 1, PDB code: 1JR4).[14] The enzyme, the water molecules, and the Mg$^{2+}$ ion were defined as stationary in the energy minimizations. The most prominent ligand–protein interactions are a hydrogen bond ($d(N(1)···N) = 2.7 \, \text{Å}$) to the backbone NH of Ser119 and a second, weaker hydrogen bond ($d(N(6)···O) = 3.2 \, \text{Å}$) to the side chain of Gln120. The adenine undergoes π–π stacking with the side chain of Met91 ($d(S···C(5)) = 3.6 \, \text{Å}$) and edge-to-face interactions with the imidazole ring of His142 ($d(C_{His}···C(6)) = 3.7 \, \text{Å}$). The indole moiety of Trp143 also displays an edge-to-face interaction with the inhibitor nucleobase ($d(C_{His}···C(8)) = 3.6 \, \text{Å}$). The exocyclic amine of the adenine moiety forms two hydrogen bonds, one being moderately strong and the other weak ($d(N···O) = 3.0 \, \text{Å}$ and $3.4 \, \text{Å}$, respectively), to a ligand-bound water molecule[19] and one to the side chain of Gln120 ($d(N···O) = 3.2 \, \text{Å}$).

Ligands displaying a favorable binding mode by honoring the above-mentioned key interaction criteria and being synthetically viable were selected as targets. By establishing a structure–activity relationship (SAR) for adenine replacements ranging from a single hydrogen atom, to substituted five- and six-membered aromatic rings, and purine analogues, we identified potent novel bisubstrate inhibitors of COMT.

Synthesis: In contrast to earlier approaches,[14–16] in which adenine was introduced in the first step, the nucleobase substitutes in the new bisubstrate inhibitors 2 were introduced at a later stage of the synthesis (Scheme 1).[12,19] The general protocol involved amide coupling of allylic amine 3 with known activated catechol ester 4.[12] Phthalimide 5[19] was used in the key step to introduce various heterocycles by using the Vorbrüggen nucleosidation reaction,[27–39] providing 3 after phthalimide cleavage.

The Vorbrüggen nucleosidation involves reaction of an in situ activated saccharide, an appropriate Lewis acid, a silylating agent, and a heterocyclic nucleophile in nonpolar solvent. Yields are strongly dependent on the basicity, polarity, and structure of the heterocycle. The adjustment of stoichiometry and solvent polarity is crucial for success. The mechanism of the transformation is not well understood and

Figure 1. Display of the adenosine binding site in the ternary complex of 1 with COMT and Mg$^{2+}$ as seen in the X-ray co-crystal structure (resolution: 2.63 Å; PDB code: 1JR4).[14] The most prominent hydrogen-bonding and van der Waals contacts are highlighted with dashed lines. The given $K_i$ value is calculated from the experimental IC$_{50}$ value using the Cheng-Prusoff equation (Supporting Information).[13] Distances in all Figures are given in Å. Color code in all Figures: gray C COMT, green C ligand, red O, blue N, yellow S; red spheres: H$_2$O.

Scheme 1. Schematic representation of the retrosynthetic approach for bisubstrate inhibitors with various adenine substitutes (2). R = adenine substitute, Su = N-succinimidyl, Ac = acetyl.
seems to vary in a substrate-dependent fashion. Due to the high similarity of the regioisomeric nucleosides formed, purification by chromatography was challenging. The isolated pure products were characterized by 2D NMR spectroscopy or NOE measurements to assign their correct constitution (see the Supporting Information). To optimize the conditions of the Vörbrüggen nucleosidation, test reactions were conducted to obtain a series of new adenosine derivatives (see the Supporting Information).

Compounds 6–12 could be prepared by using the Vörbrüggen method by adaptation of known procedures, whereas nucleosides such as 13–17 were obtained by this protocol for the first time (Scheme 2). The heterocycles 5-azaindole and methyl 1H-pyrole-3-carboxylate were prepared as described in the literature (see the Supporting Information), while the other heterocyclic starting materials were commercially available. Yields in the nucleosidation reaction were highly dependent on the heterocyclic nucleophile used, as detailed in Scheme 2. Thus, benzimidazole derivative 6 was obtained in only 5% yield, whereas purine 9 was isolated in much higher yield (51%). 4-Pyridone afforded the N-substituted regiosomer, whereas 4-pyridinethiol yielded the S-substituted product. The different regioselectivity of the two nucleosidation reactions could originate from the formation of different reactive complexes, after activation of the heterocycles by silylation with TMSOTf. The bond dissociation energy decreases from Si/CO (ca. 128 kcal mol⁻¹), over Si-CN (ca. 100 kcal mol⁻¹), to Si-CS (ca. 99 kcal mol⁻¹), suggesting a clear preference for O- over N-silylation of 4-pyridone.

The nucleosidation of 5-azabenzimidazole proceeded only in a moderate yield to give 16 with the N(5)-substituted nonbenzenoid aromatic 10 π-electron heterobicycle. A similar nonbenzenoid aromatic system in 17 was formed in high yield starting from 5-azaindole. Despite several attempts, the desired N(1) regiosomers, featuring benzenoid heterocycles (see the Supporting Information), could not be obtained in either case.

The transformation of 6-chloropurine and 6-methylpurine gave nucleosides 10 and 12, respectively, in very high yield. Except for one reported Vörbrüggen nucleosidation, 6-methylpurine nucleosides have previously been prepared by cross-coupling of 6-chloropurine nucleosides with organometallics. A convenient way to synthesize 6-methylpurine nucleosides directly from 6-methylpurine is thus established. The reaction proceeded so smoothly that no purification was necessary after workup.

In the next step, the phthalimide groups were removed with MeNH₂ and the resulting allylic amines subjected to amide coupling with activated catechol ester 4 (Scheme 3). By this method, six novel bisubstrate inhibitors 18 to 23 were obtained.

To facilitate purification by reverse-phase HPLC following the amide coupling, in most ligand preparations the catechol subunit was protected with a 4,4-dimethoxybenzophenone protecting group. Protocols for the two protected catechol building blocks 24a and 24b (Scheme 4) had already been developed in previous work. First, the phthalimides 10–13, 16, and 17 were transformed into the allylic amines using MeNH₂. The crude products were purified by reverse-phase HPLC. Subsequently, the catechol carboxylic acids were activated with HATU or HBTU (for abbreviations, see Scheme captions) and reacted with the pri-
mary allylic amine to obtain the protected inhibitors 25–31. Several additional transformations occurred as expected during phthalimide deprotection with MeNH₂. The chloropurine nucleoside 10 was transformed into the N(6)-methyladenine derivative by nucleophilic substitution of the chlorine atom. The ester groups of nucleosides 11 and 13 were converted into N-alkylated amides, ultimately yielding protected inhibitors 26, 28, and 29.

Removal of the para-methoxybenzophenone protecting group was performed under acidic conditions (Scheme 5), and the novel bisubstrate inhibitors 32–38 were isolated in moderate to good yields.

### Biological activity: IC₅₀ values were obtained by a radioligand assay (IC₅₀ = 31600 nM) lacking a heterocycle entirely; it is as active as some of the heterocycle-bearing inhibitors, such as 37 (IC₅₀ = 6800 nM), and 19 (IC₅₀ = 33600 nM), which have more interactions with the protein inside the adenine pocket.

All inhibitors lacking a nitrogen atom at the N(1) position of adenine show a dramatically decreased activity. Attempts to replace the nitrogen with an oxygen atom as hydrogen bond acceptor were not successful. Thus, pyridine 19 (IC₅₀ = 336000 nM) binds to COMT with an IC₅₀ value in the upper micromolar range, despite featuring a vinylogous amide-type hydrogen bond accepting O-atom. Similarly, ligands 35 (IC₅₀ = 89500 nM), 36 (IC₅₀ = 70300 nM), and 37 (IC₅₀ = 46800 nM) with suitably positioned oxygen acceptors (C=O) are also weak inhibitors. These findings are in agreement with results obtained from modeling as binding of these ligands requires a cis-amide conformation which is energetically disfavored by ΔΔG ≈ 1.0 to 3.6 kcal mol⁻¹ compared to the trans-amide. Inhibitor 21 (IC₅₀ = 20900 nM) bearing a trifluoromethyl group to interact by C–F–H–N contacts with the backbone NH of Ser119 also showed a relatively weak inhibition towards COMT.

N(6)-Methyladenine inhibitor 34 (IC₅₀ = 32 nM) displayed low nanomolar activity similar to adenine derivative 1 (IC₅₀ = 9 nM). The N(6)-methyl group replaces the water molecule that is bound to adenine in the cocrystal structure of 1 (PDB code: 1JR4). 6-Methylpurine inhibitor 38 (IC₅₀ = 6 nM) is the most potent ligand, displaying an approximate fivefold higher binding affinity than reference compound 39 (IC₅₀ = 31 nM), thus demonstrating that the exocyclic NH₂ of adenine is not necessary for strong binding. Of course, ligand 38 (clogP = 1.55) also benefits from an altered partitioning compared to 39 (clogP = 1.12). A comparison to purine inhibitor 23 (IC₅₀ = 155 nM, clogP = -0.68) reveals an increase in biological activity of a factor of approximately 26 just by addition of the methyl group.

### Cocrystal structures: A total of six new X-ray crystal structures of bisubstrate inhibitors with adenine substituents in complex with soluble rat-COMT and Mg²⁺ were determined (see the Supporting Information). The ligand conformation in the catechol pocket, the ribose pocket, and in the protein region occupied by the linker, in most cases (except for 22)
closely resembles the corresponding conformation observed in the previous cocrystal structures with adenine or N(6)-alkylated adenine derivatives. [14,19] Hence, the following discussion will mainly focus on the binding characteristics of the adenine replacements. The Supporting Information contains Figures (SI to 10SI) depicting the entire ligands, including the catechol moiety, bound to the active site of COMT. Some of the cocrystal structures displayed a domain swap (see Figure 11SI).

In the ternary complex of tightly bound ligand 38 (Figure 2a, resolution: 1.65 Å, PDB-code: 3NW9), the 6-methylpurine ring adopts an orientation similar to that of the adenine ring in 1 (Figure 1). Nevertheless, some notable differences are observed in the protein environment. The most important change is the rearrangement of the crystallographically observed water molecules. For steric reasons, the 6-methyl group of 38 no longer allows the import of the ligand-bound water molecule that solvates the adenine

### Scheme 4. Synthesis of the protected bisubstrate inhibitors 25 to 31.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>R¹</th>
<th>X</th>
<th>Amine</th>
<th>R²</th>
<th>Yield [%] (2 steps)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Cl</td>
<td>O2N</td>
<td>MeNH₂</td>
<td>MeNH₂</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>11</td>
<td>MeO</td>
<td></td>
<td>MeNH₂</td>
<td>MeNH₂</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>12[α]</td>
<td>Me</td>
<td>F</td>
<td>MeNH₂</td>
<td>= R¹</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>13</td>
<td>MeO</td>
<td></td>
<td>MeNH₂</td>
<td></td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>E1NH₂</td>
<td></td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>16</td>
<td>O2N</td>
<td></td>
<td>MeNH₂</td>
<td>= R¹</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>MeNH₂</td>
<td>= R¹</td>
<td>35</td>
<td>31</td>
</tr>
</tbody>
</table>

DIEA = N,N-Diisopropylethylamine; HATU = 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DMF = N,N-dimethylformamide. a) Coupled with HBTU ((1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate).
moeity in 1. At the exit of the pocket, one H₂O molecule forms a weak hydrogen bond to the NH₂ group of the amide side chain of Gln120, which is slightly rotated relative to its location in the cocrystal structure of 1. A second H₂O molecule is tetrahedrally coordinated with four hydrogen bonds to the C═O of Gln120, the side chain of Asp150, and to two N–H donors of the side chain of Arg146. This residue adopts two alternative conformations in this structure, both of which are in electrostatic contact with Asp150. The indole ring of Trp143 is rotated by 180°, firmly bound to the reoriented side chain and the backbone NH of Gln120, as well as to the side chain of Ser119. In this structure, the indole ring of Trp143 adopts two equally populated orientations.

Removal of the 6-substituent to arrive at the purine-based ligand 23 again changes the solvation pattern at the opening of the adenine pocket towards the surface, as seen in the cocrystal structure (Figure 2b, resolution: 1.49 Å, PDB code: 3OZT). One ordered H₂O molecule is observed, again changes the solvation pattern at the opening of the adenine pocket towards the surface, as seen in the cocrystal structure (Figure 2b, resolution: 1.49 Å, PDB code: 3OZT). One ordered H₂O molecule is observed, firmly bound to the reoriented side chain and the backbone NH of Gln120, as well as to the side chain of Ser119. In this structure, the indole ring of Trp143 adopts two equally populated orientations.

The X-ray cocrystal structure of the ternary complex of 4-thiopyridine ligand 20 (Figure 2c, resolution: 1.52 Å, PDB code: 3OE5) confirms the predicted location of the heterocyclic ring in the “adenine plane”. Again, the pyridine-N atom forms an essential hydrogen bond (d(N═N) = 2.9 Å) to the backbone NH of Ser119. The thioether moiety of the ligand displays very short sulfur–π interactions (d(S–C(7α)) = 3.3 Å) with the indole ring of Trp143. A chain of three ordered H₂O molecules connected through hydrogen bonds is seen at the opening of the pocket. The water bound to the side chain and backbone NH of Gln120 and to the side chain of Ser119 adopts a similar position as in the complex of purine 23 (Figure 2b).

The cocrystal structure of pyridone derivative 19 with COMT (Figure 2d, resolution 1.48 Å, PDB code: 3OZT) could be obtained despite a rather high IC₅₀ value of 336,000 nM. The pyridone moiety is positioned approximately in the “adenine plane” and its C═O moiety engages in the predicted hydrogen bond to the backbone NH Ser119 (d(N═O) = 2.9 Å) and an additional one to a H₂O molecule bound to the side chain of Gln120 (d(O═O) = 2.6 Å). The pyridone ring is involved in edge-to-face interactions with Trp143 (d(C–C(3) = 3.4 Å) and His142 (d(C–C) = 4.0 Å). The directional interactions cannot explain the low affinity of this ligand as compared to thiopyridine derivative 20: a pyridine-O atom is at least as potent a H-bond acceptor as a 4-thiopyridine-N atom.[55] However, the adenine pocket does not seem to be well filled by the pyridone ring directly attached to the ribose and partitioning of the highly hydrophilic molecule (clogP value of −1.47) into the protein is also not favorable.

The cocrystal structure of the trifluoromethyl imidazole inhibitor 21 bound to COMT (resolution 1.44 Å, PDB code: 3OZS) is shown in Figure 2e. Its binding mode is quite intriguing given the fact that 21 does not offer a strong H-bond acceptor to interact with the NH group of Ser119. Nevertheless, the imidazole ring is oriented into the “adenine plane” to undergo interactions with the surrounding Met91, His142, and Trp143 side chains. The CF₃ moiety nicely fills the space in the active site where it harvests several favorable electrostatic (β–d+) contacts.[52,53] A linear dipolar (H-bond-type) interaction with the backbone NH of Ser119
As observed for the other ligands, His142 entertains edge-to-face interactions to the imidazole moiety of 21 (d(N=N) = 4.0 Å, omitted for clarity). The backbone NH of Ser19 is displaced by 1.3 Å in comparison to the structure of 23 to recover the missing hydrogen bond to the ligand by hydrogen-bonding to a H2O molecule. Additionally, steric repulsion between Ser19 and the CF3 moiety might be responsible for the displacement of the backbone. The water

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>9</td>
<td>2</td>
<td>−0.62</td>
<td>39</td>
<td></td>
<td>31</td>
<td>7</td>
<td>1.12</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>62800</td>
<td>14000</td>
<td>1.37</td>
<td>35</td>
<td></td>
<td>89500</td>
<td>19900</td>
<td>1.33</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>33600</td>
<td>74900</td>
<td>−1.47</td>
<td>36</td>
<td></td>
<td>70300</td>
<td>15600</td>
<td>0.77</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>596</td>
<td>132</td>
<td>0.76</td>
<td>37</td>
<td></td>
<td>46800</td>
<td>10400</td>
<td>1.85</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>20900</td>
<td>4645</td>
<td>0.75</td>
<td>38</td>
<td></td>
<td>6</td>
<td>1</td>
<td>1.55</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>31600</td>
<td>7022</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>155</td>
<td>34</td>
<td>−0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>10800</td>
<td>2400</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>10700</td>
<td>2378</td>
<td>1.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>32</td>
<td>7</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
molecule is part of a well-ordered cluster of 3 water molecules near the surface of the enzyme. The structure features a ligand-bound water molecule bound exclusively by the N(3) of the imidazole moiety of 21. Based on our previous studies,[19] we predict that the replacement of this water by a ligand substituent will lead to substantially increased binding affinity.

In summary, the solvation at the surface-exposed opening of the adenine pocket varies considerably between the complexes. It appears that the plasticity of the water hydrogen-bonding networks allows COMT to adopt to the stereo-chemical requirements of the bound ligand with all remaining hydrogen-bonding valences filled in by the solvent. A comparison of the crystal structures suggests three favored locations for water molecules (see Figure 12SI) depending on the ligand and especially the presence of a substituent on the purine moiety.

Figure 2f depicts the adenosine-binding site of the cocrystal structure of inhibitor 22, lacking the nucleobase, with COMT (resolution 1.73 Å, PDB code: 3OZR). While the catechol-binding site is occupied as expected from previous crystal structures, the anomeric center of the ribose is not directed towards the adenine-binding site but is rotated towards the surface of the protein. Despite elevated B-values of the ribose, indicating increased mobility, the ribose could be faithfully placed into the electron density. Both hydrogen bonds towards Glu90 are retained (2'-OH: (d(O•••O)) = 2.4 Å; 3'-OH: (d(O•••O)) = 2.5 Å). Importantly, the cocrystal structure reveals that the adenine-binding site is structurally conserved contain-
ing two water molecules, one saturating the backbone amide of Ser119.

It becomes clear from this structural information that the strong fixation of the ribose moiety by the ionic hydrogen bonds to the side chain of Glu90 provides a framework for the alignment of the various heterocyclic adenine substitutes, which differ strongly from adenine itself.

Conclusions

We have previously described a bisubstrate inhibitor approach for COMT where the adenosine moiety of the cofactor SAM and the substrate catechol were connected by a linker. Based on this concept, we present the synthesis of novel bisubstrate inhibitors for COMT bearing various replacements for adenine. While the majority of the novel ligands displayed IC₅₀ values in the micromolar range in a radiochemical assay, several distinct examples with IC₅₀ values in the nanomolar range were obtained. The Vorbrüggen nucleosidation reaction represented the key transformation in the multistep synthesis of all ligands.

Modeling and cocrystal structures identified the key interactions of the ligands in the adenine pocket, which can be occupied by a great diversity of heterocycles. The mapping of the molecular recognition properties of the adenine pocket of COMT, based on the biological assay and X-ray cocrystal structures, shows that proper occupancy of this site is crucial for high-affinity bisubstrate inhibitors. Due to the essential hydrogen bond of adenine N(1) to the backbone NH of Ser119, all efficient adenine substitutes need to retain a hydrogen bond acceptor at this position. By contrast, the adenine nitrogen heteroatoms N(3) and N(7) can be replaced without a large energetic penalty. Removal of the exocyclic amino group of adenine results in a moderate 17-fold loss of potency. Surprisingly, replacement of this moiety by a methyl group increased the binding strength compared to adenine-based inhibitors. Thus, 6-methylpurine derivative 38 (IC₅₀ value = 6 nM) is a better inhibitor than the adenine analogue 2 (IC₅₀ value = 31 nM).

The high-resolution cocrystal structures allowed faithful assignment of the solvation patterns at the surface-exposed opening of the adenine pocket. Solvation strongly varies with the type of bound ligand and assists in the accommodation of the various ligands. The cocrystal structure of abasic inhibitor 22 provided a possible intermediate of the binding pathway of bisubstrate inhibitors to COMT. Now that the key structural requirements for high-affinity binding into the adenine pocket of COMT have been established, we aim to extend this knowledge to ligand design in other enzyme classes that contain an adenine binding site, such as kinases, oxidoreductases, or other methyl transferases.

Experimental Section

General details and procedures: see the Supporting Information. Here, the experimental details for the syntheses of inhibitors 19, 20, 21, 23, 34, and 38 is described starting with the Vorbrüggen nucleosidation, as well as the final step and purification of all the other target molecules. All other synthetic details and experimental data are given in the Supporting Information, as well as biological and X-ray crystallographic work.

1-[5E]-2,3-Di-O-acetyl-5,6,7-trideoxy-7-phthalimido-γ-D-ribo-hept-5-enofuranosyl|pyridin-4(1H)-one (7): A dry N₂-flushed flask (25 mL), equipped with a magnetic stirrer and a septum, was charged with 4-hydroxypyridine (133 mg, 1.39 mmol), MeCN (3 mL), and N-O-bis(trimethylsilyl)acetamide (0.45 mL, 1.85 mmol). The mixture was heated to 60°C. After 30 min, the mixture was cooled to 20°C and then S (400 mg, 0.93 mmol) and trimethylsilyl trifluoromethanesulfonate (0.25 mL, 1.39 mmol) were added slowly. The mixture was heated to 60°C, stirred for 2 h, and then stirred at 20°C for 2 h. Afterwards, a saturated aqueous solution of NaHCO₃ (200 mL) and EtOAc (150 mL) were added and the obtained phases separated. The aqueous phase was extracted with EtOAc (2 x 150 mL), the combined organic phases were dried (MgSO₄),
filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO$_2$; CH$_2$Cl$_2$/MeOH 98:2).

1046 m, 944 m, 892 m, 828 w, 795 w, 720 s, 644 w cm$^{-1}$.

1599 m, 1499 w, 1466 m, 1476 w, 1394 m, 1240 s, 1185 m, 1168 w, 1125 s, 1094 w, 948 w, 804 w, 756 m, 722 m, 629 m cm$^{-1}$; HR-MALDI-MS (3-HPA): m/z (%): 483,1228 (100, calecd for C$_{26}$H$_{24}$N$_5$O$_7$Si; [M+H]$^+$; 483,1221).

5-(E)-2,3-Di-O-acetyl-5,6,7-trideoxy-7-phthalimido-β-D-ribo-hept-5-enofuranosyl (17): A dry N$_2$-flushed Schlenk tube (10 mL) equipped with a magnetic stirrer and a septum, was charged with 5-((E)-2,3-Di-O-acetyl-5,6,7-trideoxy-7-phthalimido-β-D-ribo-hept-5-enofuranosyl) S$_2$pyrrolidin-3,2-acylamine (62.4 mg, 52.89 mmol), N,O-bis(trimethylsilyl)acetamide (0.22 mL, 88.14 mmol), and 1,2-dichloroethane (7.5 mL). The mixture was heated to 60°C. After 30 min, the mixture was cooled to 20°C and then 5 (0.19 g, 4.07 mmol) in dry dichloroethane (7.5 mL), and trimethylsilyl trifluoromethanesulfonate (0.32 mL, 1.76 mmol) were added slowly. The mixture was heated to 60°C, stirred for 20 h, and then cooled to 20°C. Afterwards, H$_2$O (100 mL) and CH$_2$Cl$_2$ (100 mL) were added and the obtained phases separated. The aqueous phase was extracted with CH$_2$Cl$_2$ (2 × 100 mL), the combined organic phases were dried (MgSO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO$_2$; CH$_2$Cl$_2$/MeOH 97:3 (SiO$_2$ hexane/EtOAc 4:1): 1H NMR (400 MHz, CDCl$_3$); 1H NMR (300 MHz, CDCl$_3$): δ = 2.95, 2.74 (s, 9H, 5.6, 2.8 Hz, 2H), 8.42 ppm (d, J = 5.3 Hz, 1H) 1H NMR (1H, CDCl$_3$): δ = 0.97 mmol) and trimethylsilyl trifluoromethanesulfonate (0.97 mmol) and then 5 (420 mg, 99% mmol). The mixture was heated to 60°C. After 30 min, the mixture was cooled to 20°C and then 5 (0.19 g, 4.07 mmol) in dry dichloroethane (7.5 mL), and trimethylsilyl trifluoromethanesulfonate (0.32 mL, 1.76 mmol) were added slowly. The mixture was heated to 60°C, stirred for 20 h, and then cooled to 20°C. Afterwards, H$_2$O (100 mL) and CH$_2$Cl$_2$ (100 mL) were added and the obtained phases separated. The aqueous phase was extracted with CH$_2$Cl$_2$ (2 × 100 mL), the combined organic phases were dried (MgSO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO$_2$; CH$_2$Cl$_2$/MeOH 97:3 (SiO$_2$/hexane/EtOAc 4:1)) to give 10 as a yellow oil (342 mg, 84%).

[3259 br, 2973 w, 1670 s, 1548 w, 1474 w, 1448 w, 1327 w, 1284 s, 1197 s, 1106 s, 1049 w, 948 w, 804 w, 756 w, 722 m, 629 m cm$^{-1}$; HR-MALDI-MS (3-HPA): m/z (%): 483,1228 (100, calecd for C$_{26}$H$_{24}$N$_5$O$_7$Si; [M+H]$^+$; 483,1221).
m, 1044 s, 967 m, 878 m, 853 w, 798 w, 743 m, 720 w, 660 w, 630 w cm⁻¹; 1H NMR (300 MHz, CD3OD): δ = 3.99 (t, J = 7.2 Hz, 2H), 4.25 (t, J = 5.1 Hz, 1H), 4.47 (t, J = 4.9 Hz, 1H), 1.15 (t, J = 4.2 Hz, 3H), 7.27 (m, 1H), 8.54 (s, 2H), 13.43 (m, 2H), 16.79, 16.80 ppm; IR (neat): 3295 br, 3091 w, 1642 m, 1598 s, 1548 m, 1483 m, 1401 w, 1328 m, 1283 m, 1218 s, 1202 s, 1147 s, 1137 s, 1075 s, 591 s cm⁻¹).

Replacements for Adenine

(2F)-4,7-Anthryl-1,2,3-trideoxy-1-(2,3-dihydroxy-5-nitrobenzamido)-ribo-hept-2-enitol (22): 1H NMR (400 MHz, CDCl3): δ = 2.69 (s, 3H), 3.68 (s, 3H), 3.69 (s, 3H), 4.07–3.97 (m, 5H), 4.61–4.59 (m, 1H), 6.82 (d, J = 8.8 Hz, 2H) ppm; IR (neat): 3295 br, 3091 w, 1642 m, 1598 s, 1548 m, 1483 m, 1401 w, 1328 m, 1283 m, 1218 s, 1202 s, 1147 s, 1137 s, 1075 s, 591 s cm⁻¹.

FULL PAPER

J. Nucleoside 29 (2 mL, 50% in H2O) at 0°C. The mixture was stirred at 0°C for 40 min. Afterwards, the solvents were removed at 20°C in vacuo. The residue was dissolved in H2O/MeCN, filtered, and purified three times by reverse-phase HPLC (H2O/MeCN/HCOOH 90:10:0.01 as a yellow solid (40 mg, 69%).

Acknowledgements

We thank F. Hoffmann-La Roche (Basel) and Chugai Pharmaceuticals for the generous support of this work. We are grateful to Pia Warga and Valerie Goetschy-Meyer (both Roche) for their advice concerning the assay and Jorg Benz (Roche) for advice regarding crystallization. Bruno B. Bernet (ETH) helped with nomenclature.
