Inhibitors

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Molecular Recognition at the Active Site of Catechol-*O*-Methyltransferase: Energetically Favorable Replacement of a Water Molecule Imported by a Bisubstrate Inhibitor**

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Biologically active catechols, such as L-DOPA and the neurotransmitter dopamine, are inactivated by methylation. This reaction is catalyzed by the enzyme catechol-*O*-methyltransferase (COMT) in the presence of *S*-adenosylmethionine (SAM) and Mg^{2+} ions.[1] Small nitrocatechol-based inhibitors of COMT find application in the treatment of Parkinson disease by blocking unwanted methylation of the administered L-DOPA, thereby enhancing dopamine levels in the brain.[2,3] Recent studies have pointed towards additional therapeutic applications of COMT inhibition in other disorders of the central nervous system, such as schizophrenia[4] and depression.[5]

We have developed a series of potent bisubstrate inhibitors for COMT which are competitive for both the catechol and the SAM binding sites.[6] Based on the X-ray crystal structure of ligand **1** ($IC_{50} = 9$ nM)[7a] in a ternary complex with COMT and a Mg^{2+} ion (PDB code: 1JR4),[8] we started a detailed exploration of the molecular recognition properties of the entire active site of the enzyme.[9] Importantly, we found that potentially hepatotoxic nitro groups, which are mandatory in catechol-based monosubstrate inhibitors, are not required for high-affinity bisubstrate inhibition.[10] We substituted the nitro group in position 5 of **1** with appropriate lipophilic residues, such as the 4-fluorophenyl ring in **2** ($IC_{50} =$ 31 nM),[7] and found that the high, competitive inhibitory potency was maintained. Computer modeling studies suggested that the newly introduced lipophilic residue occupies a hydrophobic cleft near the surface of the enzyme.[11] This initial proposal is validated here experimentally by X-ray crystallography.

The crystal structure of **1** in a ternary complex with COMT and a Mg^{2+} ion shows that the adenine moiety forms two hydrogen bonds, a moderately strong and a weak one (d(N···O): 3.0 and 3.4 Å, respectively), to a water molecule

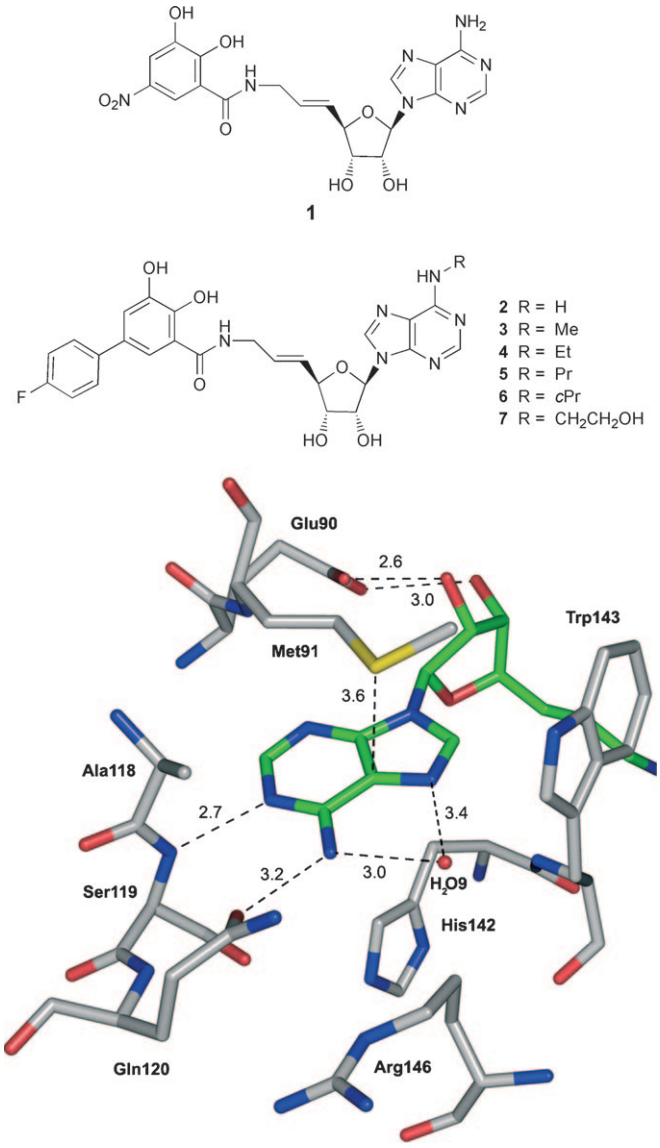

Figure 1. Top: Bisubstrate inhibitors of COMT. *c*Pr = cyclopropyl. Bottom: Structure of the adenosine binding site of the ternary complex of **1** with COMT and a Mg^{2+} ion as seen in the X-ray crystal structure.[8] Distances are given in Å (gray C_{COMT}, green C_{ligand}, red O, blue N, yellow S; PDB code: 1JR4).

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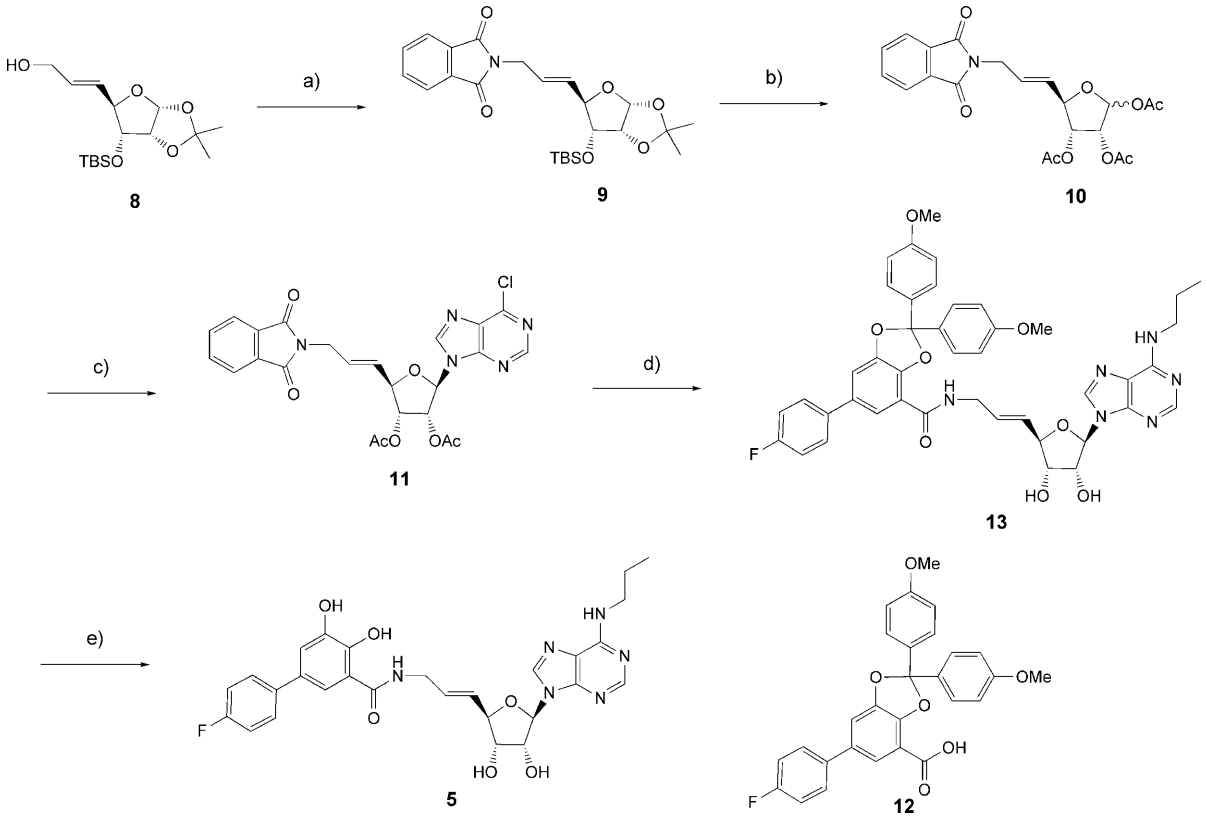
(H_2O9, Figure 1). This water molecule is apparently imported together with the ligand, as it does not undergo any hydrogen bonding to the protein, but very likely does only to dynamic bulk water.[8a] We reasoned that a replacement of this water molecule by a hydrophobic residue of the ligand would result in a favorable entropic and enthalpic gain, by freeing the solvent molecule and establishing better interactions to the protein.[12] The replacement of ordered water, as seen in cocrystal structures, to enhance protein–ligand interactions is currently a topic of intense interest in structure-based drug design, both from experimental and theoretical viewpoints.[13] The replacement of ordered, ligand-imported water molecules, which do not interact with the protein[14] has, however, not been previously addressed, despite such water being quite abundant, as revealed by a preliminary search of the Protein Data Bank (PDB; see the Supporting Information). Here, we present the novel bisubstrate inhibitors **3**–**7** and show that replacement of the ligand-imported water molecule seen in the cocrystal structure of **1** (Figure 1) is indeed an energetically favorable process.

The synthesis of the new bisubstrate inhibitors started from alcohol **8** (as illustrated in Scheme 1 for propyl derivative **5**), which was transformed via **9**[15] into the triacetylated ribose derivative **10**.[16] Nucleosidation with 6-chloropurine by a modified Vorbrüggen protocol gave nucleoside **11** in excellent yield.[17] Treatment with propylamine removed the phthalimide and acetyl protecting groups, while at the same time substituting the chlorine atom of the purine. The resulting allylic amine was coupled with the protected catechol derivative **12** to yield **13**, which was deprotected to afford the targeted bisubstrate inhibitor **5**. The other inhibitors were accessible by the same route.

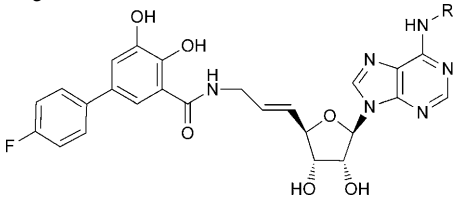
The biological activities of the novel bisubstrate inhibitors were evaluated in a radiochemical assay (see the Supporting Information);[6,18] the measured IC_{50} and calculated K_i values are given in Table 1. The novel bisubstrate inhibitors which have a substituent at the exocyclic N6 atom of the adenine moiety display a similar potency to that of the previously reported reference compound **2**.[7] A Lineweaver–Burk plot analysis[6] (see the Supporting Information) showed that all the inhibitors showed a competitive inhibition mechanism with respect to the SAM binding site.

The X-ray crystal structures of the four bisubstrate inhibitors **3**, **4**, **5**, and **7** in a ternary complex with COMT and a Mg^{2+} ion (PDB codes: 3HVH (resolution: 1.30 Å), 3HVI (1.20 Å), 3HVJ (1.79 Å), and 3HVK (1.30 Å)) were solved. The inhibitor binding mode is very similar in all four structures, as illustrated by an overlay (see the Supporting Information). Figure 2 depicts the X-ray crystal structure of ethyladenine derivative **4** in a complex with COMT and a Mg^{2+} ion.



Scheme 1. Synthesis of bisubstrate inhibitor **5**: a) phthalimide, DIAD, PPh_3, THF, 20 °C, 20 h, 95%; b) 1. AcOH (80%), 70 °C, 15 h; 2. Ac_2O, pyridine, 4-(*N*,*N*-dimethylamino)pyridine, 20 °C, 16 h, 88%; c) BSA, $Me_3SiOSO_2CF_3$, 6-chloropurine, toluene, 60 °C, 16 h, 84%; d) 1. propylamine, MeOH, 20 °C, 6 days; 2. **12**, HBTU, HOBt, iPr_2NEt, DMF, 20 °C, 25%; e) trifluoroacetic acid (50%), THF, 0 °C, 1 h, 76%. TBS = *tert*-butyldimethylsilyl, DIAD = diisopropyl azodicarboxylate, BSA = *N*,*O*-bis(trimethylsilyl)acetamide, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1*H*-benzo[*d*][1,2,3]triazol-1-ol.

Table 1: Biological results obtained from a radiochemical assay.[a]



Inhibitor	IC_{50}[a] [nM]	K_i[b] [nM]	*c*lg *P*[c]	lg *D*[d]
2 (R = H)[e]	31	7	1.12	1.64
3 (R = Me)	12	3	1.96	2.14
4 (R = Et)	21	5	2.48	2.00
5 (R = Pr)	9	2	3.01	3.15
6 (R = *c*Pr)	41	9	2.31	2.57
7 (R = CH_2CH_2OH)	15	3	1.19	1.58

[a] IC_{50} = median inhibitory concentration. [b] K_i = inhibitory constant for competitive inhibition of SAM. IC_{50} and K_i values were obtained with preincubation as described in the Supporting Information. [c] Logarithmic partition coefficient for octanol/water. [d] Logarithmic distribution coefficient for octanol/water at pH 7.4. [e] Ref. [7]

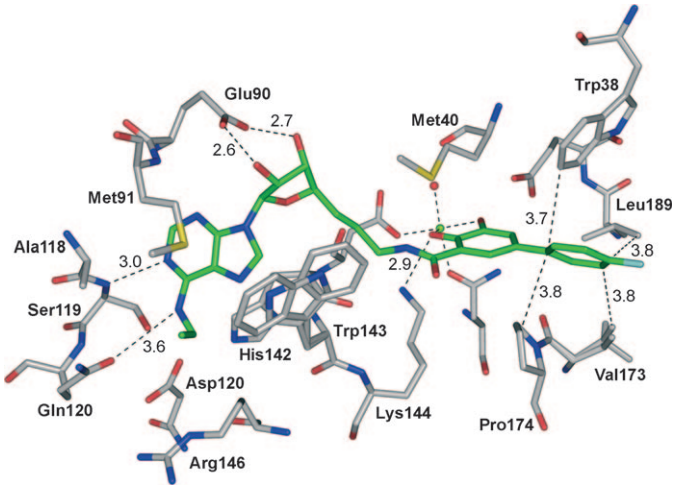


Figure 2. Binding mode of bisubstrate inhibitor **4** with a Mg^{2+} ion in the active site of COMT. The indole ring of Trp143 adopts two different orientations (red O, blue N, yellow S, turquoise F, light green Mg, green C_{ligand}, gray $C_{protein}$; PDB code: 3HVI). The numbering of the residues and atoms correspond to those in the X-ray crystal structure.

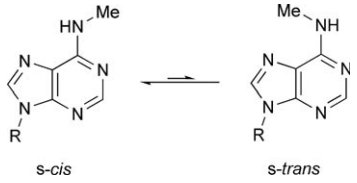
As expected, the adenosine moiety occupies the SAM binding site, while the catechol moiety coordinates to the Mg^{2+} ion. The 4-fluorophenyl substituent on the catechol ring occupies the hydrophobic cleft near the surface of the protein, as predicted by modeling studies,[10] and undergoes several van der Waals interactions (C···C contacts below 4.0 Å with Pro174, Val173, Leu189, and Trp38) with the protein (Figure 2 and the Supporting Information). These apolar contacts, together with the binding-induced entropically favorable desolvation of the cleft, contribute strongly to the high affinity of the bisubstrate inhibitors that lack a nitro group on the catechol ring.

The adenine ring of **4** forms a hydrogen bond (d(N1···N) = 3.0 Å) to the backbone N*H* atom of Ser119 and a second, weak hydrogen bond (d(N6···O) = 3.6 Å) to the side chain of Gln120. The purine ring stacks with the side chain of Met91 (d(S···C5) = 3.6 Å) and interacts in an edge-to-face arrangement with the imidazole ring of His142 ($d(CD2_{His}\cdots C6)$ = 3.8 Å). The indole ring of Trp143, which shields the adenine binding site from bulk solvent, adopts two different conformations in the complexes formed by **3**–**5**, while only one orientation is seen in the complex of **7** (see the Supporting Information). Trp143 also displays an edge-to-face interaction with the nucleobase ($d(CD2_{Trp}\cdots C8)$ = 3.4 Å in the complex of **4**).

The cocrystal structure with bound **4** clearly shows that the water molecule (H_2O9), which binds to the adenine moiety in the cocrystal structure of **1** (PDB code: 1JR4, picture 1), has been replaced by the ethyl substituent. Importantly, this replacement of the water molecule is observed in all four cocrystal structures with ligands **3**, **4**, **5**, and **7** (see the Supporting Information).

A comparison of the thermodynamic quantities (Table 1) at first sight suggests that the incorporation of the N6-alkyl substituents and the concomitant increase in the lg *P* and lg *D* values as well as the water displacement do not result in a significant gain in the binding affinity: the IC_{50} and K_i values for N6-alkylated **3**–**5** and **7** are only slightly lower than those measured for the unsubstituted ligand **2** (Table 1).

However, the new ligands bind in the energetically unfavorable s-*trans* conformation (Scheme 2). Previous kinetic analyses of N6-methylated adenine systems revealed



Scheme 2. Conformational s-*cis*–s-*trans* equilibrium of N-alkylated adenines.

activation free enthalpies ($\Delta G^{\neq}$) for the rotational barrier for the s-*cis*–s-*trans* equilibration to be around 13 kcal mol^{-1}.[19–21] This energy barrier allows observation of the conformational isomer ratio by ^{1}H NMR spectroscopy at low temperature and slow exchange rates, with the Me resonance in the s-*cis* conformer appearing around δ = 3.0 ppm and the resonance in the s-*trans* conformer around δ = 3.5 ppm. In solvents of different polarity, a strong preference for the s-*cis* isomer was obtained, which was quantified as a $\Delta G_{s\text{-}trans \rightarrow s\text{-}cis}$ value of −1.5 to −1.9 kcal mol^{-1}. We recorded the spectra of **3** in perdeuterated 2,2,2-trifluoroethanol between 238 K and 310 K and did not observe any signals for the s-*trans* isomer (see the Supporting Information). A similar result was obtained for measurements in $D_2O/(CD_3)_2SO$ between 277 K and 298 K. The accuracy of the NMR integration is about 5%, and thus a minimum isomeric ratio of 95:5 (s-*cis*/s-*trans*) can be expected. This results in a minimum $\Delta G_{s\text{-}trans \rightarrow s\text{-}cis}$ value of −1.8 kcal mol^{-1} ($\Delta G = -RT\ln K$; K = 95/5) at 310 K (the temperature at which the assay was conducted), which is in good agreement with the previous studies.[19–21] Thus, around

1.8 kcal mol^{-1} of binding free enthalpy has to be invested in forcing the N-alkylated ligand into the s-*trans* conformer in the ternary complex with COMT.

Nevertheless, non-alkylated **2** and N-alkylated **3**–**7** all form complexes of similar stabilities. The energy costs of the unfavorable conformation must be compensated by the energetic gains resulting from the replacement of water by the N6-alkyl substituent in the bound s-*trans* form, which therefore provides a gain in binding free enthalpy of at least $\Delta\Delta G = -1.8$ kcal mol^{-1}. Since the alkyl substituents point into a highly polar region near the surface, there are few additional van der Waals contacts with the protein (see the Supporting Information). Partitioning into this highly polar environment is energetically not very favorable: although the lg D value strongly increases upon changing from **3** (Me) to **5** (Pr) (Table 1), no additional binding free enthalpy is gained. Furthermore, hydroxyethyl derivative **7**, with a low lg D value similar to that of **2**, has a comparable binding affinity. Therefore, we propose that the largest portion of the gained ca. -1.8 kcal mol^{-1} originates from the displacement of the water molecule.[22] This displacement occurs as a result of the change in the N-alkyl substituent from the s-*cis* conformation in the free ligand, which presumably maintains adenine solvation as shown in Figure 1,[23] into the s-*trans* form in COMT.

In summary, we have described highly potent bisubstrate inhibitors of COMT and validated their predicted complexation mode by four X-ray cocrystal structures. The N6-alkylated inhibitors in the unfavorable s-*trans* conformation bind with similar strength as the corresponding non-alkylated derivative. Our analysis shows that the displacement of the water molecule in the s-*trans* conformation of the bound ligand contributes at least 1.8 kcal mol^{-1} to the binding affinity of the ligand to the protein, thereby compensating the similar energetic costs resulting from the unfavorable s-*trans* ligand conformation. The study clearly suggests that replacement of ligand-imported water molecules using structure-based design is worthwhile, in particular since a preliminary search of the PDB shows that such ligand-imported water molecules, which do not interact with the protein through hydrogen bonds, are frequently observed.

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