Molecular Modeling of the Metabolism of Acetaminophen and Acetaminophen-like Molecules
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ABSTRACT

Molecular Modeling of the Metabolism of Acetaminophen and Acetaminophen-like Molecules

by

Laura Rebecca Hofto

Paracetamol, or acetaminophen, is a common analgesic and fever-reducer. There are three major metabolic pathways for acetaminophen in the liver. The final products of each pathway are nontoxic and are excreted by the kidneys. Cresol and similar molecules are competitors with acetaminophen for metabolism in the gut. We can compare the metabolism of acetaminophen with alternate molecular structures—such as cresols—using a QSAR analysis of each molecule. We compute the dipole moments, hydrogen bonding capabilities, electron density and HOMO/LUMO energies of these molecules in order to understand how these molecular properties contribute to each molecule's interaction with enzymes during each possible path of metabolism. We also apply correlated quantum mechanical methods, such as MP2, and DFT methods to the interaction of acetaminophen and its cresol analogs with the active site of uridine 5'-diphosphoglucuronosyltransferase (UGT) which is responsible for its main metabolic pathway. From these calculations we can determine the strength of the binding of the ligands in the enzyme active site; based on these results, we can offer an electronic level of explanation for the known interference of *p*-cresol in the metabolism of acetaminophen. Further, we can offer predictions on how *o*-cresol and *m*-cresol might interfere in the same metabolic pathway.

1 Introduction

1.1 Acetaminophen and Acetaminophen Metabolism

Paracetamol, or acetaminophen, is a popular analgesic and fever-reducer; however, acetaminophen does not have anti-inflammatory properties like similar over-the-counter drugs.[1] Acetaminophen, known commonly as Tylenol, is an orally administered over-the-counter drug that relieves pain caused by such conditions as headaches, allergies, arthritis, cold and flu; Tylenol is beneficial because it is safe for children, and it is likely the best fever-reducer drug.[2] Additionally, Tylenol does not hold a risk factor of causing stomach ulcers like other pain relievers including non-steroidal anti-inflammatory drugs (NSAIDs). Nonetheless, acetaminophen can have adverse effects on the liver if the proper amount is exceeded. Acetaminophen can be metabolized through three pathways—glucuronidation, sulfation, and glutathione (GSH) conjugation. Glucuronidation and sulfation involve the addition of a functional group to the hydroxyl group of paracetamol resulting in nontoxic metabolites while the latter metabolic pathway creates a toxic intermediate that can lead to drug-induced hepatotoxicity if it is not detoxified with a tripeptide called glutathione (GSH).[1]

Acetaminophen is similar to aspirin in that it has pain-relieving and antipyretic qualities, but acetaminophen cannot be used for anti-inflammatory therapy.[1] In the body, lipids known as prostaglandins (PGs) are mediators of inflammation, and certain NSAIDs such as aspirin, ibuprofen, and naproxen target the synthesis of PGs to decrease inflammation.[1] The synthesis of prostaglandins is catalyzed by cyclooxygenase (COX) enzymes. COX-2 increases levels of PGs during inflammation, and COX-1 is

responsible for maintaining normal platelet and kidney functions, as well as the integrity of the stomach lining.[1] A recently discovered isozyme in the central nervous system, COX-3, is also thought to have a role in the production of prostaglandins.[3] Acetaminophen, unlike aspirin, is not a COX inhibitor in peripheral tissues; therefore, it does not exhibit significant anti-inflammatory effects.[1]

Acetaminophen metabolism, like most xenobiotic metabolism, largely occurs in the liver. In general, there are two main steps of drug metabolism. Phase I reactions are commonly oxidation reactions that add a polar functional group to the parent compound in order to produce electrophilic intermediates.[4] These metabolites may continue through phase II reactions or they may be eliminated. Acetaminophen is largely detoxified in the liver by the phase II reactions, glucuronidation and sulfation. However, a small amount of paracetamol is converted to a highly toxic metabolite by cytochrome P450-catalyzed oxidation in a Phase I reaction.[4] If excessive amounts of acetaminophen are consumed by a person, then the toxic metabolite builds up in the cells, and hepatotoxicity can occur.[4]

Glucuronidation and sulfation are the major pathways responsible for producing approximately 95% of acetaminophen's total excreted metabolites.[1] The phase II reactions are often known as conjugation reactions because they involve the addition of a group or molecule to the drug or metabolite.[5] The products or conjugates of these reactions are typically water soluble, and they are more easily excreted by the kidneys.[5] During glucuronidation, acetaminophen, or *para*-acetylaminophenol, gains a carboxylic acid group called glucuronic acid. Glucuronic acid is structurally similar to glucose, and it is attached to the –OH group of acetaminophen.[1] The enzyme, uridine 5'-diphospho-

glucuronosyltransferase (UDP-glucuronosyltransferase, UGT), catalyzes the glucuronidation reaction in the liver.[1] The resulting conjugates of the reaction are known as glucuronides, and these metabolites are nontoxic and easily excreted from the body.[1]

Sulfation is a phase II reaction that also plays a role in paracetamol metabolism. During sulfation, the sulfotransferase, 3'-Phosphoadenosine-5'-phosphosulphate (PAPS), loses a sulfate group to become 3'-Phosphoadenosine-5'-phosphate (PAP); the lost sulfate ion attaches to the –OH of acetaminophen to create a nontoxic sulfate that is removed through the kidneys.[5]

Although sulfation and glucuronidation are the most common metabolic pathways of acetaminophen, about 5% of the metabolites result from glutathione (GSH) conjugation.[1] When a person exceeds the proper dose of acetaminophen (4g/day for an adult), glucuronidation and sulfation are no longer sufficient in metabolizing the drug because these pathways are saturated. As a result, the cytochrome P-450 enzymes metabolize acetaminophen in a phase I oxidation reaction creating a toxic intermediate called N-acetylbenzoiminoquinone (NAPQI).[5] Usually glutathione-S-transferases conjugate these resulting metabolites to the detoxifying cosubstrate, glutathione (GSH).[4] As long as there is sufficient GSH for conjugation, hepatotoxicity will not occur; however, hepatic GSH is depleted quickly, and the toxic NAPQI builds up in the liver.[1] The NAPQI reacts with nucleophilic groups of cellular proteins which eventually results in liver necrosis or cell death.[1]

The liver is most vulnerable to acetaminophen poisoning because paracetamol metabolism occurs there. An overdose of acetaminophen may initially cause dizziness and disorientation, and a dose of 15g of acetaminophen can be fatal.[1] The hepatotoxicity

from the acetaminophen overdose can cause centrilobular necrosis and acute renal tubular necrosis.[1] In centrilobular necrosis, the hepatocytes around the terminal hepatic vein begin to die; massive hepatic necrosis leads to liver failure.[4] Additionally, acute tubular necrosis causes cell death in the kidneys which may result in acute renal failure.[4]

After ingesting more than the recommended dosage of acetaminophen, a person may experience nausea or vomiting; however, liver injury usually appears after 24-36 hours.[1] A serum acetaminophen concentration measurement can be used to determine the severity of the poisoning; if the patient's serum level is greater than 150-200mg/L after four hours, then he is at risk for liver damage.[1] During the first two hours after ingestion, the initial treatment for the patient is activated charcoal; activated charcoal is the most common gastrointestinal decontamination procedure because it adheres to the paracetamol, reducing its gastrointestinal absorption. The antidote, acetylcysteine, should be given at least 8-10 hours after the overdose of acetaminophen; acetylcysteine functions like glutathione (GSH) in that it binds to the toxic metabolite in the hepatocytes.[1] If the paracetamol poisoning is not properly treated, then a liver transplant could be necessary due to severe liver damage.

1.2 Interferences in Acetaminophen Metabolism

Alcohol abuse can increase the toxicity of acetaminophen; as a result, it is recommended that regular drinkers take less than the regular acetaminophen dosage of 4 g/day.[1] The metabolism of ethanol induces the cytochrome P-450 xenobiotic-metabolizing enzyme, CYP2E1.[4] As a result, the CYP2E1 enzyme increases the metabolism of acetaminophen into the toxic metabolite, NAPQI.[4] Therefore, regular consumption of alcohol with the

use of acetaminophen causes more damage to the liver because the toxic paracetamol metabolic pathway is favored.

Certain molecules can compete for the desired metabolism of acetaminophen in the gut. One such molecule, *para*-cresol, is produced by the microorganisms in the body, and it is known that *p*-cresol competes with acetaminophen for sulfation in the liver.[6] In other words, less acetaminophen will be sulfonated to non-toxic metabolites if there are large amounts of *p*-cresol in the liver.

p-Cresol, or 4-methylphenol, has a similar structure to acetaminophen—it is a phenol molecule with a methyl group attached to the benzene ring; *p*-cresol has two additional isomers, *meta*-cresol and *ortho*-cresol.[7] Cresols can be liquids or solids, and they are obtained through the distillation of coal tar or petroleum; they can be chemically synthesized as well. Cresols are present in a variety of industrial, consumer and food products; for example, *m*-cresol is used in the production of fragrances, insecticides, herbicides and antioxidants while *o*-cresol is present in disinfectants.[7] *p*-Cresol can be found in food products, and it is used in the formulation of antioxidant agents that stabilize rubber, motor fuels, lubricating oil and polymers.[7] In addition, *p*-cresol is a common intermediate in dyes and fragrances.[7]

People can absorb cresols in a variety of day-to-day activities. *p*-Cresol is endogenously derived from tyrosine inside the gut by microorganisms, but people can also be exposed to cresols from foods, beverages and through the combustion of certain materials.[7] Buckwheat honey, smoked fish, fried bacon, cheese, cow milk and whiskey are all known to contain cresols.[7] Also, humans may inhale cresols or cresol isomers

from vehicle emissions, cigarette smoke, waste incinerator emissions, disinfectant products and following the combustion of coal and wood.[7]

Cresols can also be absorbed through the skin, gastrointestinal system and respiratory tract, and they are metabolized through the same pathways as acetaminophen. Sulfate and glucuronic acid may be conjugated to the –OH group of the cresol in order to make nontoxic metabolites.[7] It is experimentally known that *p*-cresol undergoes metabolism in the same pathways as acetaminophen; thus molecular/electronic structure level calculations can be undertaken to explain the mechanism of this competition between *p*-cresol and acetaminophen.

1.3 Computational Chemistry

One of acetaminophen's main structural features is an aromatic ring; thus acetaminophen's interactions with other molecules will feature a large contribution from dispersion forces. Methods used to study these interactions must be able to accurately model dispersion interactions. Commonly used methods for investigating protein/ligand interactions where electrostatics are the most important forces include classical molecular mechanics methods, such as those employed in popular modeling packages such as AMBER,[8,9] or, occasionally, semi-empirical quantum mechanical methods such as AM1,[10] PM3,[11] and others. These classical and semi-empirical methods do an excellent job in modeling electrostatically dominated systems, but when applied to dispersion problems, these methods are usually supplemented by an empirical "dispersion potential" which is often calibrated to work with the system of interest.

Rigorous, quantitative treatment of dispersion requires use of a quantum mechanical method which takes into account electron correlation.[12] The basic molecular orbital-based quantum mechanical method used in chemistry applications is the Hartree-Fock (HF) method. This method assumes several things. First, the electrons are assumed to move in a field of static nuclei (the Born-Oppenheimer, or clamped-nucleus approach). HF also assumes that the molecular wave function (ψ) can be expressed as a single determinant of molecular orbitals (ϕ):

$$\psi_{HF}(r_1, r_2, ...r_n) = \tfrac{1}{n!}\det[\phi_1(r_1)\phi_2(r_2)...\phi_n(r_n)], \qquad 1)$$

$$\phi_i(r_i) = \sum c_j \chi_j(r_i) \qquad 2)$$

In practice, the molecular orbitals are usually expressed in a basis set of auxiliary functions (χ), commonly Gaussian functions, but sometimes exponential, or Slater-type functions. The HF model also assumes that each electron 'sees' the forces due to the other electrons only in an averaged sense. This approximation causes HF to lose electron correlation, or the instantaneous interactions of electrons, which is necessary to model dispersion. Corrections must be made to the HF model in order to add in correlation, and these methods are referred to as post-HF, or correlated methods. Of these methods, the least expensive method (in terms of computer resources and processing time) which provides meaningful data is the second order Moller Plessett theory (MP2). This method considers contributions to the molecular energy and wave function due to doubly excited determinants:

$$\psi_{MP2}(r_1, r_2, ...r_n) = \psi_{HF} + \sum_{i<j}\sum_{a<b} C_{ij}^{ab}\Phi_{ij}^{ab} \qquad 3)$$

There are more accurate and expensive post-HF methods available, such as the near-industry standard coupled cluster singles, doubles, and perturbative triples [CCSD(T)], but these are not feasible for application to large protein/ligand complexes. In this work, the MP2 method is used as the standard, and it is considered to be correct. However, it is not as accurate as CCSD(T), but in this work *differences* in energies are considered, rather than absolute energies, and so the inaccuracy is not a serious issue.

Another major focus of the work of the author's research group is in identifying accurate, inexpensive, single-determinant methods. Single determinant methods are methods which, like HF, do not make explicit reference to excited states and thus cost about as much time to run as HF, compared with the increased cost of MP2, which can take orders of magnitude more time. These methods are called Density Functional Theory (DFT) methods.[13] The DFT methods (specifically the Kohn-Sham implementation of DFT which are used[14]) use an exchange/correlation potential, ν_{XC}, to add electron correlation energy within a single determinant framework;[15]

$$E[\rho,\nabla\rho] = E_T[\rho] + E_J[\rho] + E_{ext}[\rho] + E_{XC}[\rho,\nabla\rho] \qquad 4)$$

$$\rho = 2\sum \phi_{KS}^2 \qquad 5)$$

here, ρ is the electron density obtained from summing over the squares of the Kohn-Sham orbitals, ϕ_{KS}, and the subscripts in Eq. 4 refer to T, the kinetic energy, J, the Coulomb energy, ext, the external potential, and XC, the exchange/correlation energy. While Kohn-Sham DFT is a formally exact theory, the form of the XC potential, and thus energy, is not known; different approximations are used for correlation. These

correlation functionals (functions of the electron density) work well in some cases and not others.

1.4 Previous Work

Previous work by the current author and related work has examined the ability of DFT methods to model interactions important in weakly bound protein-ligand systems (such as the system being studied here) and to replicate MP2 methods for these interactions. Godfrey-Kittle and Cafiero studied interaction energies of sandwich and T-shaped dimers of monosubstituted benzene rings using DFT methods and MP2.[16] The group of dimers included four monomers with electron-donating groups and four monomers with electron-withdrawing groups. In these systems, the interaction energies of the dimers had electrostatic and pure dispersion components; Hartree Fock calculations were performed with increasing basis set sizes in order to determine the extent of the electrostatic component of the interaction energy.[16] MP2 was the standard for the binding energies of the two orientations of the dimers. Although DFT methods usually underestimate dipole moments and polarizabilities compared with MP2, they tend to replicate MP2 for ring-ring interaction energies, as in these benzene dimer interactions.[16] Godfrey-Kittle and Cafiero used the DFT methods, TPSS, HCTH407, X3LYP, B3LYP, and P3LYP, and found that HCTH407 was the best DFT functional for predicting relative stability and binding energies.[16] Also, the DFT functionals were most accurate when representing the T-shaped orientation of the dimers.[16]

Another project by van Sickle, Culberson, Holzmacher and Cafiero used DFT and MP2 methods to evaluate the electronic interactions between indole and monosubstituted

benzene in seven different stable conformations.[17] Indole and benzene can be represented by sandwich-type conformations as well as T-shaped conformations, and the interaction between the rings contain electrostatic and dispersion energies. Again, in this study, MP2 was the standard, and the DFT methods, HCTH407, SVWN, and B3LYP, were used to determine interaction energies between indole and each substituted benzene molecule.[17] B3LYP was not accurate for predicting the energies, and while SVWN overestimated the interaction energies, it closely mimicked the trends of MP2. HCTH407 was overall the best DFT method for replicating the MP2 values for these systems.[17]

The current author, van Sickle and Cafiero studied sandwich-type interactions between benzene and fourteen polyaromatic molecules to model intercalation.[18] Intercalation occurs when a molecule becomes wedged between two other molecules; intercalation is pertinent to DNA because the intercalating molecule can result in a widening between base pairs that will cause the DNA to unwind.[18] Intercalating molecules often contain nitrogen atoms in the ring so Hofto, van Sickle and Cafiero made intercalation candidates with varied numbers of nitrogen atoms. They also calculated the interaction energy within each intercalation candidate/benzene molecule complex using MP2, HCTH407, SVWN, B3LYP and HF.[18] MP2 and SVWN produced results that were in agreement for the optimal distances between the molecules in the complexes, but HCTH407, B3LYP and HF did not model the systems well. The data showed that the interaction between molecules in the sandwich complexes increased with increasing numbers of nitrogen atoms on the intercalation candidates.[18]

Work by M. Hofto, Godfrey-Kittle and Cafiero focused on the enzyme, phenylalanine hydroxylase (PheOH), which catalyzes phenylalanine to tyrosine in the

body.[19] Tyrosine is necessary for hormones and neurotransmitters (such as dopamine), and a mutation in the active site of PheOH can disrupt tyrosine metabolism and cause the disease, phenylketonuria (PKU). The residue, Phe254, is located in the active site of PheOH, and it helps facilitate the metabolism of phenylalanine. It is known that a mutation in the active site (F254I) will induce PKU because phenylalanine hydroxylase will have decreased binding interaction between the enzyme and substrate.[19] Hofto, Godfrey-Kittle and Cafiero used MP2 and DFT methods to study the changes in protein-ligand binding due to Phe254 point mutations in order to predict which mutations might result in PKU symptoms.[19] The authors analyzed the interaction energies between Phe254 and phenylalanine as well as five other phenylalanine derivatives; then they modeled six various potential mutants at the 254 position with the phenylalanine derivatives.[19] MP2 energies were the standard model for these ligand/protein systems because they include the total electrostatic plus dispersion interactions. The DFT methods, HCTH407 and B3LYP, were used to find the aromatic interaction energies, and it was shown that HCTH407 outperformed B3LYP and actually approached the MP2 results. DFTs are useful in biological modeling because MP2 is quite expensive for large-scale modeling.[19]

M. Hofto, Cross and Cafiero continued studying how DFTs model biological systems with their work involving another enzyme in the phenylalanine metabolic pathway, tyrosine hydroxylase (TyrOH).[20] PheOH catalyzes the hydroxylation of phenylalanine to tyrosine, and TyrOH catalyzes the hydroxylation of tyrosine to L-DOPA; L-DOPA is eventually converted to dopamine and other neurotransmitters. Both PheOH and TyrOH use the aromatic cofactor, tetrahydrobiopterin (BH4), for the

hydroxylation of phenylalanine and tyrosine; BH4 forms π-stacking and T-shaped interactions with the residues Phe254 and Tyr325 in the active site of PheOH and with the residues Phe300 and Phe309 in the active site of TyrOH.[20] Mutations to any one of these residues will result in decreased binding interactions between BH4 and the enzyme resulting in disorders such as PKU, Parkinson's disease or depression. These enzyme-substrate systems contain interactions between aromatic molecules causing the binding energy to be largely dominated by dispersion-type interactions. Hofto, Cross and Cafiero used the DFT methods, B3LYP, HCTH407, SVWN, as well as MP2 and HF to calculate the changes in binding energy in the active sites of PheOH and TyrOH when the wild type residues are mutated to six different amino acids.[20] In the sandwich complex calculations of BH4/Phe300 and BH4/Phe 254 interaction energies, B3LYP and HCTH407 did not mirror the results of MP2 correctly, but SVWN followed the MP2 results and trends fairly well.[20] However, in the T-shaped complex results, HCTH407 and B3LYP were actually better than SVWN at producing results similar to MP2 for the BH4/Phe309 complex, but SVWN was again the most accurate DFT method for the BH4/Tyr325 complex.[20]

The current author, Lee and Cafiero focused on the biosynthesis pathway of serotonin in order to continue the research concerning the decrease in binding energy between aromatic ligands and aromatic residues due to mutations in the biological system.[21] Serotonin is synthesized in a two step process: dietary tryptophan is hydrolyzed to 5-hydroxy-tryptophan (5-HT) by the enzyme, tryptophan hydroxylase (TrpOH); then 5-HT is converted to serotonin by the enzyme, aromatic amino acid decarboxylase (AAAD). In each pathway, an aromatic ligand (tryptophan or 5-HT) is

held in place through induction/dispersion interactions with aromatic residues in the active site of the enzyme (TrpOH or AAAD).[21] Decreased binding in the active site of either enzyme may lead to a decrease in serotonin, and symptoms of depression, anxiety and obsessive-compulsive disorder may arise. In this project, the authors mutated the major residues in the active sites of the enzymes (Phe241/tetrahydrobioterin in TrpOH and Phe103/carbidopa in AAAD) to a variety of amino acids in order to determine any changes in binding energies of aromatic ligands.[21] They used MP2, B3LYP, HCTH407 and SVWN, as well as the scaled LRH DFT.[21] The LRH DFT includes a coefficient, n, that varies the amount of gradient correction in a GGA functional so that the DFT may range from pure SVWN (n=0) to a modified PBE functional (n=1).[21] The LRH DFT is a useful starting point for future work in creating a novel DFT method that can optimally model the dispersion forces between aromatic rings. Of the DFT methods, SVWN was best at mimicking the MP2 results, although the interaction energies were usually larger than MP2 results. The LRH DFT results showed that a larger coefficient was needed for mutations with larger side chains in order to produce results that approached MP2.[21]

Finally, more recent research by Kee, *et al*. dealt with modeling of aromatic interactions in the binding of ligands to 3-hydroxy-3-methyglutaryl-coenzyme A (HMGCoA) reductase.[22] HMGCoA reductase is a target for statin drugs because it is important in the synthesis of cholesterol. The authors studied induction/dispersion interactions between ligands and residue tyrosine 479 in the active site of HMGCoA reductase because these interactions are currently not targeted by statin drugs; it is possible that these interactions could be useful for the design of future statin drugs.[22] HMGCoA has a large coenzyme A “tail”; therefore, a truncated portion was used in

complex with tyrosine 479 to model the larger overall molecule.[22] SVWN, HCTH407, B3LYP and MP2 were the methods used to calculate the interaction energy between Y279 and HMGCoA and three mutant residue complexes. SVWN produced similar results to the standard MP2 binding energies, and it was shown that Y479 in HMGCoA reductase binds to HMGCoA better than other mutant residues.[22] Therefore, statin drug molecules, which are competitive inhibitors, could be more effective if they target the Y479/HMGCoA complex.

2 Computational Methods

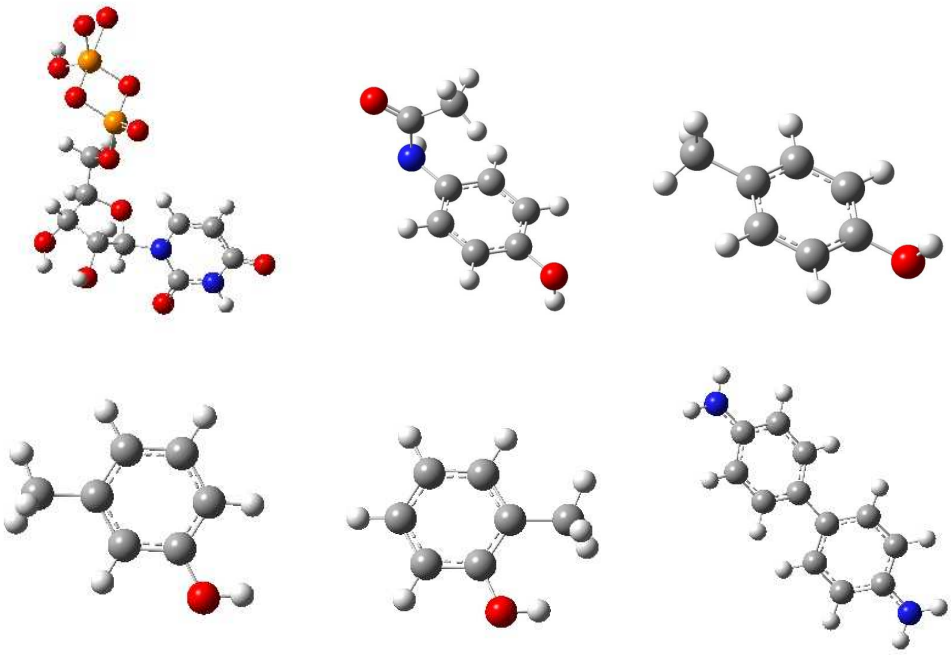
The crystal structure of the wild-type enzyme for the glucuronidation pathway, uridine 5'-diphospho-glucuronosyltransferase (UGT) was used to isolate a ligand, UDP, in the active site of the enzyme.[23] ArgusLab was used to determine the important residues in the active site by their proximity to the ligand UDP. The active site of UGT contains the following amino acids: tyrosine84, histidine308, three arginines (86, 156, 161) and two aspartic acids (194, 196). Hydrogen atoms were added to the crystal structure, and the active site amino acid residues were capped with OH to maintain electrostatic neutrality in the structure. Then, the counterpoise-corrected interaction energies between the ligand, UDP, and each amino acid in the active site were calculated using MP2, HCTH407, SVWN and B3LYP with the 6-311+G* basis set.

2.1 QSAR Methods

The structures of possible glucuronidation competitors, acetaminophen, *p*-cresol, *m*-cresol, *o*-cresol and benzidine (Figure 1), were optimized using HCTH407/6-31G in the

gas phase, aqueous phase and chloroform solution phase (COSMO used for solution calculations) in order to understand how the molecules behave in the blood and in a nonpolar environment like a cell membrane. The QSAR analysis of each molecule was computed to correlate the metabolism of acetaminophen with alternate molecular structures. In addition to solvation energies, the dipole moments and HOMO/LUMO energies for the water structures were found for each optimized structure in order to understand how the molecules might interact with UGT during glucuronidation.

Figure 1: Suite of molecules, (left to right, top to bottom) UDP, acetaminophen, *p*-cresol, *m*-cresol, *o*-cresol, benzidine.



2.2 Interaction Energy Calculations

The optimized aqueous phase structures of acetaminophen, *p*-cresol, *m*-cresol and *o*-cresol were docked in the UDP active site of UGT using the ArgusDock docking engine

and the Ascore scoring function; these molecular structures were oriented as closely to the wild-type UDP ligand pose as possible with ArgusLab (see Figures 1-4 below). Counter-poise corrected interaction energies between these ligands and each of the active site residues were calculated with the 6-311+G* basis set using MP2, SVWN, HCTH407 and B3LYP (PQS software).

3 Results and Discussion

3.1 QSAR Analysis

Molecular properties of ligands are closely related to their biological activity; for example, aqueous and chloroform solvation energies can be used to understand how molecular structure is related to movement and how a ligand electronically binds in an enzyme's active site. Also, a ligand's dipole moment shows how the molecule orients itself in the active site; HOMO/LUMO energies demonstrate a ligand's potential to bind in the active site by its tendency to donate or accept electrons. The QSAR data (Table1) for UDP, acetaminophen, *p*-cresol, *m*-cresol, *o*-cresol and benzidine helps characterize which ligands might compete for the UGT active site during glucuronidation.

UDP's large size is reflected by its overall large solvation energies and dipole moments compared to all the other ligands. Also, there appears to be a common ratio between the dipole moments and solvation energies in blood and cell membranes for acetaminophen and the cresols. The HOMO energies for all the ligands are more similar than the LUMO energies which probably implies that these molecules bound in this active site are likely to donate electrons rather than accept electrons; in other words, the ligands probably have the ability to bind in the same manner.

Table 1: QSAR data for UGT ligands calculated using HCTH407/6-31G. HOMO/LUMO energies in Hartrees.

	ΔE Solvation (H_2O, Kcal/mol)	**ΔE Solvation ($CHCl_3$, Kcal/mol)**	**Dipole (gas, debye)**	**Dipole (water, debye)**	**HOMO**	**LUMO**
Acetaminophen	-14.67	-10.03	4.20	5.69	-0.19	-0.04
p-cresol	-7.47	-5.17	1.64	2.21	-0.19	-0.03
m-cresol	-7.48	-5.17	1.19	2.54	-0.20	-0.03
o-cresol	-7.01	-4.80	1.90	2.55	-0.20	-0.03
benzidine	-13.01	-8.92	0.11	0.02	-0.15	-0.03
UDP	-31.31	-18.14	6.09	9.42	-0.22	-0.19

3.2 Interaction Energies

The wild-type ligand, UDP, is bound in the active site of UGT, and it interacts primarily with five amino acid residues (tyrosine, histidine, two arginines and one aspartic acid) in the active site. The MP2 calculations for the interaction energies (Table 2) are considered to be the standard results for the enzyme-ligand binding complex. UDP is known to be a large polar molecule which explains the large MP2 interaction energies with the residues. Also, the ligand binds well in the active site as evidenced by the large negative values for all of the interaction energies. In this case, the DFT methods, SVWN, HCTH407 and B3LYP, were not as accurate as MP2; however, the DFT methods modeled the interaction of UDP with the aspartic acid residue well because DFTs can make a good approximation of hydrogen bonds.

Table 2: Interaction energies of UDP with each amino acid residue in the active site of UGT, energies in Kcal/mol.

	MP2	SVWN	B3LYP	HCTH
tyr	-66.07	-19.88	-13.78	-5.78
his	-41.63	-23.25	-22.87	-18.85
arg1	-66.87	-38.78	-27.85	-26.28
arg2	-16.16	-10.77	-9.23	-11.43
asp	-29.63	-30.00	-21.94	-20.12

Acetaminophen is a much smaller molecule than UDP (Figure 2); therefore, it has noticeably smaller interaction energies (Table 3) with each residue in the UGT active site. MP2 results are considered to be the standard for the binding energies, and the DFTs do not mimic the MP2 results for acetaminophen. DFT methods tend to model hydrogen bonding and dipole-dipole interactions best which means that there is probably predominantly induction and dispersion forces in the active site when acetaminophen is present. Due to acetaminophen's structure, the molecule is oriented slightly differently from UDP in the active site, and it binds with two other amino acid residues: arginine and aspartic acid. These additional amino acids are also pertinent to the active site of UGT when the cresols are bound to the enzyme.

Table 3: Interaction energies of acetaminophen with each amino acid residue in the active site of UGT, energies in Kcal/mol.

	MP2	SVWN	B3LYP	HCTH
tyr	13.25	2.98	24.92	30.13
his	-0.07	-0.05	-0.07	-0.06
arg1	1.03	-4.77	1.85	2.49
arg2	-1.43	-4.23	2.02	0.71
arg3	-0.32	-0.18	-0.13	-0.25
asp1	-1.99	-1.86	-1.26	-2.19
asp2	2.02	-2.79	3.17	3.70

p-Cresol is a known competitor of acetaminophen metabolism in the liver so it is essential to compare the binding of both molecules in the UGT active site (Table 4, Figure 3). Both ligands have repulsive interactions with tyrosine84, and the MP2 values are quite similar for acetaminophen/Tyr84 and *p*-cresol/Tyr84. Also, the interaction energy with histidine308 is approximately zero for both ligands, and the binding energies between the ligands and the arginine residues are mainly small, negative (binding) values for acetaminophen and *p*-cresol. Finally, both molecules appear to interact with the aspartic acid residues in the same way because they have the same trends for these residues—aspartic acid1 is an attractive interaction while asp2 is repulsive. SVWN is almost an acceptable method for the *p*-cresol binding interactions because *p*-cresol is almost a pure ring, and SVWN can model these kinds of structures well. It is apparent by the similar trends of acetaminophen and *p*-cresol binding that the structures were docked in the same orientation in the active site of UGT.

Table 4: Interaction energies of *p*-cresol with each amino acid residue in the active site of UGT, energies in Kcal/mol.

	MP2	**SVWN**	**B3LYP**	**HCTH**
tyr	17.17	10.21	30.44	33.35
his	0.03	0.05	0.04	0.04
arg1	-2.31	-3.40	0.81	-1.41
arg2	-1.74	-3.85	-0.47	-0.84
arg3	-3.78	0.21	0.24	-0.29
asp1	-0.53	-1.05	0.24	-0.63
asp2	4.66	2.05	6.07	5.68

The QSAR data and interaction energies show that *o*-cresol and *m*-cresol will probably behave like *p*-cresol in terms of binding. These molecules were docked in the active site of UGT like *p*-cresol so that predictions could be made about these ligands'

ability to compete with acetaminophen in the metabolic pathway of glucuronidation (Figure 4-5). *o*-Cresol and *m*-cresol have similar interaction energy trends as *p*-cresol in the active site of the enzyme (Table 5-6); for *o*-cresol, the MP2 results are almost identical to those of *p*-cresol, with the exception of the tyrosine, arginine3, and aspartic acid2 residues. *m*-Cresol also follows the MP2 trends of *p*-cresol, although the strength of binding with the arg2 and arg3 residues differs from *p*-cresol; additionally, *m*-cresol, unlike *p*-cresol, actually forms an attractive interaction with the asp2 residue.

Table 5: Interaction energies of *o*-cresol with each amino acid residue in the active site of UGT, energies in Kcal/mol.

	MP2	**SVWN**	**B3LYP**	**HCTH**
tyr	-2.74	-4.74	4.13	4.57
his	0.02	0.05	0.05	0.04
arg1	-2.38	-4.75	0.70	-0.49
arg2	-1.05	-0.70	-0.11	-1.04
arg3	0.23	0.40	0.42	0.27
asp1	-0.44	-0.31	-0.16	-0.44
asp2	-3.21	-5.96	-1.82	-2.07

Table 6: Interaction energies of *m*-cresol with each amino acid residue in the active site of UGT, energies in Kcal/mol.

	MP2	**SVWN**	**B3LYP**	**HCTH**
tyr	33.66	20.92	49.06	53.63
his	0.08	0.10	0.09	0.09
arg1	-1.38	-4.97	1.76	1.42
arg2	-11.00	-19.21	-10.78	-10.68
arg3	-0.03	0.13	0.20	0.01
asp1	-0.58	-0.48	-0.17	-0.71
asp2	-7.74	-11.36	-7.10	-6.50

Figure 2: Acetaminophen in the active site of UGT.

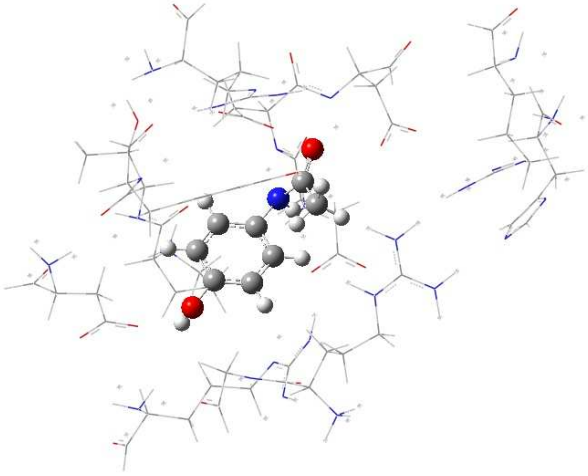


Figure 3: *p*-Cresol in the active site of UGT.

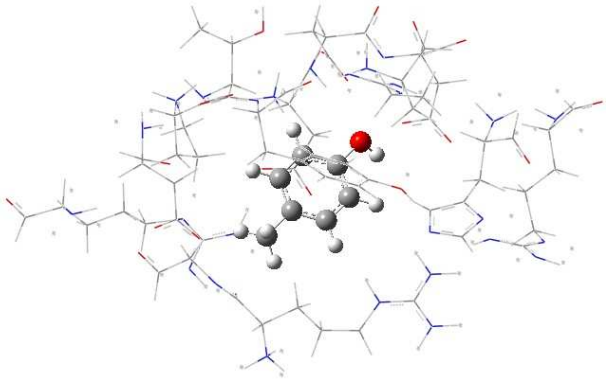


Figure 4: *o*-Cresol in the active site of UGT.

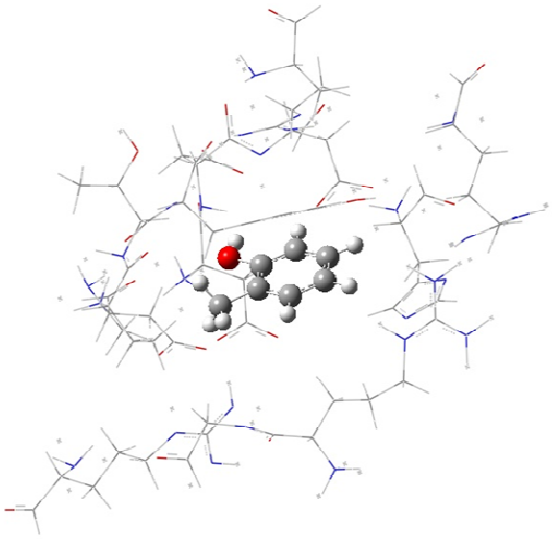
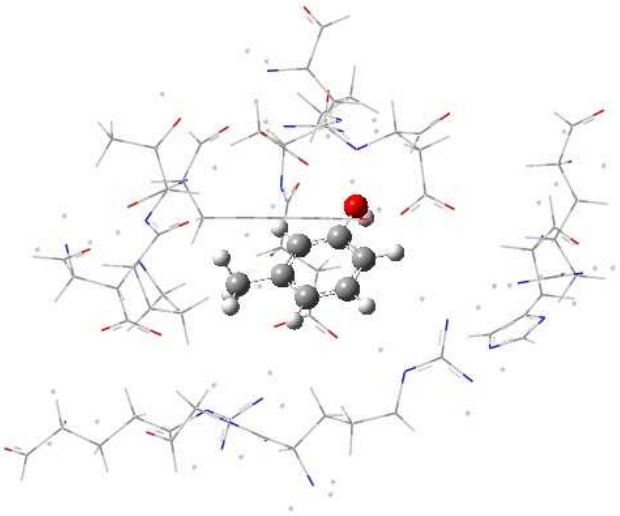


Figure 5: *m*-Cresol in the active site of UGT.



3.3 Physiological Implications

The interaction energies of each ligand with all the residues in the active site of UGT can be combined to find the total binding energy for the ligand in the active site. These total interaction energies can be compared to determine how well a particular molecule binds with UGT, and they can be used to predict which structures would compete for the active

site and therefore the metabolic pathway of glucuronidation. Acetaminophen and *p*-cresol have almost the same total interaction energies (Table 7) which explains why *p*-cresol is a known competitor for acetaminophen metabolism.

m-Cresol has effectively the same total interaction energy in the enzyme active site meaning it will probably act much like *p*-cresol during glucuronidation. It will likely form the same strength of interactions with the amino acids in the active site as acetaminophen, and *m*-cresol will likely compete with acetaminophen for metabolism. *o*-Cresol is probably an extremely strong competitor of acetaminophen because it binds very well in the active site of UGT; the large difference in binding is likely due to the overall dipole of the *o*-cresol molecule. The proximity of the electron-withdrawing OH group to the electron-donating methyl group of *o*-cresol causes a localized dipole that wants to interact strongly with the polar residues in the active site of UGT.

Table 7: Total interaction energies of each ligand with all residues in the active site of UGT, energies in Kcal/mol.

	MP2
acet	12.48
pcresol	13.51
ocresol	-9.57
mcresol	13.01

4 Conclusions

The total binding energies for acetaminophen and *p*-cresol are extremely close values which supports the idea that *p*-cresol is a competitor for acetaminophen metabolism. Due to the similar trends in the QSAR analysis and total binding energies of *m*-cresol and *o*-cresol, it is likely that these molecules will also compete for the active site of the enzyme, UGT. Competition with acetaminophen for the glucuronidation pathway may cause

acetaminophen to build up to toxic levels in the liver so it is important to be wary of cresol exposure while using paracetamol.

Overall the DFT methods, SVWN, HCTH407, B3LYP, did not provide interaction energy results that completely mimicked MP2; SVWN performs well when dispersion forces dominate the interaction while B3LYP performs well when electrostatic forces dominate the interaction. HCTH407 is seemingly caught in the middle of these extremes. However, the DFT methods were reasonable approximations for the much more expensive MP2 method.

References

1. Katzung, Bertram G. Basic and Clinical Pharacology, 2nd Ed. McGraw-Hill: New York, 2007.
2. From http://www.tylenol.com/. Accessed on 1 May 2010.
3. Cannon, Joseph G. Pharmacology for Chemists, 2nd Ed. Oxford University Press: Oxford, 2007.
4. Kumar, V., Abbas, A.K., Fausto, N. Robbins and Cotran Pathologic Basis of Disease, 7th Ed. Elsevier: Philadelphia, 2005.
5. Thomas, Gareth. Medicinal Chemistry: An Introduction, 2nd Ed. Wiley and Sons Ltd: Chichester, 2007.
6. Clayton,T.A., Baker, D., Lindon, J.C., Everett, J.R., Nicholson, J.K., *Proc. Nat. Acad. Sci. USA*, **106**, 14728 (2009).
7. Sanders, J.M., et al, NIH Publication No. 08-5891 (2008).
8. Case, D.A., Cheatham, T.E., III, Darden, T., Gohlke, H., Luo, R., Merz, Jr., K. M., Onufriev, A., Simmerling, C., Wang, B., and Woods. R., *J. Computat. Chem.* **26,** 1668-1688 (2005).
9. Ponder J. W., and Case, D. A., *Adv. Prot. Chem.* **66,** 27-85 (2003).
10. Dewar, M. J. S., Zoebisch, E. G., Healy, E. F. and Stewart, J. J. P., *Journal of the American Chemical Society*, **107**, 3902, (1985).
11. Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209.
12. Discussion of the quantum mechanics methods described here, including the Hartree-Fock, MP2, and CCSD(T) methods may be found in any introductory computational chemistry text, such as: Jensen, F., *Introduction to Computational Chemistry*, John Wiley and Sons, 1999.
13. Hohenberg, P., and Kohn, W., *Phys. Rev.*, **136**, B864, (1964).
14. Kohn, W., Sham, L.J., *Phys Rev.*, **140**, A1133, (1965).
15. LDA, GGA, and hybrid DFT methods are discussed at length in: Koch, W., and Holthausen, M.C., *A Chemist's Guide to Density Functional Theory*, Wiley-VCH, 1999.
16. Godfrey-Kittle, A., and Cafiero, M., *Int. J. Quantum Chem*, **106**, 2035 (2006).
17. Van Sickle, K., Culberson, L.M, Holzmacher, J.L., Cafiero, M., *Int. J. Quantum Chem.*, **107**, 1523 (2007).
18. Hofto, L.R., Van Sickle, K., Cafiero, M., *Int. J. Quantum Chem.*, **107**, 112 (2007).
19. Hofto, M.E., Godfrey-Kittle, A., Cafiero, M., *THEOCHEM*, **809**, 125 (2007).
20. Hofto, M.E., Cross, J.N., Cafiero, M., *J. Phys. Chem. B*, **111**, 9651 (2007).
21. Hofto, L.R., Lee, C.E., Cafiero, M., *J. Comp. Chem.*, **30**, 1111 (2008).
22. Kee, E.A., Livengood, M.C., Carter, E.E., McKenna, M., Cafiero, M., *J. Phys. Chem. B*, **113**, 14810 (2009).
23. Y. Tone, *et al J.BIOL.CHEM*. **283**, 16801 (2008).