JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

pubs.acs.org/jcim

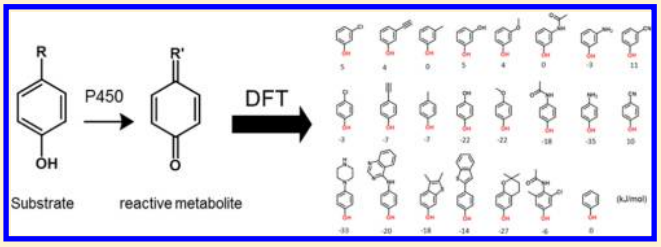
Density Functional Theory Study on the Formation of Reactive Benzoquinone Imines by Hydrogen Abstraction

Rasmus Leth, Patrik Rydberg,† Flemming Steen Jørgensen, and Lars Olsen*

Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Supporting Information

ABSTRACT: Many drug compounds are oxidized by cytochrome P450 (CYP) enzymes to form reactive metabolites. This study presents density functional theory calculations of the CYP-mediated metabolism of acetaminophen and a series of related compounds that can form reactive metabolites by hydrogen abstraction. The substitution pattern affects the activation barrier for hydrogen abstraction by up to 30 kJ/mol. A correlation (R^2 = 0.72) between the transition-state energies and the corresponding substrate radical energies has been established. Using this correlation is significantly less time-demanding than using the porphyrin model to determine the activation energies. We have used this correlation on monosubstituted phenols to rationalize the effect of the various substituents in the drug compounds. In addition to facilitating a chemical interpretation, the approach is sufficiently fast and reliable to be used as an *in silico* method in the design of new compounds with improved metabolic stability.



INTRODUCTION

Cytochrome P450 (CYP) is a large superfamily of enzymes involved in the oxidative metabolism of endogenous and exogenous compounds. The CYP family plays an important role in drug metabolism and is therefore highly relevant in the drug discovery and development process. An understanding of CYP metabolism can give important insights into the effects and side-effects of drugs.

One of the side-effects from CYP-mediated drug metabolism is the generation of reactive metabolites that can be toxic. Acetaminophen (paracetamol), a widely used over-the-counter analgesic and antipyretic, is an example on a drug forming reactive metabolites which can cause fatal kidney and liver damage.[1] Cytochrome P450 enzymes oxidize acetaminophen to form the metabolites 3-hydroxyacetaminophen and *N*-acetyl-*p*-benzoquinone imine (NAPQI) (see Figure 1). NAPQI and the much more reactive tautomeric forms of the semiquinone, *N*-acetyl-*p*-benzosemiquinone imine (NAPSQI), can covalently bind to and inactivate intracellular proteins.[2−4] This mechanism is the principal cause of acetaminophen hepatotoxicity.

Reactive metabolites formed by metabolic bioactivation of drug molecules, as well as the parent drug itself, can be responsible for hepatoxicity and adverse drug reactions. The reactive metabolites can inactivate CYPs or react with intracellular proteins. However, it remains a challenge to accurately predict the precise mechanism for the metabolism and likelihood of hepatotoxicity. Many known drugs, both marketed and withdrawn, produce reactive quinone and semiquinone imine species upon bioactivation in humans.

Detection of intermediate acetaminophen radical metabolites (NAPSQI) has been proven very difficult to perform experimentally.[2] The transformation from NAPSQI to

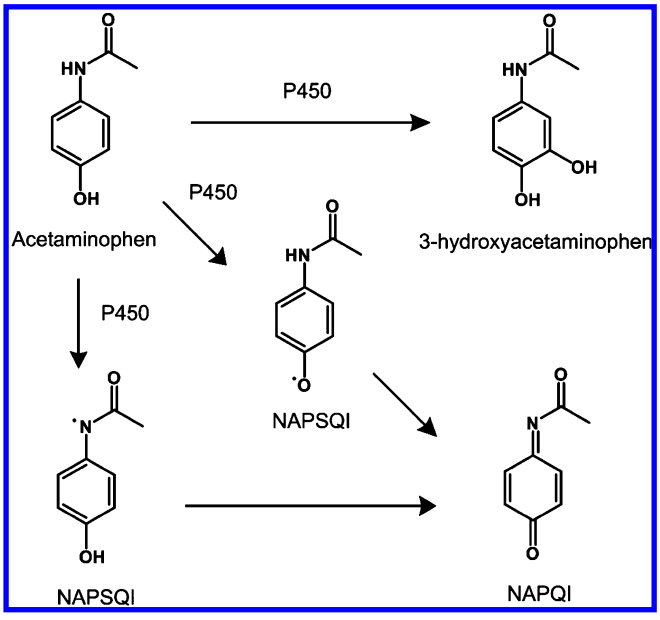


Figure 1. Acetaminophen is metabolized by cytochromes P450 to produce two metabolites, 3-hydroxyacetaminophen and NAPQI. The tautomeric NAPSQI intermediates involved in the formation of the hepatotoxic NAPQI are shown.

NAPQI is not necessarily enzyme-mediated, and in principle various redox systems present in the cells could be involved.[5−8] Therefore, the mechanism of oxidation of acetaminophen to

Received: October 29, 2014
Published: February 6, 2015

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666

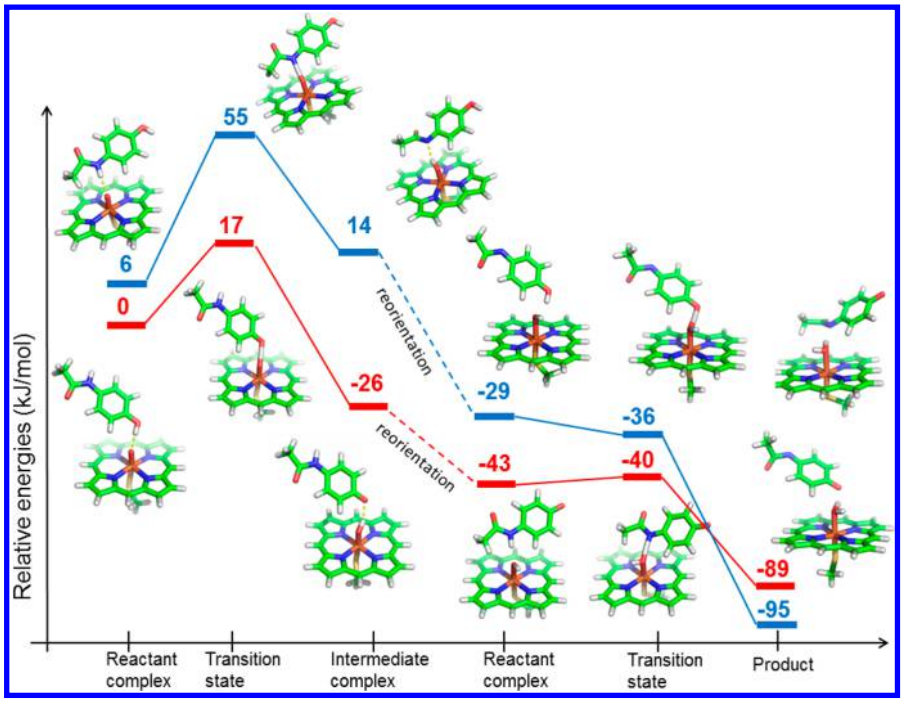


Figure 2. Reaction profile for acetaminophen doublet spin state metabolism, showing the relative energies for the initial and subsequent hydrogen abstraction from the oxygen (red) and nitrogen (blue) atoms of acetaminophen leading to the quinone imine product. The energies were obtained with UB3LYP/Bs2, including the zero-point vibrational energy.

NAPQI has been investigated in several theoretical studies using semiempirical, Hartree−Fock (HF), and density functional theory (DFT) methods. They show that formation of the phenoxy radical is energetically favored over the *N*-acetamide radical, based on the stability of the radical or the intermediate of the reaction.[9−13]

In this study, we use DFT calculations to investigate the hydrogen abstraction mechanism of acetaminophen and related drug compounds to form the reactive quinone imine compounds reviewed by Orr et al.[14] (see the Supporting Information, Table S1). These types of calculations have previously been used for studies on other CYP-mediated reaction types, like the aliphatic and aromatic hydroxylations and heteroatom oxidations, to explain trends in activation energies.[15,16] However, the trends in the N−H and O−H hydrogen abstraction reactions that potentially form reactive metabolites have not, to our knowledge, been studied in detail before. Based on a series of activation energies, computationally faster methods are developed.[17−19] We use the correlation between the transition-state (TS) energies and the corresponding substrate radical energies to rationalize the substituent effects on the activation energies.

METHODS

Compound I (Cpd I) was modeled as porphyrin without side chains and with CH_3S^- and O^{2-} as axial ligands.[18] All calculations were done at the DFT/B3LYP level of theory,[20−22] to determine geometries and energies. For calculations with dispersion correction, the B3LYP-D3 was used.[23]

Three different states along the hydrogen abstraction reaction pathway were studied: reactant complex, transition state, and intermediate complex. A potential subsequent hydrogen abstraction leading to the quinone imine has also been investigated. Both doublet and quartet spin states were calculated for all complexes. Transition-state structures were confirmed by frequency calculations. The geometry optimizations, frequency calculations, and solvent calculations were performed with the Aldrichs VDZ basis set of Schäfer et al.,[24] enhanced with a p function on the Fe atom and the 6-31G(d) basis set on the other atoms (Bs1).[25−27]

Final energies were calculated with a larger 6-311++G(2d,2p) basis set,[28,29] except for Fe, for which we used the Aldrichs VDZ basis set of Schäfer et al.,[24] enhanced with s, p, d, and f functions (Bs2).[30] Energies were corrected for zero-point vibrational energy and solvation with the continuum conductor-like screening model COSMO,[31] with a dielectric constant of 4 and an atomic radius of 2.0 Å for Fe, which have been used in previous studies as well.[17,32,33] Unless otherwise stated, we report the Bs2 energies with zero-point vibrations at the Bs1 level. The spin distributions were obtained from the calculation with the Bs2 basis set from a Natural Bond Order analysis.[34]

All TS barrier activation energies are given relative to the reactant complex. Most calculations were performed using the Turbomole 6.3 package,[35] except those using the M06 and M06-L functionals and the LACVP* basis set, which were done with Jaguar.[36−39]

Bond dissociation energy (BDE) was calculated as the energy difference between the optimized substrate and the radical (after removal of the phenolic hydrogen atom). Unless otherwise stated, the energies are reported at the Bs2 level, with zero-point vibrations at the Bs1 level.

RESULTS AND DISCUSSION

Formation of Reactive Metabolites of Acetaminophen. The TS energy barriers for the initial hydrogen abstraction either from the phenolic hydroxyl group or from the nitrogen atom in the acetylamide moiety were determined (cf. Figure 2 and Supporting Information, Table S2). The hydrogen abstraction from the phenolic hydroxyl group is

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666

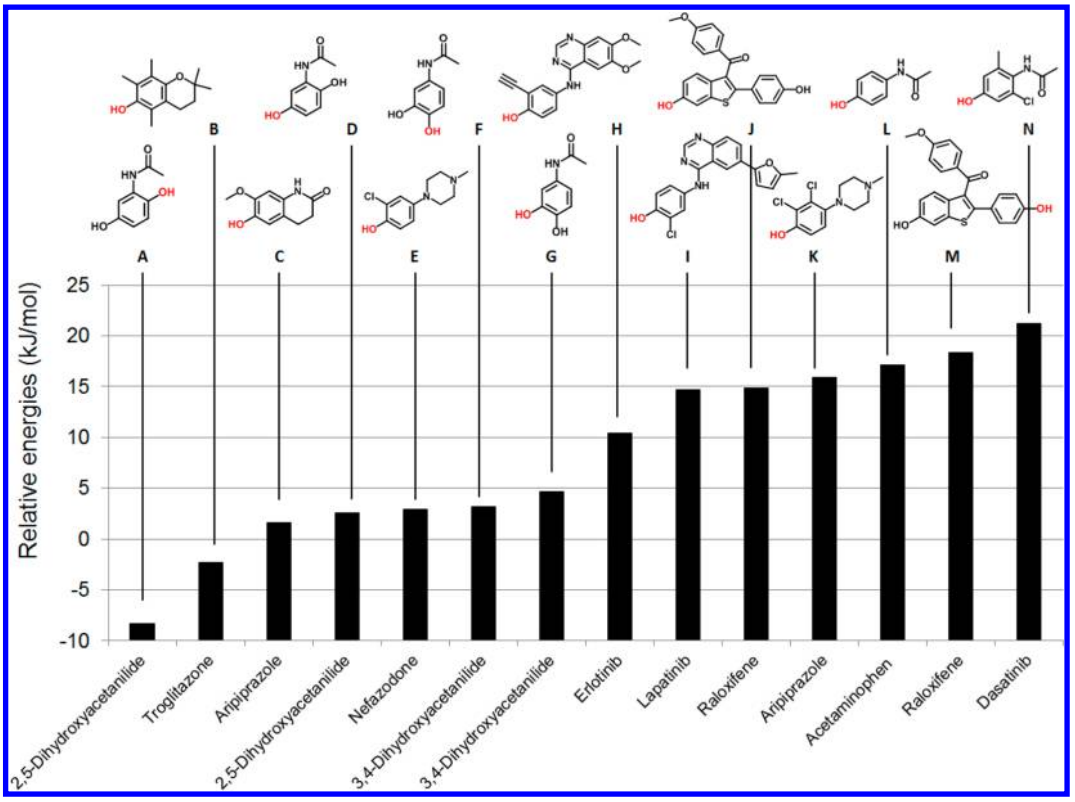


Figure 3. Transition-state energies of the investigated drug metabolites relative to the respective reactant complex. Displayed results are based on the lowest energy of the doublet and quartet spin states. The red hydroxyl groups indicate the site where the hydrogen abstraction occurs. Substituents are considered relative to these hydroxyl groups. The names of the drugs containing the shown drug metabolites are listed below the bars.

favored by 35 kJ/mol over hydrogen abstraction from the amide nitrogen in the doublet spin state.

The difference in the intermediate energies of 54 kJ/mol is in fair agreement with the value of 50 kJ/mol found by Alves et al.[12] Energies for the quartet spin state vary from the doublet spin state values by ∼1 kJ/mol (see the Supporting Information, Table S2). Distances in the TS between the oxygen of Cpd I, the abstracted hydrogen, and the heteroatom (O or N atom in the substrate) are O(Cpd I)−H = 1.21 Å and H−O = 1.20 Å for the phenolic hydrogen abstraction. For the hydrogen abstraction from the nitrogen atom, the corresponding distances are O(Cpd I)−H = 1.19 Å and H−N = 1.30 Å. This indicates that the TS for the abstraction from the nitrogen is later than the phenolic hydrogen abstraction, in agreement with the higher energy barrier for the former reaction. In the TS for the hydrogen abstraction from the nitrogen atom, the spin density is larger on the substrate than for the phenolic hydrogen abstraction (0.75 and 0.50, respectively; see the Supporting Information, Table S3). It has previously been observed for aliphatic hydrogen abstraction that reactions with larger activation energies have more radical character of the substrate in the TS, which also seems to be the case for the hydrogen abstraction from the O and N atoms.[18] In the case of abstraction from the nitrogen, the spin is more similar to that of the intermediate, which also indicates a later TS and accounts for the larger activation barrier.

Phenolic hydrogen abstraction of acetaminophen leads to NAPSQI. However, a second hydrogen abstraction must occur before NAPQI is formed. This second hydrogen abstraction might be CYP-mediated but could also result from interactions with various other redox systems present in the cells. We have calculated the activation barrier for a second hydrogen abstraction of NAPSQI from both the phenolic oxygen and the nitrogen atom. The second activation barriers are significantly lower for both reactions (see Figure 2). Thus, the initial phenolic hydrogen abstraction seems to be rate-limiting.

There are several competing CYP-mediated reactions that may occur. Instead of the hydrogen abstraction of the phenolic hydrogen atom, acetaminophen can also be hydroxylated in the 3 position. 3-Hydroxyacetaminophen, the nontoxic hydroxylated metabolite of acetaminophen, has been observed as a product of CYP enzymes 2E1 and 2A6, albeit to a much lesser degree than NAPQI.[40] The activation energy for 3-hydroxylation of acetaminophen has an activation barrier (86 kJ/mol) which is larger than that for the phenolic hydrogen abstraction (see Supporting Information, Table S5). This is a relatively large activation energy compared to other aromatic hydroxylation reactions studied previously in the literature.[17,18] With the low barrier of the phenolic hydrogen abstraction in mind, this reaction seems to be preferred, if the binding of substrate is in a favorable position for this reaction to occur.

We also tested other DFT functionals to check whether they were consistent with B3LYP (see Supporting Information, Figure S2). With the B3LYP-D3 functional, which has dispersion included, the hydrogen atom abstraction energy for the reaction with the NH group is also larger than that for the OH group. The TS structure from the phenolic hydrogen abstraction differs somewhat from that with the B3LYP functional. A larger part of the substrate interacts with the porphyrin model, which may cause some strain from the atoms directly involved in the reaction. Accordingly, the activation energy is not stabilized by the same amount for the phenolic hydrogen abstraction compared to that for the NH group. A

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666

similar trend is seen for TS complexes solved with M06 and M06-L functionals (see Supporting Information, Table S2). Thus, the direct binding of the substrate to the porphine model seems to play a significant role, which has also been observed by others.[41,42] However, it is still the hydrogen abstraction from the OH group that is most favorable.

Formation of Reactive Metabolites of Known Drug Compounds. The activation barriers for hydrogen abstraction for the compounds **A**–**N** were determined (see Supporting Information, Table S1). These compounds may all lead to reactive benzoquinone imines, and the fragments are either present in or formed by metabolic hydroxylation of known drug compounds (see Figure 3). The doublet and quartet spin states for the compounds show very similar activation energies (within 5 kJ/mol). There is a correlation between the activation barriers and the distance between the abstracted hydrogen atom and Cpd I. This distance is shorter, corresponding to a later TS, for the higher energy reactions (see the Supporting Information, Table S4). The different substitution patterns on the phenol rings give rise to about 30 kJ/mol difference in activation energies. Interestingly, issues with reactive metabolites have been observed experimentally for most of the compounds in this study with low (<5 kJ/mol) activation energies, either by depletion of glutathione (GSH), leading to hepatotoxicity, or by inactivation of CYP enzymes, leading to drug–drug interactions.[14,43−45]

Correlation between Substrate Radicals and the Transition-State Energies and Geometries. In previous studies, a linear correlation between BDEs and TS barrier energies has been found.[17,18,46] This is an interesting observation, as this allows a chemical interpretation and provides a fast estimate of the activation energies in CYP-mediated drug metabolism. Locating the TSs for hydrogen abstraction is considerably more complex and requires more CPU time than the calculation of BDEs.

We find a linear correlation between the TS energy barriers and the respective O–H BDEs, with an R^2 value of 0.72 for compounds **A**–**N** (cf. Figure 4). In general, the outliers from this correlation have a substituent *ortho* to the hydroxyl group, from which the hydrogen is abstracted. For example, compounds **B** and **F** have BDEs that seem too favorable compared to the TS energy (i.e., they are above the correlation trend line in Figure 4), because the H atom of the OH group is forced out the plane of the phenyl ring in the TS due to steric hindrance in the TS (destabilizing the TS, see Supporting Information, Figure S3). Compounds **C** and **E**, on the other hand, have BDEs that seem too high (below the trend line in the correlation), because the substrate in the BDE calculation is stabilized with an intramolecular H-bond (see Supporting Information, Figure S3). This intramolecular H-bond cannot be present in the TS structure, because the H atom being abstracted points away from the H-bonding acceptor in both the reactant and the TS. Otherwise, this acceptor group would come too close to the oxo-ferryl group of the heme. The correlation could easily be improved by using this knowledge about the structure of the TS for substrates with H-bonding acceptor in the *ortho*-position, by determining the BDE with the hydrogen of the OH group pointing away from the neighboring acceptor. If this is done for compounds **C** and **E**, the correlation is improved to $R^2 = 0.85$. Finally, it should be noted that the BDEs were all obtained with the Bs2 basis set and corrected for zero-point vibrations. The implementation of a BDE model using the Bs1 energies would be significantly faster (see Supporting Information, Figure S4).

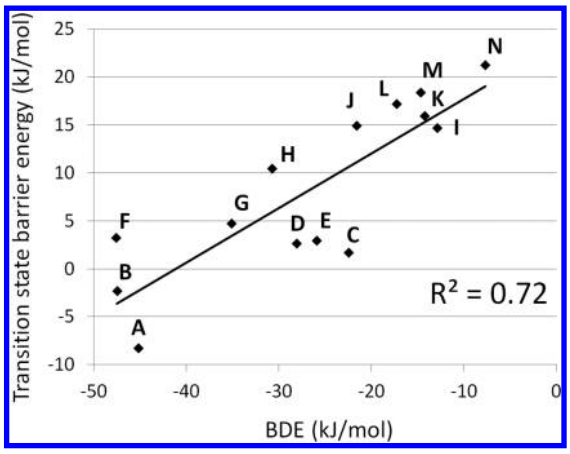


Figure 4. Correlation of BDE for formation of the phenolic radical and transition-state energy for all the metabolites in this study. The BDEs are relative to that of the unsubstituted phenol. Equation for the correlation line: $y = 0.57x + 23.4$.

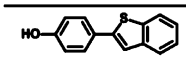
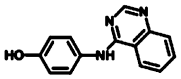
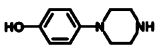
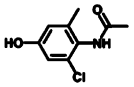
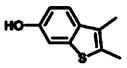
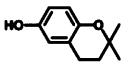
Using a Fast Prediction Method To Rationalize Formation of Reactive Metabolites. To see if the model also can be used to interpret the trends of the individual substituent effects on the BDEs, a range of phenols substituted in the *ortho*-, *meta*-, *para*-positions have been calculated. Table 1 displays results for substitution at the *meta*- and *para*-positions and a range of more complex substituents representing the compounds investigated in this study. Generally, substituents in the *ortho*-position have a similar effect as substituents in the *para*-position (all energies are reported in the Supporting Information, Figure S1). The electron-donating groups in the *para*-position reduce the BDE and, thus, the activation barrier for phenolic hydrogen abstraction. The effect of electron-donating groups is generally larger than the effect of the electron-withdrawing groups. The BDEs have also been determined for the electron-withdrawing cyano and nitro groups, which increase the BDEs. These observations are in agreement with the experimental data on the strength of the O–H bonds in phenols.[47]

Generally, it cannot be assumed that the individual effects from substituents are additive, e.g., because of interactions between the substituents. However, in cases where very similar compounds are compared, the effect of adding a particular substituent on the BDE may be explained. For example, comparing compounds **A** and **D** that both have an OH substituent in the *para*-position, it is seen that moving acetamide from the *ortho*- (compound **A**) to the *meta*-position (compound **D**) increases the BDE significantly, in agreement with the substituent effects observed in Table 1 and Figure S1. Another example is the comparison of **F** with **G**, where both compounds have an OH group in the *ortho*-position. However, compound **F** has an acetamide in the *para*-position, whereas **G** has it in the *meta*-position, explaining why **F** has a more favorable BDE.

It is not only the electronic effects from the substituents that affect the BDEs. For example, substituents in the *ortho*-position relative to the acetamino group induce a rotation out-of-plane of the acetamide group. The conformational effect on the activation barrier of reducing the coplanarity, and thereby the overlap between the acetamino and the aromatic systems, is significant (cf. Figure 5). This effect is observed in the case of

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666

Table 1. BDEs for the Phenolic Hydrogen Abstraction of a Range of Substituted Phenols, Relative to the BDE of the Unsubstituted Phenol

Substitution	BDE (kJ/mol) *meta*	*para*
NO_2	11	17
CN	11	8
Cl	4	-3
F	4	-8
C≡CH	4	-8
CH_3	-1	-9
$NHCOCH_3$	-1	-17
OH	3	-21
OCH_3	3	-23
NH_2	-4	-35
	-18	
	-20	
	-35	
	-6	
	-21	
	-27	

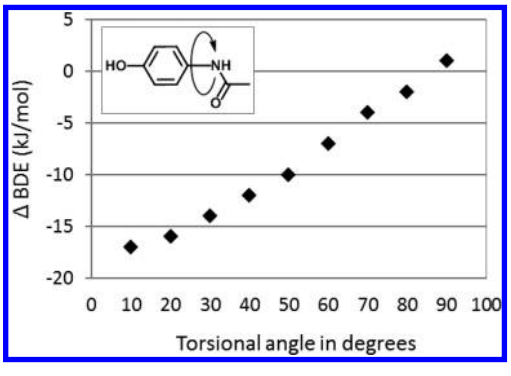


Figure 5. Effect of rotating the acetamino group out of the plane of the aromatic ring on the BDEs for phenolic hydrogen abstraction. The BDEs are relative to that of the unsubstituted phenol.

compound **N**, the fragment present in dasatinib, as the addition of the chloro and methyl substituents forces the acetamide moiety ~65° out-of-plane and reduces the conjugation between the aromatic system and the acetamide moiety. A compound with this deformation corresponds to an energy difference of −6 kJ/mol (cf. Figure 5), which is similar to the value for the *N*-(2-chloro-4-hydroxy-6-methylphenyl)acetamide substitution pattern in Table 1.

Compound **B** has a very small activation barrier, which is consistent with the high rate constant for the hydrogen abstraction for troglitazone found by Franchi et al.[47] A methyl group is a weak electron-donating group, but for compound **B** as well as troglitazone, both *ortho*-positions are occupied by methyl substituents. One methyl alone in the *ortho*-position lowers the activation energy by 8 kJ/mol. However, the biggest effect is due to the 2,2-dimethylchroman-6-ol moiety of troglitazone, which in itself gives a very low activation barrier (−27 kJ/mol). Troglitazone has been shown to produce hepatotoxicity, and as a consequence of this, it has been withdrawn from the market.[43]

Aripiprazole undergoes hydroxylation on the 2,3-dichlorophenylpiperazine moiety, which by oxidation can produce a benzoquinone imine. Nevertheless, GSH adducts derived from conjugation with this metabolite have not been observed experimentally, suggesting that the oxidation does not occur.[44] This is consistent with the relatively high activation barrier found for the 2,3-dichlorophenylpiperazine metabolite (**K**) in this study. The activation barrier for hydrogen abstraction is lowered relative to that for a *para*-piperazinyl substitution, but the addition of a chlorine substituent in the *meta*-position not only increases the barrier but also reduces the conjugation with the nitrogen atom in the piperazinyl moiety in the *para*-position.

The acetanilide motif of aripiprazole is capable of undergoing *ortho*- and *para*-hydroxylation followed by oxidation to a quinone imine similar to acetaminophen. This oxidation, via compound **C**, is associated with one of the lowest activation barriers found in this study, which is explained by the donating effect from the methoxy and amino substituents in the *ortho*- and *para*-positions, respectively.

Nefazodone (compound **E**) undergoes hydroxylation on the 3-chlorophenylpiperazine moiety, which by oxidation produces a benzoquinone imine. The TS energy barrier for this oxidation is low, primarily due to the piperazinyl in the *para*-position. This might explain why nefazodone gives rise to the GSH adducts in human liver microsomes.[45] Therapeutic use of nefazodone has been associated with acute hepatoxicity and fatality, resulting in the withdrawal of the drug from the market in some countries.[48]

We have also determined the activation energies for aromatic hydroxylation for the compounds that undergo hydroxylation prior to hydrogen abstraction (compounds **C**, **E**, **H**, **K**, and **N**). The activation energies for the aromatic hydroxylation follow the same order as the hydrogen abstraction activation barriers (see the Supporting Information, Table S5). For these compounds, it is the same CYP isoform, CYP3A4, which performs the hydroxylation and hydrogen abstraction.[14] The fact that the aromatic hydroxylation and phenolic hydrogen abstraction energies correlate indicates that the formation of reactive benzoquinone imines and the relative ordering of reactive metabolites can be studied, to a large degree, by considering only the hydrogen abstraction step.

■ CONCLUSION

In this study, we have used DFT to study the activation energies for compounds that can form benzoquinone imine by hydrogen abstraction. Phenolic hydrogen abstraction is energetically more favorable than hydrogen abstraction from the acetamide nitrogen atom of acetaminophen. Different substituents affect the activation barrier by up to 30 kJ/mol. A correlation between the activation barrier energy for the hydrogen abstraction from a drug compound and the stability of the related radical was established. This computationally less demanding DFT-based method was used to rationalize the

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666

relative ordering of activation barriers for known drug compounds. This fast approach makes it feasible to consider various substituents and substituent patterns prior to synthesizing the compounds and, accordingly, could prove useful in the design of compounds with optimized metabolic properties.

■ ASSOCIATED CONTENT

Supporting Information

Molecular structures, DFT energies, spin densities, vibrational frequencies, and Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: lo@sund.ku.dk.

Author Contributions

R.L. performed all calculations. All authors participated in defining the study, in ongoing discussions, and in analyzing the results. R.L., F.S.J., and L.O. wrote the manuscript.

Notes

The authors declare no competing financial interest.

†Deceased.

■ ABBREVIATIONS

B3-LYP, Becke three-parameter Lee−Yang−Parr; BDE, bond dissociation energy; COSMO, continuum conductor-like screening model; Cpd I, compound I; CYP, cytochrome P450; DFT, density functional theory; DFT-D3, empirical dispersion correction for DFT calculations; GSH, glutathione; HF, Hartree−Fock; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NAPSQI, *N*-acetyl-*p*-benzosemiquinone imine; TS, transition state

■ REFERENCES

(1) Black, M. Acetaminophen Hepatotoxicity. *Annu. Rev. Med.* **1984**, *35*, 577−593.

(2) Dahlin, D. C.; Miwa, G. T.; Lu, A. Y.; Nelson, S. D. N-Acetyl-P-Benzoquinone Imine: A Cytochrome P-450-Mediated Oxidation Product of Acetaminophen. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1327−1331.

(3) Rogers, L. K.; Moorthy, B.; Smith, C. V. Acetaminophen Binds to Mouse Hepatic and Renal DNA at Human Therapeutic Doses. *Chem. Res. Toxicol.* **1997**, *10*, 470−476.

(4) Obach, R. S.; Kalgutkar, A. S.; Soglia, J. R.; Zhao, S. X. Can in Vitro Metabolism-Dependent Covalent Binding Data in Liver Microsomes Distinguish Hepatotoxic from Nonhepatotoxic Drugs? An Analysis of 18 Drugs with Consideration of Intrinsic Clearance and Daily Dose. *Chem. Res. Toxicol.* **2008**, *21*, 1814−1822.

(5) de Vries, J. Hepatotoxic Metabolic Activation of Paracetamol and Its Derivatives Phenacetin and Benorilate: Oxygenation or Electron Transfer? *Biochem. Pharmacol.* **1981**, *30*, 399−402.

(6) Keller, R. J.; Hinson, J. A. Mechanism of Acetaminophen-Stimulated Nadph Oxidation Catalyzed by the Peroxidase-H2O2 System. *Drug Metab. Dispos.* **1991**, *19*, 184−187.

(7) Bisby, R. H.; Tabassum, N. Properties of the Radicals Formed by One-Electron Oxidation of Acetaminophen—a Pulse Radiolysis Study. *Biochem. Pharmacol.* **1988**, *37*, 2731−2738.

(8) Bessems, J. G.; de Groot, M. J.; Baede, E. J.; te Koppele, J. M.; Vermeulen, N. P. Hydrogen Atom Abstraction of 3,5-Disubstituted Analogues of Paracetamol by Horseradish Peroxidase and Cytochrome P450. *Xenobiotica* **1998**, *28*, 855−875.

(9) Loew, G. H.; Goldblum, A. Metabolic Activation and Toxicity of Acetaminophen and Related Analogs. A Theoretical Study. *Mol. Pharmacol.* **1985**, *27*, 375−386.

(10) Koymans, L.; van Lenthe, J. H.; van de Straat, R.; Donne-Op den Kelder, G. M.; Vermeulen, N. P. A Theoretical Study on the Metabolic Activation of Paracetamol by Cytochrome P-450: Indications for a Uniform Oxidation Mechanism. *Chem. Res. Toxicol.* **1989**, *2*, 60−66.

(11) Koymans, L.; den Kelder, G. D.; van Lenthe, J. H. Quantum Chemical Calculations on the Metabolic Activation of Paracetamol. *Supercomputer* **1992**, *9*, 16−22.

(12) Alves, C. N.; Borges, R. S.; Da Silva, A. B. F. Density Functional Theory Study of Metabolic Derivatives of the Oxidation of Paracetamol. *Int. J. Quantum Chem.* **2006**, *106*, 2617−2623.

(13) Borges, R. S.; do Nascimento, J. L. M.; Alves, C. N. A Theoretical Study for Oxidative Metabolism of Acetaminophen. *J. Comput. Theor. Nanosci.* **2010**, *7*, 1968−1972.

(14) Orr, S. T.; Ripp, S. L.; Ballard, T. E.; Henderson, J. L.; Scott, D. O.; Obach, R. S.; Sun, H.; Kalgutkar, A. S. Mechanism-Based Inactivation (Mbi) of Cytochrome P450 Enzymes: Structure-Activity Relationships and Discovery Strategies to Mitigate Drug-Drug Interaction Risks. *J. Med. Chem.* **2012**, *55*, 4896−4933.

(15) Shaik, S.; Kumar, D.; de Visser, S. P.; Altun, A.; Thiel, W. Theoretical Perspective on the Structure and Mechanism of Cytochrome P450 Enzymes. *Chem. Rev.* **2005**, *105*, 2279−2328.

(16) Rydberg, P.; Jorgensen, F. S.; Olsen, L. Use of Density Functional Theory in Drug Metabolism Studies. *Exp. Opin. Drug Metab. Toxicol.* **2014**, *10*, 215−227.

(17) Bathelt, C. M.; Ridder, L.; Mulholland, A. J.; Harvey, J. N. Mechanism and Structure-Reactivity Relationships for Aromatic Hydroxylation by Cytochrome P450. *Org. Biomol. Chem.* **2004**, *2*, 2998−3005.

(18) Olsen, L.; Rydberg, P.; Rod, T. H.; Ryde, U. Prediction of Activation Energies for Hydrogen Abstraction by Cytochrome P450. *J. Med. Chem.* **2006**, *49*, 6489−6499.

(19) Rydberg, P.; Ryde, U.; Olsen, L. Prediction of Activation Energies for Aromatic Oxidation by Cytochrome P450. *J. Phys. Chem. A* **2008**, *112*, 13058−13065.

(20) Becke, A. D. Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, *38*, 3098−3100.

(21) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B, Condens. Matter* **1988**, *37*, 785−789.

(22) Becke, A. D. Density-Functional Thermochemistry. III. The Role of Exact Exchange. *J. Chem. Phys.* **1993**, *98*, 5648.

(23) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A Consistent and Accurate Ab Initio Parametrization of Density Functional Dispersion Correction (DFT-D) for the 94 Elements H-Pu. *J. Chem. Phys.* **2010**, *132*, 154104.

(24) Schäfer, A.; Horn, H.; Ahlrichs, R. Fully Optimized Contracted Gaussian Basis Sets for Atoms Li to Kr. *J. Chem. Phys.* **1992**, *97*, 2571.

(25) Hehre, W. J. Self—Consistent Molecular Orbital Methods. XII. Further Extensions of Gaussian—Type Basis Sets for Use in Molecular Orbital Studies of Organic Molecules. *J. Chem. Phys.* **1972**, *56*, 2257.

(26) Hariharan, P. C.; Pople, J. A. The Influence of Polarization Functions on Molecular Orbital Hydrogenation Energies. *Theor. Chim. Acta* **1973**, *28*, 213−222.

(27) Francl, M. M.; Pietro, W. J.; Hehre, W. J.; Binkley, J. S.; Gordon, M. S.; Defrees, D. J.; Pople, J. A. Self-Consistent Molecular-Orbital Methods. XXIII. A Polarization-Type Basis Set for Second-Row Elements. *J. Chem. Phys.* **1982**, *77*, 3654−3665.

(28) McLean, A. D.; Chandler, G. S. Contracted Gaussian Basis Sets for Molecular Calculations. I. Second Row Atoms, Z=11−18. *J. Chem. Phys.* **1980**, *72*, 5639.

(29) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. Self-Consistent Molecular Orbital Methods. XX. A Basis Set for Correlated Wave Functions. *J. Chem. Phys.* **1980**, *72*, 650.

(30) Rulisek, L.; Jensen, K. P.; Lundgren, K.; Ryde, U. The Reaction Mechanism of Iron and Manganese Superoxide Dismutases Studied by Theoretical Calculations. *J. Comput. Chem.* **2006**, *27*, 1398−1414.

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666

(31) Klamt, A.; Schuurmann, G. Cosmo—A New Approach to Dielectric Screening in Solvents with Explicit Expressions for the Screening Energy and Its Gradient. *J. Chem. Soc., Perkin Trans. 2* **1993**, 799−805.
(32) Rydberg, P.; Sigfridsson, E.; Ryde, U. On the Role of the Axial Ligand in Heme Proteins: A Theoretical Study. *J. Biol. Inorg. Chem.* **2004**, *9*, 203−223.
(33) Rydberg, P.; Ryde, U.; Olsen, L. Sulfoxide, Sulfur, and Nitrogen Oxidation and Dealkylation by Cytochrome P450. *J. Chem. Theory Comput.* **2008**, *4*, 1369−1377.
(34) Reed, A. E.; Weinstock, R. B.; Weinhold, F. Natural-Population Analysis. *J. Chem. Phys.* **1985**, *83*, 735−746.
(35) *Turbomole*, V6.3, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989−2007; TURBOMOLE GmbH, 2011; http://www.turbomole.com.
(36) *Jaguar*; Schrödinger, LLC: New York, 2014.
(37) Zhao, Y.; Truhlar, D. G. A New Local Density Functional for Main-Group Thermochemistry, Transition Metal Bonding, Thermochemical Kinetics, and Noncovalent Interactions. *J. Chem. Phys.* **2006**, *125*, 194101.
(38) Zhao, Y.; Truhlar, D. G. The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06-Class Functionals and 12 Other Function. *Theor. Chem. Acc.* **2007**, *120*, 215−241.
(39) Hay, P. J.; Wadt, W. R. Ab Initio Effective Core Potentials for Molecular Calculations. Potentials for K to Au Including the Outermost Core Orbitals. *J. Chem. Phys.* **1985**, *82*, 299.
(40) Chen, W.; Koenigs, L. L.; Thompson, S. J.; Peter, R. M.; Rettie, A. E.; Trager, W. F.; Nelson, S. D. Oxidation of Acetaminophen to Its Toxic Quinone Imine and Nontoxic Catechol Metabolites by Baculovirus-Expressed and Purified Human Cytochromes P450 2e1 and 2a6. *Chem. Res. Toxicol.* **1998**, *11*, 295−301.
(41) Rydberg, P.; Lonsdale, R.; Harvey, J. N.; Mulholland, A. J.; Olsen, L. Trends in Predicted Chemoselectivity of Cytochrome P450 Oxidation: B3lyp Barrier Heights for Epoxidation and Hydroxylation Reactions. *J. Mol. Graph. Model.* **2014**, *52*, 30−35.
(42) Lonsdale, R.; Harvey, J. N.; Mulholland, A. J. Inclusion of Dispersion Effects Significantly Improves Accuracy of Calculated Reaction Barriers for Cytochrome P450 Catalyzed Reactions. *J. Phys. Chem. Lett.* **2010**, *1*, 3232−3237.
(43) Gale, E. A. Lessons from the Glitazones: A Story of Drug Development. *Lancet* **2001**, *357*, 1870−1875.
(44) Bauman, J. N.; Frederick, K. S.; Sawant, A.; Walsky, R. L.; Cox, L. M.; Obach, R. S.; Kalgutkar, A. S. Comparison of the Bioactivation Potential of the Antidepressant and Hepatotoxin Nefazodone with Aripiprazole, a Structural Analog and Marketed Drug. *Drug Metab. Dispos.* **2008**, *36*, 1016−1029.
(45) Kalgutkar, A. S.; Vaz, A. D.; Lame, M. E.; Henne, K. R.; Soglia, J.; Zhao, S. X.; Abramov, Y. A.; Lombardo, F.; Collin, C.; Hendsch, Z. S.; Hop, C. E. Bioactivation of the Nontricyclic Antidepressant Nefazodone to a Reactive Quinone-Imine Species in Human Liver Microsomes and Recombinant Cytochrome P450 3a4. *Drug Metab. Dispos.* **2005**, *33*, 243−253.
(46) de Visser, S. P.; Kumar, D.; Cohen, S.; Shacham, R.; Shaik, S. A Predictive Pattern of Computed Barriers for C-H Hydroxylation by Compound I of Cytochrome P450. *J. Am. Chem. Soc.* **2004**, *126*, 8362−8363.
(47) Franchi, P.; Lucarini, M.; Pedulli, G. F.; Valgimigli, L.; Lunelli, B. Reactivity of Substituted Phenols toward Alkyl Radicals. *J. Am. Chem. Soc.* **1999**, *121*, 507−514.
(48) Choi, S. Nefazodone (Serzone) Withdrawn Because of Hepatotoxicity. *Can. Med. Assoc. J.* **2003**, *169*, 1187.
(49) Chen, Q.; Ngui, J. S.; Doss, G. A.; Wang, R. W.; Cai, X.; DiNinno, F. P.; Blizzard, T. A.; Hammond, M. L.; Stearns, R. A.; Evans, D. C.; Baillie, T. A.; Tang, W. Cytochrome P450 3a4-Mediated Bioactivation of Raloxifene: Irreversible Enzyme Inhibition and Thiol Adduct Formation. *Chem. Res. Toxicol.* **2002**, *15*, 907−914.

DOI: 10.1021/ci500653b
J. Chem. Inf. Model. 2015, 55, 660−666