Graphical Abstract

DA optimized in the COMT active site using the VR model.

DA with Water Model 4 (13 water molecules) optimized using the aug-cc-pvdz basis set.

***Ab initio* study of electronic interaction energies and desolvation energies for dopaminergic ligands in the catechol-O-methyltransferase active site**

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Abstract

Catechol-O-methyltransferase (COMT) deactivates dopamine and other catecholic substrates via the methylation of a hydroxyl group. The targeted inhibition of COMT can prevent premature metabolism of both endogenous and exogenous ligands; for example, it can prolong the effectiveness of L-DOPA treatments for Parkinson's disease. The inhibitory ability of a suite of dopaminergic derivatives against COMT has been studied using electronic interaction energies between each ligand and the enzymatic active site as well as desolvation energies for each ligand. A crystal structure of the COMT active site in complex with a known COMT inhibitor, BIA 8-176, was isolated from the Protein Data Bank (PDB ID: 2CL5). The positions of a suite of dopaminergic derivatives in this same active site were optimized using M062X/6-31G with three different model chemistries: *in vacuo* with rigid amino acid side chains, with implicit solvation and rigid amino acid side chains, and with implicit solvation and flexible amino acid side chains. Electronic interaction energies between the ligands and the active site residues were calculated for each model using M06L and MP2 with the 6-311+G* basis set. Ligands with a nitrile substituent were favored over other substituent *in vacuo*, but this preference was not retained when the same ligands were optimized with implicit solvation; the preference was regained when using implicit solvation and a relaxed active site. Desolvation energies of the ligands were calculated using a series of hydration shells ($n=7, 9, 11$ and 13) with M062X and the aug-cc-pvdz, cc-pvdz, and cc-pvtz basis sets. Ligands with carboxyl and nitro substituents exhibited the least favorable desolvation energies, whereas ligands with the nitrile substituents exhibited the most favorable desolvation energies.

1. Introduction

Catechol-O-methyltransferase (COMT) is involved in the metabolism of catecholamine molecules, affecting the ability of their metabolites to act as hormonal signals in the body or as neurotransmitters in the brain. Inhibition of COMT can prevent the metabolism of catecholamines, thus prolonging the molecules' signaling ability. In order to identify viable inhibitors of COMT, a computational study of systematically varied dopaminergic derivatives has been performed to determine the relative inhibitory ability of the proposed ligands. The electronic binding energies (ΔE_{EBE}) of the ligands to the active site are assumed to be a sum of three contributions:

$$\Delta E_{EBE} = \Delta E_{int} + \Delta E_{desolv} + \Delta E_{rearrange} , \quad \text{EQ. 1}$$

where ΔE_{int} is the electronic interaction energy between the ligand and the active site residues, ΔE_{desolv} is the desolvation energy of the ligand and the active site, and $\Delta E_{rearrange}$ is the rearrangement energy of the entire molecular system upon ligand binding. Since this study seeks only to identify *relative* EBE's, we can assume that the only significant contributor to desolvation energy will be the desolvation of the ligand (see EQs 3 and 4 below). This assumes that the ligands will displace an equal number of

water molecules upon binding to the active site. Further, the rearrangement energies are also taken to be constant across all ligands. Thus, for the relative EBE ($\Delta\Delta E_{\text{EBE}}$) the following expression is obtained:

$$\Delta\Delta E_{\text{EBE}} = \Delta E_{\text{int}} + \Delta E_{\text{desol,lig}} \quad \text{EQ. 2}$$

The relative inhibitory ability of the ligand is thus assumed to be proportional to the $\Delta\Delta E_{\text{EBE}}$. This study uses two computational methods, M06L¹ and MP2², to analyze ligand electronic interaction energies with the active site and uses the M062X^{3,4} method to calculate the desolvation energies for the ligands.

The metabolic activity of COMT occurs in an active site located between the two macromolecule subunits of the enzyme. Within the active site, a magnesium ion serves as the primary anchor for the bound ligands through coordination. The system is further stabilized by residues Asp141, Asp169, and Asn170, which are coordinated with the magnesium ion, and by Glu199 and Lys144, which interact with the ligand.^{5,6,7} The lysine residue, Lys144, deprotonates a phenolic hydroxyl on the catecholineric ligand, and the deprotonated substituent interacts with an electron-deficient region of the S-adenosyl-L-methionine (SAM) co-factor. This interaction results in the transfer of a methyl group from SAM onto the catecholamine ligand,⁶ altering the ability of the molecules to act as hormonal or neural signals. The crystal structure of catechol-O-methyltransferase was initially confirmed by Rodrigues et al.⁸ and was further studied by Palma et al.⁶

Inhibition of the catechol-O-methyltransferase enzyme prevents the metabolism of catecholamines, and COMT inhibition can be favorably applied in the treatment of conditions characterized by catecholamine deficiencies. Parkinson's disease, for example, results from the degradation of the substantia nigra, causing a loss of dopaminergic signaling in downstream regions such as the basal ganglia. The loss of dopaminergic signaling is traditionally treated by oral administration of an dopamine precursor, 3,4-dihydroxy-L-phenylalanine, or L-DOPA.⁹ Dopamine cannot be directly administered as a Parkinson's treatment because it cannot cross the blood brain barrier (BBB). L-DOPA treatment is preferential due to its BBB permeability, which occurs via the large, neutral amino acid transporter LAT1, part of transporter system L.^{5,10} Orally-administered L-DOPA is not a targeted treatment for neurological disorders but a small portion does cross the BBB to enter the brain, acting to correct for the loss of dopaminergic nigra cells.⁵ L-DOPA is activated by aromatic amino acid decarboxylase, which converts the molecule to dopamine.¹¹ L-DOPA is commonly administered in conjunction with a decarboxylase inhibitor, such as carbidopa or benserazide, which prolongs the half-life of L-DOPA.¹²

COMT interferes with the effectiveness of L-DOPA treatments because L-DOPA can be prematurely metabolized by COMT into 3-O-methyldopa, preventing its conversion to dopamine by aromatic amino acid decarboxylase.¹¹ COMT is also involved, in conjunction with monoamine oxidase and aldehyde dehydrogenase, in the metabolism of dopamine into homovanillic acid.¹³ The effect of COMT in the pharmacological fate of L-DOPA is shown in Figure 1. Because of the direct and indirect role of COMT in L-DOPA activity, inclusion of a COMT inhibitor in the treatment regimen can further prolong the efficacy of the L-DOPA treatment by preventing the premature metabolism of L-DOPA itself and of dopamine derived from L-DOPA.

Dopamine is characterized by two phenolic hydroxyl groups in the 3- and 4-positions on the benzene ring with respect to its aminoethyl substituent (Figure 2). When metabolizing dopamine, COMT has

shown a preference for the methylation of the deprotonated 3-hydroxyl group but no distinct preference has been shown between the deprotonation of the 3- or 4-position of various dopaminergic derivatives.^{6,14} This study examines a suite of strategically varied dopaminergic derivatives (Figure 2), in which different substituents and combinations of substituents were chosen to maximize the stereoelectronic interactions between the ligand and the COMT active site residues.

The positions of the suite of dopaminergic derivatives, bound in the COMT active site were optimized using M062X, a DFT method, both *in vacuo* and with solvation using the Polarizable Continuum Model (PCM) of Tomasi et al.¹⁵ M062X was chosen for the optimizations for its ability to accurately model a wide range of interactions^{3,4}, and it has been shown to perform well in similar ligand/active site models¹⁷. Electronic interaction energies for the optimized conformations of the ligand in the active site were determined with both MP2 and the M06L DFT functional. MP2 was chosen as the most economical post-HF correlation method that can perform well for non-bonded interactions¹⁶ while M06L has been shown to be accurate for calculations involving transition metals.¹ The method was switched from M062X for optimizations to M06L for interaction energies because, in the optimization, there are many different types of intermolecular forces to describe, and M062X was deemed to be the most widely applicable, whereas in the interaction energies, the ligand-Mg²⁺ interaction is the most important, and so it was necessary to capture that interaction as accurately as possible. M06L was then used for the other interactions so that the individual interactions could be summed for a total interaction energy. The MP2 results obtained here agree well with the M06L method even though MP2 has been shown to overestimate non-bonded interactions.^{2,16} Inclusion of the Polarizable Continuum Model in the optimization of the ligand-active site complex provides a better analysis of intermolecular forces involving charge distribution by providing improved charge distribution and dipole moments in the system. Riley et al. and Bigler et al. found that PCM, when used with TPSS and MP2 + CCSD(T) (Riley) and with MP2 and M062X (Bigler), showed good agreement between the methods for O-based and N-based hydrogen bonds.^{17,18}

When considering the ligand-protein complex in solvation, it should be noted that the PCM cannot accurately describe desolvation of the ligand and active site upon formation of the complex; this is due to the fact that specific interactions between the ligand and solvent are not explicitly modelled. Thus, this study includes solvation effects in two ways: using implicit solvation to find the structures of the ligand-protein complexes, and using explicit solvation to calculate the desolvation energies of the ligands. Friesner et al. discusses the importance of considering desolvation effects on the binding free energy of the system.¹⁹

Energy may be lost or gained when shells of solvation are stripped from the ligand and active site and are displaced into the bulk solution, which must occur to allow the ligand and active site to associate with one another. The total binding process including solvation/desolvation may be written as:

This can be broken down into the cycle:

When ligands are similar in size, as they are in this study, $\Delta E_{\text{desolv,AS}}$ and ΔE_{bulk} are constant across all complexes, and may thus be ignored. Favorable desolvation effects occur when a non-polar, hydrophobic group from the ligand or active site releases waters of solvation and associates with another, newly desolvated hydrophobic group in the active site. Unfavorable desolvation effects take place when a polar group in the complex releases waters of solvation and makes contact with a non-polar group in the active site. The ligands in this study range from more polar (catecholaminic ligands) to less polar (catecholic ligands), and both desolvation effects can be seen in the results below.

A series of hydration shells that increase in size ($n=7, 9, 11$ and 13) were used to explicitly model the desolvation of the dopamine analogs. Structures of the solvated ligands, the water clusters and free ligands were optimized using M062X and a series of increasingly larger basis sets. Different basis sets were used for desolvation calculations (Dunning basis sets)^{20,21} than for the interaction energy calculations (Pople basis sets).^{22,23} The more extended systems in the water clusters called for the greater flexibility of the Dunning basis sets, and, since the desolvation energy is taken to a somewhat converged point, its addition to the Pople-basis-based interaction energies should be consistent.

2. Computational Details

2.1 Ligand-active site electronic interaction energies

The ligands in this study include dopamine, BIA 8-176,⁶ resveratrol, and a suite of dopaminergic derivatives. BIA 8-176 is a known COMT inhibitor,^{6,24,25} and dopamine is an endogenous ligand of the COMT enzyme. These molecules serve as a basis of comparison for this study because the most viable inhibitors will behave more favorably as ligands of COMT than both dopamine and BIA 8-176, providing an alternative and more favorable inhibitor. Various proposed ligands, including 6-hydroxydopamine, 6-nitrodopamine, 6-bromodopamine, 6-cyanodopamine, 6-ethenyldopamine, 6-carboxydopamine retain the ethylamine substituent seen on the dopamine molecule (the catecholamine core), with the addition of various substituents in the 6-position. The other molecules investigated retain the two phenolic hydroxyl groups (the catechol core) but have combinations of other substituents in place of the aminoethyl group. All ligands investigated are shown in Figure 2.

The catechol-O-methyltransferase active site, with BIA 8-176 bound, was isolated from the crystal structure (PDB ID: 2CL5). The active site residues were selected by proximity to the ligand (within 4.0 angstroms of BIA 8-176). The active site has a hydrophilic region surrounding the magnesium ion which consists of Asp169, Asn170, Asp141 and Glu199. The other region of the active site is more hydrophobic and includes Trp38, Met40, Val42, Pro174, Trp253, Val388, Leu413 and Pro174. In order to maintain the charge and polarity found in peptide bonds in a complete enzyme, the amino acid residues were capped with hydroxyl and amino groups. Every amino acid residue included in the active site and the ligands in question were adjusted to the protonation state at physiological pH.

Three ligand-active site complex models were investigated in this study and will be referred to by the designations VR, SR and SX. The VR model assumes *in vacuo* conditions and optimizations were performed with rigid amino acid side chains. The SR model applies the Polarizable Continuum Model¹⁵ for implicit solvation in the optimization of the ligand-active site complex, and uses rigid amino acid side chains. The SX model, like the SR, applies the Polarizable Continuum Model¹⁵ for implicit solvation but allows for the amino acid side chains, in addition to the ligand, to optimize. The structure of each ligand-active site complex was optimized using M062X with the 6-31G basis set²² for each of the three models.

For each optimized structure, the interaction energy of the ligand-active site complex was determined from a summation of counterpoise corrected electronic interaction energies between the ligand and each individual amino acid residue (i) in the active site.

$$\Delta E_{\text{int}i}^{\text{CP}} = E_{\text{ligand}+i} - E_{\text{ligand}} - E_i \quad \text{EQ. 5}$$

$$\Delta E_{\text{int}} = \sum_i \Delta E_{\text{int}i}^{\text{CP}}, \quad \text{EQ. 6}$$

where i runs over the 12 amino acids mentioned above. For the VR and SR models, electronic interaction energies were calculated with both M06L and MP2 methods, each with the 6-311+G* basis set.²³ In the VR and SR models reported in this study, the M06L and MP2 showed an average difference of 3.81% showing that M06L can reliably provide relative electronic interaction energies for the ligand-active site complexes. The consistency of M062X and MP2 has also been shown by Bigler et al. in an investigation performed with similar methods.¹⁷ Thus, for the SX model, only M06L was used to calculate electronic interaction energies. The values of ligand interactions with every residue in the active site are provided in the supplementary data, while summary tables that highlight the residues that contribute most strongly to the overall interaction energy are provided below.

All ligands were optimized in two orientations, modeling deprotonation of either the hydroxyl group in the 3- or 4- position relative to the aminoethyl tail. The optimization positions are designated as either 3-dp or 4-dp, indicating deprotonation in the 3- and 4- position, respectively.

2.2 Desolvation energies

Desolvation energies were calculated by optimizing ligands solvated by varying numbers of explicit water molecules (n=7,9,11,13) and then calculating the energy needed to remove the explicit water molecules, leaving a desolvated ligand and a water cluster. The different sizes of explicit shells are referred to as water models 1-4 (WM1-4). Starting structures of water clusters used to model the bulk water in this work were taken from previous work; for each cluster size, the lowest energy structural isomer was selected as the starting point for optimization. The initial heptamer and nonamer water cluster structures were taken from Pérez et al.^{26,27} The undecamer water cluster structure was taken from Lee et al.²⁸ The tredecamer water cluster structure was adapted from Lee et al.'s dodecamer water cluster. For each of the explicitly solvated ligands in each water model, the water clusters and the free ligands were optimized using M062X with the basis sets aug-cc-pvdz, cc-pvdz, and cc-pvtz.^{20,21}

The initial structures of the solvated ligands were made with water molecules placed near the most polar moieties on the ligands. These polar regions include hydroxyl groups of all ligands, as well as amino-, bromo-, nitro-, cyano-, and carboxy- substituents. The fused, heterocyclic ring (isoquinoline moiety) also provided a slight polar region for moderate interaction between the water molecules and ligand 1,2,3,4-tetrahydroisoquinoline-6,7-diol. Once polar regions were saturated, additional water molecules were placed in the areas adjacent to already existing water molecules to allow coordination between the molecules. Placement of water molecules in the initial structures was consistent across all ligands. Consistency of water molecule placement of the solvated ligands was desired above having global minima across all solvated ligands.

The desolvation energies were calculated by subtracting the electronic energies of the free ligand and the water cluster from the energy of the complex:

$$\Delta E_{desolv,lig} = E_{complex} - E_{ligand} - E_{cluster} \quad \text{EQ. 7}$$

Counterpoise corrections were not used in these calculations as the basis sets are large enough that the correction is minimal with DFT methods. The water clusters and solvated ligands were then optimized using the PCM and M062X/aug-cc-pvdz in order to place an additional “shell” of implicit water around the explicit water.

All calculations were performed with Gaussian 09.²⁹

3. Discussion

3.1 Optimization and Structure

The optimized structures of the ligands in the VR model are shown in Figure 3, with the hydroxyl group in the 3-position positioned nearer to Lys144, thus modeling the deprotonation of the ligand in the 3-position. Ligands BIA 8-176 and resveratrol are included in Figure 3 but were only investigated in the position shown in the figure.

Figure 4 shows the optimized structures of the ligands in the VR model with the hydroxyl group in the 4-position nearer to Lys144, thus modeling the deprotonation of the ligand in the 4-position. BIA 8-176 and resveratrol are not included in this figure.

The optimized structures of the ligands in the SR model are depicted in Figure 5 with the hydroxyl group in the 3-position nearer to Lys144, thus modeling the deprotonation of the ligand in the 3-position.

The optimized structures of the ligands in the SR model are provided (Fig. 6) with the hydroxyl group in the 4-position nearer to Lys144, thus modeling the deprotonation of the ligand in the 4-position.

The optimized structures of the ligands in the SX model are depicted in Figure 7 with the hydroxyl group in the 3-position nearer to Lys144, thus modeling the deprotonation of the ligand in the 3-position.

The optimized structures of the ligands in the SX model are provided (Fig. 8) with the hydroxyl group in the 4-position nearer to Lys144, thus modeling the deprotonation of the ligand in the 4-position.

3.2 VR Model Electronic Interaction Energies

The counterpoise corrected interaction energies for the ligands are shown in Table 1 and Table 2, with Table 1 providing the overall interaction energy for each ligand-active site complex and with Table 2 highlighting the Mg^{2+} interaction and the amino acid residues that contributed most strongly to the overall interaction energy. The complete set of counterpoise corrected electronic interaction energies for every amino acid in the active site is shown in the supplementary data. The VR model, in which the active site was optimized with rigid amino acid side chains and *in vacuo* conditions, showed Mg^{2+} , the Asp169/Asn170 dipeptide, Lys144, Asp149, and Glu199 to be the strongest contributors to the overall interaction energy of the ligand-active site complex. These residues and the metal ion are centralized on one side of the active site, creating a charged, hydrophilic pocket. The opposite part of the active site, which consists of neutral amino acids, did not contribute as strongly to the overall interaction energy. The magnesium ion contributed the most strongly to the total interaction energy.

In this model, BIA 8-176 was shown to be the most favorable inhibitor, with an interaction energy of -345.6 and -346.4 kcal/mol with methods M06L and MP2, respectively. This interaction energy showed that BIA 8-176 has a high enough affinity to be preferred as a ligand for the active site over dopamine, which was shown to have electronic interaction energies of -235.6 and -234.6 kcal/mol with M06L and MP2, respectively. BIA 8-176, a known COMT inhibitor, was bound in the active site of the protein when the crystal structure was elucidated, and it is therefore possible that its high affinity may be an artifact of the conditions under which the crystal structure was determined. The least favorable electronic interaction energies were seen between the active site and the ligand resveratrol.

Though BIA 8-176 was shown to be the most favorable inhibitor of COMT, several ligands were shown to have higher affinities than dopamine in the active site. Ligands that had higher affinities include 6-hydroxydopamine, 2-(3,4-dihydroxyphenyl)acetonitrile and 4-(3,4-dihydroxyphenyl)butanenitrile in the 4-dp position as well as 6-carboxydopamine and 3-(3,4-dihydroxyphenyl)propanenitrile in both the 3-dp position and 4-dp position. Dopamine yielded electronic interaction energies of -235.6 and -234.6 kcal/mol with M06L and MP2 in the 3-dp position and -249.6 and -242.3 kcal/mol in the 4-dp position. The interaction energy of 6-hydroxydopamine in the 4-dp position was -267.8 and -264.5 kcal/mol with methods M06L and MP2, respectively. 6-hydroxydopamine was not more favorable than dopamine when bound in the 3-dp position. Ligand 2-(3,4-dihydroxyphenyl)acetonitrile was also more favorable than dopamine in the 4-dp position with electronic interaction energies of -258.4 and -254.1 kcal/mol with M06L and MP2, respectively. Ligand 4-(3,4-dihydroxyphenyl)butanenitrile, favorable in the 4-dp position as well, had electronic interaction energies of -275.3 and -269.5 kcal/mol with M06L and MP2. The remaining favorably inhibitory ligands had comparable affinities in both the 3- and 4-dp positions, with the following electronic interaction energies as determined with M06L and MP2 respectively: -265.4 and -259.1 kcal/mol (6-carboxydopamine, 3-dp); -259.7 and -264.1 kcal/mol (6-carboxydopamine, 4-dp); -259.6 and -255.0 kcal/mol (3-(3,4-dihydroxyphenyl)acetonitrile, 3dp); -268.3 and -264.2 (3-(3,4-dihydroxyphenyl)acetonitrile, 4-dp).

3.3 SR Model Electronic Interaction Energies

The results for the SR model, which retains the rigid active site seen in the VR model, and includes the PCM model of implicit solvation in the ligand-active site complex, are shown in Tables 1 and 3. Interestingly, results from the counterpoise corrected interaction energy calculations showed that BIA 8-176 was no longer the most favorable ligand in the COMT active site. BIA 8-176, which had electronic interaction energies of -345.6 and -346.4 kcal/mol with M06L and MP2 in the VR model, showed energies of -260.6 and -253.7 kcal/mol with M06L and MP2, respectively. Ligands 6-hydroxydopamine and 6-carboxydopamine were shown to be equally favorable as compared to BIA 8-176 in the SR model. 6-Hydroxydopamine is comparably favorable in the 3-dp and 4-dp positions, with 3-dp electronic interaction energies of -267.1 and -284.4 kcal/mol and 4-dp electronic interaction energies of -270.9 and -264.5 kcal/mol with M06L and MP2. 6-Carboxydopamine showed 3-dp interaction energies of -265.7 and -262.6 kcal/mol with M06L and MP2 and 4-dp electronic interaction energies of -274.5 and -265.0 kcal/mol with M06L and MP2, respectively.

Overall, two trends of energetic changes were identified in the transition from *in vacuo* simulation to the model with implicit solvation. First, select ligands retained roughly the same overall interaction energy in the active site. This trend was seen with dopamine, for example, which had an average change of 3.81% for either method between the VR and SR models. This trend was also seen for ligands resveratrol, 6-hydroxydopamine, 1,2,3,4-tetrahydroisoquinoline-6,7-diol, 6-nitrodopamine, 6-bromodopamine, 6-cyanodopamine and 6-ethenyldopamine. The retention of overall affinity in the active site results from the ligands optimizing in the same overall protonation state in the SR model that was seen in the VR model. Therefore, the addition of solvation stabilized the charges seen in the VR model and the affinity of these ligands remained comparably constant.

The second trend seen in the transition from the VR model to the SR model includes ligands that showed a significant decrease in affinity with the addition of solvation to the active site optimization model. A decrease in affinity was seen for the derivatives 3,4-dihydroxybenzonitrile, 2-(3,4-dihydroxyphenyl)acetonitrile, 3-(3,4-dihydroxyphenyl)propanenitrile and 4-(3,4-dihydroxyphenyl)butanenitrile due to their lack of an ionizable tail moiety. The decrease in affinity results from a change in protonation state of the ligand during optimization with the presence of implicit solvent. The deprotonated hydroxyl group on the ligands would, in some optimizations, extract a proton from Asn170, changing the overall charge of the ligand. When the ligand gained a proton and thus became more positively charged, the interactions with charged amino acids changed, becoming either more attractive if the charged amino acid was positive (i.e. Lys144) or more repulsive if the charged amino acid was negative (i.e. Asp169 or Glu199). The most significant interaction change seen as a result of the different optimized protonation state was between the ligand and the magnesium ion. When the ligand became more positively charged, its interaction with magnesium decreased significantly, reducing the overall interaction energy of the ligand in the active site. For the decreased affinity trend, the electronic interaction energies between the ligand and the residues present in the hydrophobic portion of the active site remained comparably constant and did not contribute to the changes seen in total affinity.

3.4 SX Model Electronic Interaction Energies

The results from the SX model for active site optimization, which applied PCM for implicit solvation and allows for the optimization of the amino acid side chains as well as the ligand, are shown in Tables 1

and 4. The electronic interaction energies determined for the complexes optimized in the SX model show resveratrol to be the most favorable ligand with an electronic interaction energy of -309.8 kcal/mol. Dopamine yielded an electronic interaction energy of -292.5 kcal/mol in the 4-dp position, but was less favorable in the 3 position. Of the novel derivatives proposed, 6-hydroxydopamine (4-dp) and 4-(3,4-dihydroxyphenyl)butanenitrile (4-dp) were the most favorable ligands, followed by 6-carboxydopamine in both the 3-dp and 4-dp positions and 6-hydroxy dopamine in the 3-dp position.

The most significant changes in ligand favorability seen in the transition from the SR model to the SX model were seen in resveratrol, which got significantly more favorable (-267.1 kcal/mol in SR model, -309.8 kcal/mol in SX model), and 1,2,3,4-tetrahydroisoquinoline-6,7-diol, which became significantly less favorable (-210.2 kcal/mol in SR model, -62.4 kcal/mol in SX model). The derivatives with nitrile substituents became more favorable in the SX model with the exception of 2-(3,4-dihydroxyphenyl)acetonitrile in the 3-dp position, which became less favorable. The nitrile derivatives in the SR model yielded electronic interaction energies, with M06L, ranging between -150.1 kcal/mol and -175.8 kcal/mol. In the SX model, however, the nitrile derivatives, excluding 2-(3,4-dihydroxyphenyl)acetonitrile (3-dp), had electronic interaction energies ranging between -221.5 and -288.7 kcal/mol.

An artifact was identified in the crystal structure when analyzing the active site in preparation for the SX model optimizations. Asn170, which is peptide bonded to Asp169 and coordinated with the magnesium ion, was established in the crystal structure with the nitrogen on the side chain coordinating to the Mg^{2+} rather than the oxygen; this means that the oxygen on the side chain was protonated—taking a proton from the NH_2 moiety—while the NH_2 moiety was left with one proton. This conformation is not favorable at physiological pH, so, to further analyze the situation, two pre-optimization structures were established with dopamine as the ligand: one with the asparagine in its conformation from the original crystal structure and another with the conformation of asparagine corrected to its normal state. Both models were optimized, and it was found that the corrected model was 36.9 kcal/mol more favorable than the uncorrected model. Based on these findings, all SX model structures were optimized with the structure of asparagine corrected. The corrected asparagine side chain was allowed to optimize with the other amino acid side chains.

3.5 Deprotonation in the 3-position vs the 4-position

In both the VR and SR models, the binding affinities of several analyzed ligands were comparably favorable in both deprotonation positions investigated. In the VR model, ligands 1,2,3,4-tetrahydroisoquinoline-6,7-diol, 6-bromodopamine, 3,4-dihydroxybenzonitrile, 2-(3,4-dihydroxyphenyl)acetonitrile and 3-(3,4-dihydroxyphenyl)propanenitrile maintained comparable electronic interaction energies in both positions. Ligands 6-bromodopamine and 1,2,3,4-tetrahydroisoquinoline-6,7-diol did not retain this trend in the SR model. In the SR model, lack of position preference was retained in ligands 3,4-dihydroxybenzonitrile, and 2-(3,4-dihydroxyphenyl)acetonitrile, and 3-(3,4-dihydroxyphenyl)propanenitrile. Ligands 6-hydroxydopamine and 6-nitrodopamine, which were not equally favorable in both positions in the VR model, were comparably favorable in both positions in the SR model.

When ligands interacted more strongly in one deprotonation position, it was shown that deprotonation in the 4-OH position was consistently preferred. In the VR model, ligands dopamine, 6-

hydroxydopamine, 6-cyanodopamine, 6-ethenyldopamine and 6-carboxydopamine yielded stronger electronic interaction energies when optimized in the 4-OH deprotonation position than in the 3-OH deprotonation position. In the SR model, dopamine, 1,2,3,4-tetrahydroisoquinoline-6,7-diol, 6-bromodopamine, 6-cyanodopamine, 6-ethenyldopamine, 6-carboxydopamine, and 4-(3,4-dihydroxyphenyl)butanenitrile bound more favorably in the 4-OH deprotonation position.

Though several ligands bound more favorably in the position that modeled deprotonation in the 4-position, the preferential binding difference was not significant enough that the ligands could not bind competitively in either position. The preferential deprotonation for all ligands except 6-hydroxydopamine was slight, with differences ranging between 6 and 23 kcal/mol in total electronic interaction energies between the 3- versus 4-position. 6-Hydroxydopamine was shown to be 37.5 kcal/mol and 44.9 kcal/mol more favorable in the 4-position using M06L and MP2 methods, respectively.

In the SX model, dopamine and ligands 6-hydroxydopamine, 1,2,3,4-tetrahydroisoquinoline-6,7-diol, 6-nitrodopamine, 6-cyanodopamine, 2-(3,4-dihydroxyphenyl)acetonitrile, and 4-(3,4-dihydroxyphenyl)benzonitrile showed preference for deprotonation in the 4-dp position. These trends are similar to those seen in the SR model with the exception of 2-(3,4-dihydroxyphenyl)acetonitrile, which showed no strong preference in the SR model, and 6-bromodopamine and 6-cyanodopamine, which no longer showed a preference for deprotonation in the 4-dp position in the SX model.

The deprotonation preference in the 4-OH position for the ligands described above is contrary to experimental results, which show that dopamine is deprotonated and subsequently methylated in the 3-position.¹⁰ Experimental studies have shown no strong preference for deprotonation position in dopaminergic derivatives studied thus far.¹⁰ Results from this investigation are consistent, with either no preference for deprotonation position or slight preference for the 4-dp position, but no strong preferences shown for the proposed derivatives.

3.6 Desolvation energies

Figures 9-20 (all in the supplementary data) show the structures of the optimized solvated ligand complexes and water clusters for all basis sets and water models. All water models and basis sets exhibited water molecules interacting most strongly with polar regions (more likely to surround polar regions) of the ligands. These polar regions include hydroxyl groups of all ligands, as well as amino, bromo, nitro, cyano, and carboxy substituents. The pi electrons of the fused, heterocyclic ring, isoquinoline, also provided a slight polar region for moderate interaction between the water molecules and ligand (1,2,3,4-tetrahydroisoquinoline-6,7-diol). Water clusters were not found around the non-polar ethenyl group. As the water models increased in size, a greater number of water molecules surrounded these polar regions in addition to forming a network of waters that bridge between hydroxyl groups and polar substituents on the opposite side of the ligands. (These trends are consistent among all basis sets and water models.)

Water clusters, and solvated ligand structures were optimized in larger basis sets using the corresponding optimized structure from the smaller basis set; therefore the optimized ligand and water cluster structures are very similar among all basis sets.

The desolvation energies of the water clusters in the four different explicit water models with the three different basis sets are given in Table 5. With few exceptions, the desolvation process is unfavorable, with a positive energy value indicating that energy must be put in to the system to desolvate the ligands. This is not surprising considering the relatively strong polarities of most of the ligands studied. Despite the basis set or water model used, the desolvation energies of the catecholaminic ligands range between 30 and 50 kcal/mol, though most cluster around 40 kcal/mol. The catecholic ligands (those without the aminoethyl tail) have more favorable desolvation energies (between -20 and +20 kcal/mol) with some having negative energies, indicating energy is released upon desolvation.

In general, the desolvation energies decrease with increasing basis set size and with increasing water model size. The exception to this trend is the tredecamer water model (WM4), which shows desolvation energies increasing compared with the smaller water models. It is believed this may be due to the water molecules beginning to interact with each other more than the ligand, as a second solvation shell is beginning to form. This may be supported by the fact that when an implicit water model is used around the explicit models (see Table 5), the desolvation energy increases again.

3.7 Total electronic binding energies

The total electronic binding energies calculated as the sum of the SX model M06L/6-311+G* electronic interaction energies (considered the most accurate model used here for electronic interaction energies) and the undecamer explicit water model desolvation energies (M062X/aug-cc-pvdz) are shown in Table 7. The desolvation energies from the undecamer were used as they are considered the converged first solvation shell—using the tredecamer water model would include an unconverged second solvation shell. The notable result here is that, when electronic interaction energies alone are considered, the catecholaminic and catecholic molecules show similar binding with the active site, with energies averaging -233 kcal/mol for the catecholamines and -239 for the catechols. When desolvation energies are added, the catecholaminic molecules fall to an average of -196 kcal/mol (with two outliers between -30 and -72 kcal/mol) while the catecholic molecules' average ends up at -234 kcal/mol. Thus, when desolvation is included, the catechols are significantly more favorable than the catecholamines.

4. Conclusions

The work presented herein describes the interaction between a suite of ligands and catechol-O-methyltransferase, an enzyme involved in the metabolism of catecholamines and structurally similar molecules. Ultimately, this work will help guide the design of COMT inhibitors, which may prove to be beneficial in prolonging the effectiveness of L-DOPA and subsequently, in the treatment of diseases, such as Parkinson's disease.

The relative EBE's were determined for a set of dopaminergic derivatives, BIA 8-176, a known COMT inhibitor and resveratrol using three different models: *in vacuo* and with implicit solvation, with rigid and non-rigid amino acid side chains. We assumed the EBE's were a sum of the electronic interaction energies and the energy involved in the desolvation of the ligand. The electronic interaction energies for two models were calculated with both M06L and MP2 and were in good agreement. The solvation effects were examined by calculating the desolvation of each ligand with a series of hydration shells.

In the vacuum rigid model, 6-hydroxydopamine and 6-carboxydopamine showed the most favorable electronic interaction energy among the catecholaminic derivatives, while the catecholic derivatives exhibited slightly more favorable electronic interaction energies than the catecholaminic derivatives and were some of the most favorable in this model aside from BIA 8-176. In the VR model, BIA 8-176, the known COMT inhibitor, had a significantly more favorable interaction energy than any of the ligands examined. However, in the SR model, 6-hydroxydopamine and 6-carboxydopamine exhibited the strongest binding in the COMT active site of all ligands examined, and the affinity of BIA 8-176 significantly decreased. Implicit solvation strongly affects the electronic interaction energies of the catecholic derivatives, as observed by the decrease in interaction energy from the VR to SR model. This is likely due to the lack of the amino ethyl tail. In both models, resveratrol gives the least favorable electronic interaction energies. Ligands either showed no preference between the 3- and 4-deprotonation positions or showed a slight preference for deprotonation in the 4-position. In the SX model, resveratrol became much more favorable, as did the catecholic ligands lacking the charged tail moiety, suggesting that the structure of the active site from the crystal structure was biased towards more polar, catecholaminic ligands.

Using three basis sets and four different water models, the desolvation energy was determined for each ligand. Aside from a few exceptions the desolvation process is unfavorable with the catecholaminic derivatives having a larger desolvation energy versus the catecholic ligands, again likely due to the more hydrophilic nature of the ligands more closely resembling dopamine and therefore the more energy required to desolvate the ligand.

The SX model M06L electronic interaction energies and desolvation energies from the undecamer model were used to calculate the total electronic binding energies. Although very small differences were observed between the catecholaminic and catecholic derivatives in the electronic interaction energies, when combined with the desolvation energies as total EBE, the catecholic ligands were found to be more favorable inhibitors for COMT.

5. Acknowledgements

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6. References

1. Zhao, Y.; Truhlar, D. G., A new local density functional for main-group thermochemistry, transition metal bonding, thermochemical kinetics, and noncovalent interactions. *Journal of Chemical Physics* **2006**, *125* (19), 194101/1-194101/18.
2. Moller, C.; Plesset, M. S., Note on the approximation treatment for many-electron systems. *Physical Review* **1934**, *46*, 618-22.
3. Zhao, Y.; Truhlar, D. G., Density Functionals for Noncovalent Interaction Energies of Biological Importance. *Journal of Chemical Theory and Computation* **2007**, *3* (1), 289-300.
4. Zhao, Y.; Truhlar, D. G., Design of Density Functionals That Are Broadly Accurate for Thermochemistry, Thermochemical Kinetics, and Nonbonded Interactions. *Journal of Physical Chemistry A* **2005**, *109* (25), 5656-5667.
5. Bonifacio, M. J.; Palma, P. N.; Almeida, L.; Soares-da-Silva, P., Catechol-O-methyltransferase and its inhibitors in Parkinson's disease. *CNS Drug Reviews* **2007**, *13* (3), 352-379.

6. Palma, P. N.; Rodrigues, M. L.; Archer, M.; Bonifacio, M. J.; Loureiro, A. I.; Learmonth, D. A.; Carrondo, M. A.; Soares-da-Silva, P., Comparative study of ortho- and meta-nitrated inhibitors of catechol-O-methyltransferase: interactions with the active site and regioselectivity of O-methylation. *Molecular Pharmacology* **2006**, *70* (1), 143-153.
7. Axelrod, J.; Tomchick, R., Enzymatic O-methylation of epinephrine and other catechols. *The Journal of biological chemistry* **1958**, *233* (3), 702-5.
8. Rodrigues, M. L.; Bonifacio, M. J.; Soares-da-Silva, P.; Carrondo, M. A.; Archer, M., Crystallization and preliminary x-ray diffraction studies of a catechol-O-methyltransferase/inhibitor complex. *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications* **2005**, *61* (1), 118-120.
9. Baas, H.; Beiske, A. G.; Ghika, J.; Jackson, M.; Oertel, W. H.; Poewe, W.; Ransmayr, G., Catechol-O-methyltransferase inhibition with tolcapone reduces the "wearing off" phenomenon and levodopa requirements in fluctuating parkinsonian patients. *Neurology* **1998**, *50* (5, Suppl. 5), S46-S53.
10. Ohtsuki, S.; Terasaki, T., Contribution of Carrier-Mediated Transport Systems to the Blood-Brain Barrier as a Supporting and Protecting Interface for the Brain; Importance for CNS Drug Discovery and Development. *Pharmaceutical Research* **2007**, *24* (9), 1745-1758.
11. Cedarbaum, J. M., Clinical pharmacokinetics of anti-parkinsonian drugs. *Clinical pharmacokinetics* **1987**, *13* (3), 141-78.
12. Iwaki, H.; Nishikawa, N.; Nagai, M.; Tsujii, T.; Yabe, H.; Kubo, M.; Ieiri, I.; Nomoto, M., Pharmacokinetics of levodopa/benserazide versus levodopa/carbidopa in healthy subjects and patients with Parkinson's disease. *Neurology and Clinical Neuroscience* **2015**, *3* (2), 68-73.
13. Mamisto, P. T.; Kaakkola, S., Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. *Pharmacology & Toxicology (Oxford, United Kingdom)* **1990**, *66* (5), 317-23.
14. Creveling, C. R.; Morris, N.; Shimizu, H.; Ong, H.; Daly, J., Catechol O-methyltransferase. IV. Factors affecting m- and p-methylation of substituted catechols. *Molecular Pharmacology* **1972**, *8* (4), 398-409.
15. Tomasi, J.; Mennucci, B.; Cammi, R., Quantum Mechanical Continuum Solvation Models. *Chemical Reviews (Washington, DC, United States)* **2005**, *105* (8), 2999-3093.
16. Sinnokrot, M. O.; Sherrill, C. D., High-Accuracy Quantum Mechanical Studies of π - π Interactions in Benzene Dimers. *Journal of Physical Chemistry A* **2006**, *110* (37), 10656-10668.
17. Bigler, D. J.; Peterson, L. W.; Cafiero, M., Effects of implicit solvent and relaxed amino acid side chains on the MP2 and DFT calculations of ligand-protein structure and electronic interaction energies of dopaminergic ligands in the SULT1A3 enzyme active site. *Computational & Theoretical Chemistry* **2015**, *1051*, 79-92.
18. Riley, K. E.; Vondrasek, J.; Hobza, P., Performance of the DFT-D method, paired with the PCM implicit solvation model, for the computation of interaction energies of solvated complexes of biological interest. *Physical Chemistry Chemical Physics* **2007**, *9* (41), 5555-5560.
19. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T., Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. *J. Med. Chem.* **2006**, *49* (21), 6177-6196.
20. Dunning Jr., T.H., Gaussian basis sets for use in correlated molecular calculations. I. The atoms boron through neon and hydrogen. *Journal of Chemical Physics* **1989**, *90*, 1007-23.
21. Woon, D.E.; Dunning Jr., T.H., Gaussian-basis sets for use in correlated molecular calculations. 3. The atoms aluminum through argon. *Journal of Chemical Physics* **1993**, *98*, 1358-71.
22. Hehre, W.J.; Ditchfield, R.; Pople, J.A., Self-Consistent Molecular Orbital Methods. 12. Further extensions of Gaussian-type basis sets for use in molecular-orbital studies of organic-molecules. *Journal of Chemical Physics* **1972**, *56*, 2257.
23. Raghavachari, K.; Trucks, G.W., Highly correlated systems: Excitation energies of first row transition metals Sc-Cu. *Journal of Chemical Physics* **1989**, *91*, 1062-65.
24. Learmonth, D. A.; Freitas, A. P., Chemical Synthesis and Characterization of Conjugates of a Novel Catechol-O-methyltransferase Inhibitor. *Bioconjugate Chemistry* **2002**, *13* (5), 1112-1118.

25. Learmonth, D. A.; Bonifacio, M. J.; Soares-da-Silva, P., Synthesis and Biological Evaluation of a Novel Series of "Ortho-Nitrated" Inhibitors of Catechol-O-methyltransferase. *J. Med. Chem.* **2005**, *48* (25), 8070-8078.
26. Perez, C.; Lobsiger, S.; Seifert, N. A.; Zaleski, D. P.; Temelso, B.; Shields, G. C.; Kisiel, Z.; Pate, B. H., Broadband Fourier transform rotational spectroscopy for structure determination: the water heptamer. *Chemical Physics Letters* **2013**, *571*, 1-15.
27. Perez, C.; Zaleski, D. P.; Seifert, N. A.; Temelso, B.; Shields, G. C.; Kisiel, Z.; Pate, B. H., Hydrogen Bond Cooperativity and the Three-Dimensional Structures of Water Nonamers and Decamers. *Angewandte Chemie, International Edition* **2014**, *53* (52), 14368-14372.
28. Lee, H. M.; Suh, S. B.; Kim, K. S., Structures, energies, and vibrational spectra of water undecamer and dodecamer: An ab initio study. [Erratum to document cited in CA135:201986]. *Journal of Chemical Physics* **2001**, *115* (15), 7331.
29. Gaussian 09, Revision C.01, M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E., Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A., Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J., Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J., Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A., Montgomery, Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N., Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian Inc., Wallingford CT, 2009.

Figure 1: Metabolic pathways of L-DOPA, commonly administered in the pharmacological treatment strategy for Parkinson's disease^{7,9}

Figure 2: The suite of dopaminergic derivatives analyzed as inhibitors of catechol-O-methyltransferase and in desolvation models. BIA 8-176 a known COMT inhibitor and resveratrol was studied to investigate the importance of position of phenolic hydroxyl groups. All other ligands were derived from dopamine, with systematically varied substituents to allow for different electron-donating and electron-withdrawing properties.

Dopamine (3-dp)

6-hydroxydopamine (3-dp)

1,2,3,4-
tetrahydroisoquinoline-6,7-
diol (3-dp)

6-nitrodopamine (3-dp)

6-bromodopamine (3-dp)

6-cyanodopamine (3-dp)

6-ethenyldopamine (3-dp)

6-carboxydopamine (3-dp)

3,4-dihydroxybenzonitrile
(3-dp)

Figure 3: Optimized models of the dopaminergic ligands, with dopamine, BIA 8-176 and resveratrol, in the vacuum rigid active site model. Ligands are positioned with the 3-position hydroxyl group nearer to Lys144 and represent metabolism via deprotonation in the 3-position.

Dopamine (4-dp)

6-hydroxydopamine (4-dp)

1,2,3,4-tetrahydroisoquinoline-6,7-diol (4-dp)

6-nitrodopamine (4-dp)

6-bromodopamine (4-dp)

6-cyanodopamine (4-dp)

6-ethenyldopamine (4-dp)

6-carboxydopamine (4-dp)

3,4-dihydroxybenzonitrile (4-dp)

2-(3,4-
dihydroxyphenyl)acetonitrile
(4-dp)

3-(3,4-
dihydroxyphenyl)propanenitrile
(4-dp)

4-(3,4-
dihydroxyphenyl)butanenitrile
(4-dp)

Figure 4: Optimized models of the dopamine and the dopaminergic derivatives in the vacuum rigid active site model. Ligands are positioned with the 4-position hydroxyl group nearer to Lys144 and therefore represent the deprotonation in the 4-position.

Dopamine_(aq) (4-dp)

6-hydroxydopamine_(aq) (3-dp)

1,2,3,4-tetrahydroisoquinoline-6,7-diol_(aq) (3-dp)

6-ethenyldopamine_(aq) (3-dp)

6-bromodopamine_(aq) (3-dp)

6-cyanodopamine_(aq) (3-dp)

6-nitrodopamine_(aq) (3-dp)

6-carboxydopamine_(aq) (3-dp)

3,4-dihydroxybenzonitrile_(aq) (3-dp)

Figure 5: Optimized models of the dopaminergic ligands, with dopamine, BIA 8-176 and resveratrol in the solvated rigid active site model. Ligands are positioned with the 3-position hydroxyl group nearer to Lys144 and therefore represent the deprotonation in the 3-position.

Dopamine_(aq) (4-dp)

6-hydroxydopamine_(aq) (4-dp)

1,2,3,4-tetrahydroisoquinoline-6,7-diol_(aq) (4-dp)

6-nitrodopamine_(aq) (4-dp)

6-bromodopamine_(aq) (4-dp)

6-cyanodopamine_(aq) (4-dp)

6-ethenyldopamine_(aq) (4-dp)

6-carboxydopamine_(aq) (4-dp)

3,4-dihydroxybenzonitrile_(aq)

2-(3,4-
dihydroxyphenyl)acetonitrile_(aq)
(4-dp)

3-(3,4-
dihydroxyphenyl)propanenitrile_(aq)
(4-dp)

4-(3,4-
dihydroxyphenyl)butanenitrile_(aq)
(4-dp)

Figure 6: Optimized models of the ligands in the solvated rigid active site model. Ligands are positioned with the 4-position hydroxyl group nearer to Lys144 and therefore represent the deprotonation in the 4-position.

Dopamine_(aq) (3-dp)

6-hydroxydopamine_(aq) (3-dp)

1,2,3,4-
tetrahydroisoquinoline-6,7-
diol_(aq) (3-dp)

6-ethenyldopamine_(aq) (3-dp)

6-bromodopamine_(aq) (3-dp)

6-cyanodopamine_(aq) (3-dp)

6-nitrodopamine_(aq) (3-dp)

6-carboxydopamine_(aq) (3-dp)

3,4-dihydroxybenzonitrile_(aq)
(3-dp)

Figure 7: Optimized models of the dopaminergic ligands, with dopamine, BIA 8-176 and resveratrol in the solvated, non-rigid active site model. Ligands are positioned with the 3-position hydroxyl group nearer to Lys144 and therefore represent the deprotonation in the 3-position.

Figure 8: Optimized models of the ligands in the solvated non-rigid active site model. Ligands are positioned with the 4-position hydroxyl group nearer to Lys144 and therefore represent the deprotonation in the 4-position.

Table 1. Summary table for counterpoise-corrected M06L and MP2 calculations of the electronic interaction energies of the ligands optimized using the vacuum rigid (VR), solvated rigid (SR) and solvated relaxed (SX) models. Interaction energies in kcal/mol.

Molecule	Deprotonation	VR Model		SR Model		SX Model
		M06L	MP2	M06L	MP2	M06L
Dopamine	3dp	-235.6	-234.6	-216.9	-218.4	-224.9
	4dp	-249.6	-242.3	-248.0	-239.4	-292.5
BIA 8-176	--	-345.6	-346.4	-260.6	-253.7	-232.9
resveratrol	--	-109.6	-146.1	-111.7	-145.1	-309.8
6-hydroxydopamine	3dp	-230.3	-219.6	-267.1	-284.4	-275.6
	4dp	-267.8	-264.5	-270.9	-264.5	-293.6
1,2,3,4-tetrahydroisoquinoline-6,7-diol	3dp	-228.3	-227.9	-209.9	-210.2	-62.4
	4dp	-226.5	-221.4	-214.0	-216.5	-249.3
6-nitrodopamine	3dp	-202.9	-197.9	-237.3	-229.3	-117.8
	4dp	-217.9	-223.5	-219.9	-225.4	-198.1
6-bromodopamine	3dp	-218.8	-207.5	-201.0	-194.9	-258.4
	4dp	-237.7	-209.6	-240.5	-235.6	-251.1
6-cyanodopamine	3dp	-201.5	-200.1	-202.4	-199.5	-215.8
	4dp	-225.2	-222.1	-231.3	-228.5	-248.8
6-ethenyldopamine	3dp	-223.5	-214.8	-208.0	-209.9	-245.6
	4dp	-232.4	-230.2	-236.1	-257.3	-245.5
6-carboxydopamine	3dp	-265.4	-259.1	-265.7	-262.6	-271.6
	4dp	-259.7	-262.5	-274.5	-265.0	-278.7
3,4-dihydroxybenzonitrile	3dp	-246.8	-245.2	-171.3	-166.2	-245.0
	4dp	-249.8	-246.8	-165.7	-162.2	
2-(3,4-dihydroxyphenyl)acetonitrile	3dp	-248.3	-253.5	-165.1	-161.0	-138.2
	4dp	-258.4	-254.1	-169.5	-165.4	-263.4
3-(3,4-dihydroxyphenyl)propanenitrile	3dp	-259.6	-255.0	-168.2	-164.0	-265.9
	4dp	-268.3	-264.2	-169.2	-163.9	-221.5
4-(3,4-dihydroxyphenyl)butanenitrile	3dp	-172.7	-168.1	-150.1	-162.9	-251.4
	4dp	-275.3	-269.5	-175.8	-169.4	-288.7

Table 2. Counterpoise-corrected M06L and MP2 calculations of the electronic interaction energies of the ligands optimized *in vacuo* with rigid amino acid residues in the active site (vacuum rigid model, VR). Energies in kcal/mol.

Molecule		Method	Mg ²⁺	Asp169/Asn 170	Lys144	Glu199	Asp141	Total
Dopamine	3dp	M06L	-217.1	12.6	-59.0	14.8	31.4	-235.2
		MP2	-220.7	14.2	-57.4	15.5	32.7	-234.6
	4dp	M06L	-208.0	22.0	-55.9	-0.3	23.0	-249.6
		MP2	-211.7	24.8	-55.5	0.5	32.0	-241.1
BIA 8-176	--	M06L	-310.9	-36.9	-93.4	57.5	52.3	-345.6
		MP2	-314.4	-37.5	-91.5	61.1	53.7	-346.4
Resveratrol	--	M06L	-94.9	2.8	-13.9	-3.8	0.4	-109.6
		MP2	-123.3	3.5	-12.2	-4.9	1.6	-146.1
6-hydroxydopamine	3dp	M06L	-216.5	25.7	-57.5	11.0	23.3	-230.3
		MP2	-216.7	26.0	-55.9	12.4	31.4	-219.6
	4dp	M06L	-193.9	13.0	-61.7	-47.7	27.1	-267.8
		MP2	-198.4	14.5	-60.1	-44.9	29.7	-264.5
1,2,3,4-tetrahydroisoquinoline-6,7-diol	3dp	M06L	-183.1	9.9	-53.2	-17.4	25.8	-228.3
		MP2	-186.6	11.3	-51.6	-15.9	26.3	-227.9
	4dp	M06L	-196.8	19.0	-54.4	-10.0	27.5	-226.5
		MP2	-195.4	20.3	-53.1	-8.5	28.4	-221.4
6-nitrodopamine	3dp	M06L	-190.3	15.1	-30.5	15.1	8.5	-202.9
		MP2	-193.9	19.0	-31.4	18.8	10.6	-197.9
	4dp	M06L	-198.9	16.3	-37.3	6.1	18.4	-217.9
		MP2	-204.5	14.4	-37.5	7.5	18.3	-223.5
6-bromodopamine	3dp	M06L	-214.4	18.7	-39.5	22.6	18.2	-218.8
		MP2	-217.2	23.7	-38.7	29.2	20.8	-207.5
	4dp	M06L	-223.7	14.9	-43.7	17.5	23.8	-237.7
		MP2	-226.3	19.0	-38.7	29.2	25.3	-209.6
6-cyanodopamine	3dp	M06L	-205.6	18.3	-33.7	19.7	14.7	-201.5
		MP2	-209.4	22.4	-34.5	22.5	16.3	-200.1
	4dp	M06L	-209.8	13.6	-40.1	13.0	20.9	-225.2
		MP2	-214.1	17.5	-40.1	15.0	21.9	-222.1
6-ethenyldopamine	3dp	M06L	-206.3	24.4	-58.9	12.4	23.6	-223.5
		MP2	-212.5	26.7	-57.5	13.7	31.1	-214.8
	4dp	M06L	-207.2	24.4	-55.1	-0.3	24.9	-232.5
		MP2	-209.1	26.7	-54.8	0.5	27.3	-230.2
6-carboxydopamine	3dp	M06L	-325.5	68.4	-120.5	55.4	69.7	-265.4
		MP2	-324.1	69.5	-118.1	55.7	70.5	-259.1
	4dp	M06L	-320.4	64.2	-116.2	45.8	75.5	-259.7
		MP2	-319.5	64.8	-114.1	46.3	67.5	-262.5
3,4-dihydroxybenzonitrile	3dp	M06L	-327.5	51.4	-83.7	65.6	56.9	-246.8
		MP2	-331.7	55.2	-82.8	67.8	58.1	-245.2
	4dp	M06L	-334.1	56.1	-81.4	70.3	57.4	-249.8
		MP2	-337.4	61.3	-81.0	72.3	58.6	-246.8
2-(3,4-dihydroxyphenyl)acetone	3dp	M06L	-341.2	50.3	-85.5	80.7	61.0	-248.3
		MP2	-344.9	55.1	-84.8	74.5	62.0	-253.5
	4dp	M06L	-341.0	56.9	-86.7	60.8	70.5	-258.4
		MP2	-344.0	62.0	-85.3	61.5	72.6	-250.7
3-(3,4-dihydroxyphenyl)propanenitrile	3dp	M06L	-342.4	59.4	-87.9	68.3	59.6	-259.6
		MP2	-345.1	64.0	-86.3	70.6	60.9	-255.0
	4dp	M06L	-342.1	51.3	-87.5	69.2	58.8	-268.3
		MP2	-344.8	55.1	-86.0	71.0	59.8	-264.2
4-(3,4-dihydroxyphenyl)butanenitrile	3dp	M06L	-125.6	-17.4	-16.1	-3.6	1.3	-172.7
		MP2	-126.6	-11.8	-16.6	-1.9	2.2	-168.1
	4dp	M06L	-346.4	50.7	-88.2	69.8	60.2	-275.3
		MP2	-348.8	54.8	-86.8	71.6	61.2	-269.5

Table 3. Counterpoise-corrected M06L and MP2 calculations of the electronic interaction energies of the ligands optimized with implicit solvation and rigid amino acid residues in the active site (solvated rigid, SR model). Energies in kcal/mol.

Molecule		Method	Mg ²⁺	Asp169/Asn170	Lys144	Glu199	Asp141	Total
Dopamine	3dp	M06L	-216.3	30.6	-60.6	13.8	33.0	-216.9
		MP2	-221.8	32.2	-59.1	14.3	34.6	-218.4
	4dp	M06L	-210.0	21.3	-53.5	0.5	22.6	-248.0
		MP2	-213.7	24.2	-53.3	1.2	31.7	-239.4
BIA 8-176	--	M06L	-320.5	52.4	-93.4	59.2	59.2	-260.6
		MP2	-324.2	57.8	-91.7	63.0	63.0	-253.7
Resveratrol	--	M06L	-96.8	6.4	-15.9	-1.4	1.2	-111.7
		MP2	-129.4	7.5	-0.5	-2.3	2.3	-145.1
6-hydroxydopamine	3dp	M06L	-201.7	13.6	-55.9	-44.6	26.9	-267.1
		MP2	-205.4	-4.9	-55.5	-42.7	30.0	-284.4
	4dp	M06L	-200.4	11.3	-55.6	-44.7	24.3	-270.9
		MP2	-204.1	13.3	-54.4	-42.2	29.7	-264.5
1,2,3,4-tetrahydroisoquinoline-6,7-diol	3dp	M06L	-188.2	11.9	-49.8	-0.3	26.2	-209.9
		MP2	-191.7	13.1	-48.5	0.5	26.8	-210.2
	4dp	M06L	-197.6	23.8	-52.2	-2.7	27.9	-214.0
		MP2	-202.2	25.4	-51.1	-2.8	28.9	-216.5
6-nitrodopamine	3dp	M06L	-214.2	15.9	-31.4	20.1	11.8	-237.3
		MP2	-218.6	22.2	-32.4	25.4	14.1	-229.3
	4dp	M06L	-208.9	18.6	-38.6	10.4	20.7	-219.9
		MP2	-214.3	22.7	-38.4	11.8	16.2	-225.4
6-bromodopamine	3dp	M06L	-15.0	-110.5	28.1	-45.3	-39.5	-201.0
		MP2	-16.2	-103.6	28.1	-44.0	-38.5	-194.9
	4dp	M06L	-237.6	19.3	-43.3	18.7	27.0	-240.5
		MP2	-240.7	26.2	-42.5	22.8	23.4	-235.6
6-cyanodopamine	3dp	M06L	-2.5	-118.7	30.2	-50.7	-44.1	-202.4
		MP2	-4.8	-114.1	29.3	-49.0	-42.3	-199.5
	4dp	M06L	-221.5	19.5	-40.3	15.4	13.0	-231.3
		MP2	-225.8	24.8	-40.0	17.9	19.2	-228.5
6-ethenyldopamine	3dp	M06L	-202.5	26.5	-53.0	5.3	28.1	-208.0
		MP2	-207.4	28.4	-52.0	5.8	29.8	-209.9
	4dp	M06L	-208.1	20.6	-57.7	-0.4	25.5	-236.1
		MP2	-211.0	0.0	-57.3	0.4	28.4	-257.3
6-carboxydopamine	3dp	M06L	-325.5	68.4	-120.6	55.4	69.7	-265.7
		MP2	-324.1	69.5	-118.1	55.6	70.5	-262.6
	4dp	M06L	-332.4	64.0	-113.9	46.1	68.9	-274.5
		MP2	-325.3	65.0	-112.0	46.5	69.8	-265.0
3,4-dihydroxybenzonitrile	3dp	M06L	-117.1	-30.9	-10.6	-5.3	-1.6	-171.3
		MP2	-120.1	-23.5	-10.9	-3.6	-0.2	-166.2
	4dp	M06L	-116.5	-27.5	-9.0	-2.8	-2.0	-165.7
		MP2	-119.6	-21.0	-9.6	-1.5	-0.4	-162.2
2-(3,4-dihydroxyphenyl)acetonitrile	3dp	M06L	-124.3	-21.5	-12.6	-0.3	2.2	-165.1
		MP2	-126.8	-15.4	-12.7	1.1	3.2	-161.0
	4dp	M06L	-123.7	-22.6	-14.1	-2.8	2.1	-169.5
		MP2	-126.0	-16.7	-13.7	-1.5	3.1	-165.4
3-(3,4-dihydroxyphenyl)propanenitrile	3dp	M06L	-128.1	-17.7	-13.0	-1.7	0.0	-167.6
		MP2	-130.2	-12.2	-12.8	-0.6	1.5	-164.0
	4dp	M06L	-127.1	-18.0	-13.4	-1.3	-0.3	-169.2
		MP2	-129.3	-12.4	-13.1	-0.2	1.2	-163.9
4-(3,4-dihydroxyphenyl)butanenitrile	3dp	M06L	-132.0	-16.7	-13.8	-0.2	2.1	-172.2
		MP2	-133.6	-11.0	-13.9	1.2	3.2	-168.4
	4dp	M06L	-131.7	-17.2	-14.9	-1.4	1.6	-175.8
		MP2	-133.3	-11.5	-14.6	-0.2	2.8	-169.4

Table 4. Counterpoise-corrected M06L calculations of the electronic interaction energies of the ligands optimized with implicit solvation and non-rigid amino acid residues in the active site (solvated non-rigid, SX model). Energies in kcal/mol.

Molecule		Method	Mg ²⁺	Asp169/Asn170	Lys144	Glu199	Asp141	Total
Dopamine	3dp	M06L	-219.8	26.4	-63.2	13.5	31.3	-224.9
	4dp	M06L	-215.7	19.4	-75.5	-2.3	24.0	-292.5
BIA 8-176	--	M06L	-288.7	60.2	-90.9	52.2	50.0	-232.9
Resveratrol	--	M06L	-337.1	-337.1	-337.1	-337.1	-337.1	-337.1
6-hydroxydopamine	3dp	M06L	-225.0	11.4	-71.8	-11.5	33.3	-275.6
	4dp	M06L	-209.5	11.6	-64.3	-55.2	31.4	-293.6
1,2,3,4-tetrahydroisoquinoline-6,7-diol	3dp	M06L	-200.4	-9.3	-56.0	-9.4	226.2	-62.4
	4dp	M06L	-211.8	21.6	-69.3	0.5	32.0	-249.3
6-nitrodopamine	3dp	M06L	-185.3	27.5	-25.0	16.1	9.5	-177.8
	4dp	M06L	-178.9	21.5	-36.7	1.5	11.9	-198.1
6-bromodopamine	3dp	M06L	-232.8	25.8	-42.5	-1.7	17.4	-258.4
	4dp	M06L	-222.3	25.2	-42.9	-3.0	19.1	-251.1
6-cyanodopamine	3dp	M06L	-204.7	25.8	-33.0	1.8	15.7	-215.8
	4dp	M06L	-208.8	18.6	-40.1	-4.9	12.9	-248.8
6-ethenyldopamine	3dp	M06L	-207.7	23.6	-73.3	2.8	29.3	-245.6
	4dp	M06L	-212.8	20.0	-62.1	-2.6	27.7	-245.5
6-carboxydopamine	3dp	M06L	-343.8	70.1	-116.4	61.1	74.5	-271.6
	4dp	M06L	-334.8	60.4	-116.4	47.2	69.8	-278.7
3,4-dihydroxybenzotrile	3dp	M06L	-303.2	58.7	-102.7	53.0	65.4	-245.0
	4dp	M06L						
2-(3,4-dihydroxyphenyl)acetonitrile	3dp	M06L	-127.7	-8.1	-10.5	3.3	5.6	-138.2
	4dp	M06L	-350.0	59.9	-85.3	65.5	62.4	-263.4
3-(3,4-dihydroxyphenyl)propanenitrile	3dp	M06L	-352.1	61.0	-85.0	64.0	61.9	-265.9
	4dp	M06L	-314.0	60.3	-72.1	51.9	61.4	-221.5
4-(3,4-dihydroxyphenyl)butanenitrile	3dp	M06L	-318.4	59.4	-100.4	52.3	67.0	-215.4
	4dp	M06L	-357.0	65.3	-92.9	66.8	47.0	-288.7

Table 5. A comparison of desolvation energies for the twelve ligands using the four explicit water models (WM*n*) with M062X and various basis sets. Energies are in kcal/mol.

		WM1	WM2	WM3	WM4
cc-pvdz	Dopamine	49.5	41.9	47.2	41.3
	6-hydroxydopamine	47.3	31.8	37.1	49.8
	1,2,3,4-tetrahydroisoquinoline-6,7-diol	31.9	26.1	28.9	43.7
	6-nitrodopamine	54.7	44.8	50.4	57.8
	6-bromodopamine	50	38.2	37.4	48.8
	6-cyanodopamine	47.1	38.1	38.4	43.7
	6-ethenyldopamine	49.3	41.9	36.7	44.3
	6-carboxydopamine	52.9	45.9	45.4	56.5
	3,4-dihydroxybenzonitrile	15.5	2	5.7	21.5
	2-(3,4-dihydroxyphenyl)acetonitrile	18.4	4.3	-2.4	-1.8
	3-(3,4-dihydroxyphenyl)propanenitrile	-0.4	-17.7	1.4	0.5
	4-(3,4-dihydroxyphenyl)butanenitrile	-0.6	7.6	14.4	17.2
cc-pvtz	Dopamine	44.3	36.8	40.9	40
	6-hydroxydopamine	42.2	28.7	33.9	44
	1,2,3,4-tetrahydroisoquinoline-6,7-diol	32	24.8	28.5	40.7
	6-nitrodopamine	48.7	41.3	44.8	51.9
	6-bromodopamine	44.8	34.3	34	43.4
	6-cyanodopamine	41.2	33	34.2	39.2
	6-ethenyldopamine	43.7	37.4	34.1	47.4
	6-carboxydopamine	48.4	40.5	40.8	50
	3,4-dihydroxybenzonitrile	13.5	0.8	4.7	17.6
	2-(3,4-dihydroxyphenyl)acetonitrile	15.2	2.7	-3.1	3.4
	3-(3,4-dihydroxyphenyl)propanenitrile	10.2	11.5	1.6	8.3
	4-(3,4-dihydroxyphenyl)butanenitrile	9.9	6.2	4.9	18.4
aug-cc-pvdz	Dopamine	42.7	32.9	35.1	41.6
	6-hydroxydopamine	40.3	28.5	35	44.4
	1,2,3,4-tetrahydroisoquinoline-6,7-diol	32.4	26.2	31.6	41.5
	6-nitrodopamine	43.5	39.8	45.4	52.2
	6-bromodopamine	43.4	34.1	35.4	49.3
	6-cyanodopamine	39.8	33.1	35.5	36.5
	6-ethenyldopamine	41.9	35.8	35	39
	6-carboxydopamine	46.7	40.2	41.4	50
	3,4-dihydroxybenzonitrile	13.2	-3.4	6.6	15
	2-(3,4-dihydroxyphenyl)acetonitrile	14.2	2.9	-0.8	3.9
	3-(3,4-dihydroxyphenyl)propanenitrile	10.7	10.7	7.2	8.2
	4-(3,4-dihydroxyphenyl)butanenitrile	17.2	6.7	7.5	18.7

Table 6 . A comparison of desolvation energies for the twelve ligands using the four explicit water models (WM n) and implicit solvation (PCM) with M062X/aug-cc-pvdz. Energies are in kcal/mol.

	WM1	WM2	WM3	WM4
Dopamine	77.8	72.7	71.6	73.7
6-hydroxydopamine	77.4	69.1	66.3	76.4
1,2,3,4-tetrahydroisoquinoline-6,7-diol	67.3	62.4	65.3	73
6-nitrodopamine	85.1	79.4	80.6	86.5
6-bromodopamine	77.8	69.4	69.6	81.4
6-cyanodopamine	78.1	70.5	68.9	71.9
6-ethenyldopamine	76.7	71.4	69	64.2
6-carboxydopamine	83	76.6	75.4	84.6
3,4-dihydroxybenzonitrile	19.2	8.9	13.8	8.5
2-(3,4-dihydroxyphenyl)acetonitrile	18.9	10.5	6	13.6
3-(3,4-dihydroxyphenyl)propanenitrile	17	17.2	14.5	17.5
4-(3,4-dihydroxyphenyl)butanenitrile	21.9	16.3	16	27.3

Table 7. Total electronic binding energies (EBE) for twelve ligands calculated as the sum of the M06L/6-311+G* electronic interaction energies and the M062X/aug-cc-pvdz electronic desolvation energies using an undecamer water model. Energies are in kcal/mol. *used nonamer water model.

Molecule	Deprotonation	EBE
Dopamine	3dp	-189.8
	4dp	-257.4
6-hydroxydopamine	3dp	-240.6
	4dp	-258.6
1,2,3,4-tetrahydroisoquinoline-6,7-diol	3dp	-30.8
	4dp	-217.7
6-nitrodopamine	3dp	-72.4
	4dp	-152.7
6-bromodopamine	3dp	-223
	4dp	-215.7
6-cyanodopamine	3dp	-180.3
	4dp	-213.3
6-ethenyldopamine	3dp	-210.6
	4dp	-210.5
6-carboxydopamine	3dp	-230.2
	4dp	-237.3
3,4-dihydroxybenzonitrile	3dp	-238.4
	4dp	-
2-(3,4-dihydroxyphenyl)acetonitrile	3dp	-139
	4dp	-264.2
3-(3,4-dihydroxyphenyl)propanenitrile	3dp	-258.7
	4dp	-214.3
4-(3,4-dihydroxyphenyl)butanenitrile	3dp*	-244.7*
	4dp*	-282*