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Using Transition State Modeling To Predict Mutagenicity for Michael Acceptors

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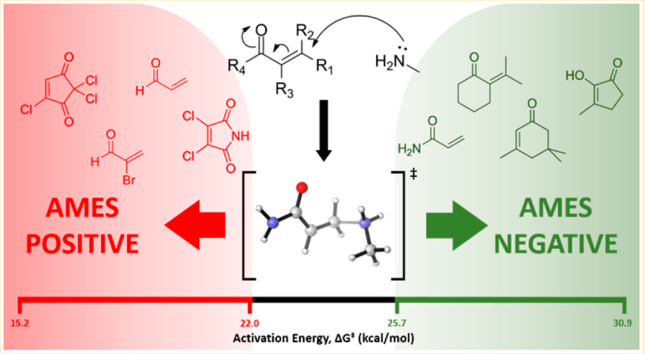
Supporting Information

ABSTRACT: The Ames mutagenicity assay is a long established in vitro test to measure the mutagenicity potential of a new chemical used in regulatory testing globally. One of the key computational approaches to modeling of the Ames assay relies on the formation of chemical categories based on the different electrophilic compounds that are able to react directly with DNA and form a covalent bond. Such approaches sometimes predict false positives, as not all Michael acceptors are found to be Ames-positive. The formation of such covalent bonds can be explored computationally using density functional theory transition state modeling. We have applied this approach to mutagenicity, allowing us to calculate the activation energy required for α,β-unsaturated carbonyls to react with a model system for the guanine nucleobase of DNA. These calculations have allowed us to identify that chemical compounds with activation energies greater than or equal to 25.7 kcal/mol are not able to bind directly to DNA. This allows us to reduce the false positive rate for computationally predicted mutagenicity assays. This methodology can be used to investigate other covalent-bond-forming reactions that can lead to toxicological outcomes and learn more about experimental results.



INTRODUCTION

Cancer is a toxicological end point of much concern in the development of new chemical compounds. Cancer can be caused by mutagenic chemicals, those that have the ability to directly alter the DNA of an exposed organism.[1−3] The ability of a compound to be mutagenic is typically assessed in vitro using methods including the Ames mutagenicity assay.[1−4] This assay reports chemicals as mutagens if they produce a dose-dependent increase in the number of revertant *Salmonella* colonies. In order to test this, a *Salmonella* strain lacking the ability to generate histidine required for growth is incubated in a medium containing minimal histidine both in the presence of the possible mutagen and without it as a control. In the presence of a mutagenic substance, the *Salmonella* are able to revert to a state in which they are able to synthesize the required histidine, resulting in an increased growth rate. As such, the test plate is compared to the control to establish the number of revertants. The Ames test is widely used as an early screen in compound development.

The formation of a covalent bond between a toxicant and a DNA molecule can be considered a molecular initiating event (MIE).[5,6] These MIEs are chemical triggers that can lead to toxicological effects via adverse outcome pathways (AOPs).[7] One potential MIE for mutagenicity is the reaction between a guanine nucleobase on DNA and an electrophilic xenobiotic.[8]

Some in silico approaches for predicting mutagenicity use structural alerts to group chemicals on the basis of their perceived reactivity with DNA molecules.[9] These categories include Michael acceptors, Schiff base formers, etc. Notably, a number of chemicals that conform to these chemical categories are not found to be mutagenic in the Ames test. Therefore, broad structural alerts are an oversimplification of the real situation. Quantum-mechanical calculations offer a more scientifically rigorous way to examine these reactions, such as those used by Leach et al.[10] to examine the relative stabilities of nitrenium ions for the prediction of Ames test results. Furthering our understanding of the chemical reaction that occurs between the toxicant and DNA molecule will allow us to provide improved computational predictions.

Transition state modeling using density functional theory (DFT) has been successfully used to elucidate the mechanisms of many chemical reactions.[11] This research often involves the analysis of competing reaction pathways, with the lowest activation energy barrier dictating which one is most likely to

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dominate a reaction. In an analogous fashion, the transition state of a reaction between a DNA nucleophile and a potential toxicant electrophile in this MIE could be found and its activation energy calculated. A higher activation energy would result in a chemical being less able to react with DNA. Could an energy barrier be established at which chemicals that might react with DNA do not?

MATERIALS AND METHODS

To answer this question, Ames test data were collected from the OECD QSAR Toolbox,[12] a toxicological database and prediction tool, for a number of α,β-unsaturated carbonyls. This chemical category was chosen as a prototypical collection of Michael acceptors.[9] A total of 20 compounds were assessed, 13 of which are recorded in the Toolbox as Ames-positive and seven as Ames-negative. The reactions between these Michael acceptors and a model of the guanine nucleobase were modeled using DFT. Transition states for the N−C bond-forming reaction were located using the B3LYP density functional[13,14] and the 6-31+G(d) basis set within the IEFPCM model (water).[15] Single-point energies were calculated using the M06-2X functional[16,17] and the polarized triple-ζ valence-quality def2-TZVPP basis set of Weigend and Ahlrichs[18] within the IEFPCM model (water). The resulting energies were used to correct the energies obtained from the B3LYP optimizations given the limitations of B3LYP, particularly in its description of dispersion interactions. This approach has previously been shown to give reliable results.[19,20] Computed structures are illustrated with CYLView.[21] These calculations were performed using Gaussian 09, revision D.01.[22] To simplify these calculations, a methylamine nucleophile was used as a model system for the guanine base, and two of the carbonyl compounds were truncated. The generalized reaction modeled is shown in Figure 1, and an example reaction, transition state, and calculated activation energy are shown in Figure 2.

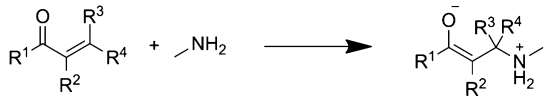

Figure 1. Generalized reaction between an α,β-unsaturated carbonyl and methylamine model for DNA modeled in this work.

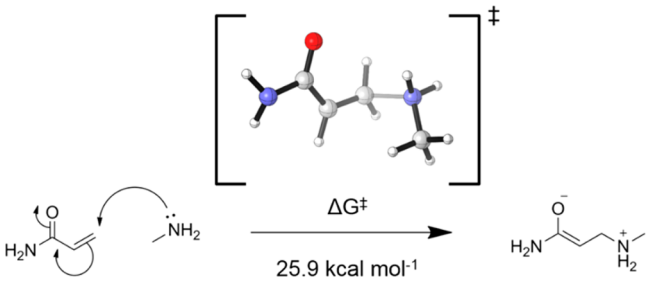

Figure 2. An example reaction modeled in this work. Its transition state and calculated activation energy are also shown.

RESULTS AND DISCUSSION

The results of our study are shown in Table 1. The 20 compounds analyzed have activation energies ranging from 15.2 to 30.9 kcal/mol. The average activation energy for the 13 Ames-positive compounds is 19.9 kcal/mol, and the average for the seven Ames-negative compounds is 26.6 kcal/mol. This shows a reasonably large energy gap of 6.7 kcal/mol between the average Ames-positive and Ames-negative compounds. Of the Ames-positive compounds, only two showed activation energies higher than 22.0 kcal/mol: 4-hydroxy-5-methyl-3-furanone and 3-(dichloromethylene)-2,5-pyrrolidinedione. These compounds show an overlap with the Ames-negative compounds, all of which have activation energies greater than or equal to 24.2 kcal/mol. Why are these compounds mutagenic and yet show such high activation energies?

From a search of the literature reports that feed into the OECD QSAR Toolbox, some insight can be gained. 4-Hydroxy-5-methyl-3-furanone is reported to be able to generate active oxygen radicals via hydrolysis of the lactone ring and subsequent oxidation of the free hydroxyl groups generated, as shown in Figure 3.[23] These active oxygen radicals are able to go on to react directly with DNA, making this a different toxicological pathway and MIE from the one modeled in this study. As such, this result is unsuitable for this study and was therefore removed. Literature reports for other studied compounds were subsequently checked, and none of the other compounds were found to be able to undergo a similar process.

No such competing toxicity mechanism was found for 3-(dichloromethylene)-2,5-pyrrolidinedione. However, it shows a very low molar mutagenicity compared with other compounds we have studied, as shown in Table 2.[24] The molar mutagenicity values quoted correlate well with the calculated activation energies in our study, showing a clear link between the mutagenicity potentials of chemicals and their activation energies.

From consideration of these cases, it appears that activation energies between 22.0 and 25.7 kcal/mol display a change in mutagenicity potential for α,β-unsaturated carbonyls. We can predict with confidence that α,β-unsaturated carbonyls with activation energies of 22.0 kcal/mol or lower will be Ames-positive and that those with activation energies of 25.7 kcal/mol or higher will not activate the MIE for covalent modification of DNA. As the 4-hydroxy-5-methyl-3-furanone example shows, these compounds may be mutagenic via other toxicity pathways.

Between these values, it is sensible at this time to consider the compounds unknown and possibly Ames-positive or -negative. Additional calculations on compounds in this range may eventually shrink this unknown region, providing a more clear-cut model. On the basis of the evidence presented here, compounds predicted as Ames-positive because of the presence of an α,β-unsaturated carbonyl may now be corrected to Ames-negative if they are found to have an activation energy equal to or greater than 25.7 kcal/mol. These findings are summarized in Figure 4.

To explore the possibility that the energy of the zwitterionic intermediate that forms from the transition state would provide a good estimate of the activation barrier, this intermediate was optimized from the lowest-energy transition state for the three compounds with activation barriers of 30.9, 24.2, and 15.2 kcal/mol. It was found that the barriers from the reactants to these intermediates were 30.6, 22.7, and 8.5 kcal/mol, respectively, indicating a lack of consistency in the ability to approximate the activation barrier from this intermediate.

To compare this methodology to existing computational tools, bacterial mutagenicity in vitro has been predicted using Derek Nexus,[25,26] an expert knowledge-based system developed using data implemented by Lhasa Limited. Derek correctly

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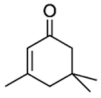
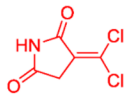
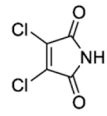
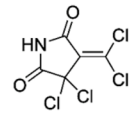
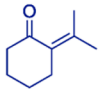
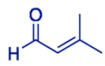
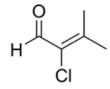
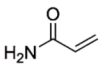
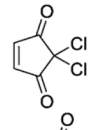
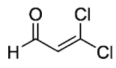
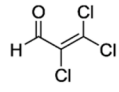
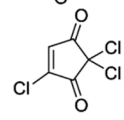
Table 1. Table of Results for This Study in Order of Decreasing Activation Energy[a]

Compound	Ames Test	ΔG‡ (kcal/mol)	Compound	Ames Test	ΔG‡ (kcal/mol)	Compound	Ames Test	ΔG‡ (kcal/mol)	Compound	Ames Test	ΔG‡ (kcal/mol)
	Negative	30.9		Negative	25.7		Positive	21.6		Positive	18.8
	Negative	29.2		Positive	24.4		Positive	21.2		Positive	16.7
	Negative	26.0		Negative	24.3		Positive	21.0		Positive	16.1
	Negative	25.9		Negative	24.2		Positive	19.8		Positive	16.1
	Positive	25.8		Positive	22.0		Positive	19.8		Positive	15.2

[a]Ames-positive chemicals with unexpectedly high activation energies are shown in red and discussed in the text. Compounds that have been truncated are shown in blue (the one in the first column has been truncated from pulegone and the one in the second column from citral).

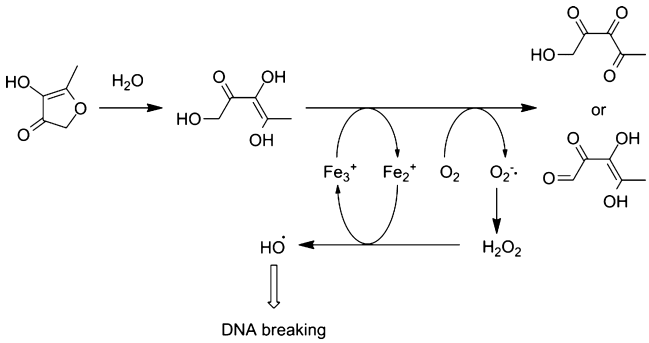


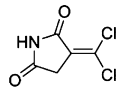
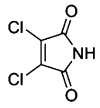
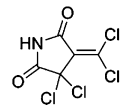
Figure 3. Possible mechanism for DNA breaking by 4-hydroxy-5-methyl-3-furanone proposed by Hiramoto et al. Reprinted with permission from ref 23. Copyright 1996 Elselvier.

identified all seven of the Ames-negative chemicals as inactive, and 11 of the 12 positives were correctly identified as plausible (this excludes 4-hydroxy-5-methyl-3-furanone; the incorrect prediction was for 2,2-dichloro-4-cyclopentene-1,3-dione). While Derek predicts these compounds extremely well, our model provides an improved understanding of the chemical–biological interaction that is the MIE by providing an image of the transition state as well as the activation energy. It also has a large applicability domain, incorporating any α,β-unsaturated carbonyl because it models the chemical interaction directly, while structural alerts require constant refinement as new data are obtained. Finally, negatives with activation energies greater than 25.7 kcal/mol can be confidently predicted not to have this MIE and hence to be experimental negatives. Our methodology can also be used in conjunction with other models, as ICH M7 guidelines for in silico Ames predictions require two complementary methods.[27]

CONCLUSIONS

In this work, activation energy barriers have been calculated for the reaction between a model nitrogen nucleophile, representing a DNA base, and electrophilic Michael acceptors. These barriers have been used to show that Ames mutagenicity assay results correlate well with these data, with Ames-negative compounds generally having higher activation energies than Ames-positive compounds. This methodology can be used to predict the Ames test results for new α,β-unsaturated carbonyls, with those having activation energies greater than 25.7 kcal/mol expected to be unable to directly bind to DNA and hence to be Ames-negative. α,β-Unsaturated carbonyls with activation energies less than 22.0 kcal/mol would be expected to bind directly to DNA and to be Ames-positive. By modeling this MIE directly, we provide a new in silico methodology for the prediction of genotoxicity that can be used to complement

Table 2. Mutagenicities of Three Chlorinated Imides in *Salmonella typhimurium* TA100, All Measured by Haddon et al.,[24] and Their Respective Activation Energies; The Data Show a Clear Correlation between Increasing Activation Energy and Decreasing Molar Mutagenicity

Compound	Dose (nmol/plate)	Revertants /plate	Molar Mutagenicity (rev/nmol)	ΔG‡ (kcal/mol)
	694	280; 318; 325	0.24	24.4
	278	190; 230; 218		
	56	142; 151; 128		
	6	351; 365	28	21.2
	3	244; 254		
	0.75	233; 201		
	0.4	687; 590; 623	1450	16.7
	0.1	260; 295; 278		

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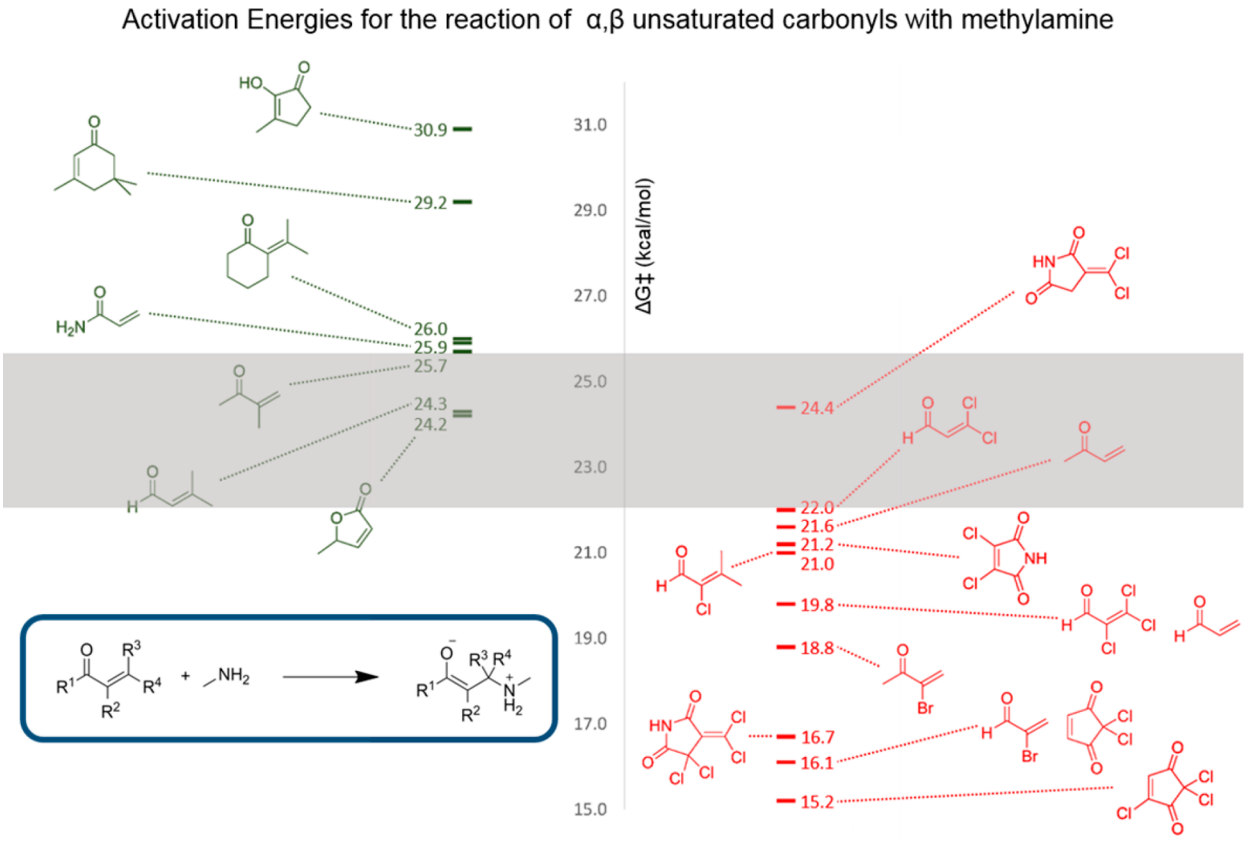

Figure 4. Finalized results graphic outlining the findings of this work. Compounds to the left of the *y* axis and shown in green are Ames-negative, and compounds to the right and shown in red are Ames-positive. The generalized modeled reaction is shown in the bottom left for reference. All of the *α,β*-unsaturated carbonyls with activation energies of 25.7 kcal/mol or higher were found to be Ames-negative. All of those with activation energies of 22.0 kcal/mol or lower were found to be Ames-positive. The area indicated in gray between 22.0 and 25.7 kcal/mol is the crossover point, containing two Ames-negative compounds and one Ames-positive compound. Novel molecules that fall into this area would be considered unknown using this model.

more traditional computational methods such as structural alerts in order to meet toxicology risk assessment guidelines.

This methodology could be applied to further chemical categories of electrophilic compounds expected to be mutagens, such as Schiff base formers or reactive aromatic rings.[28] It is likely in these other cases that the activation energy barrier will differ from the one established here for *α,β*-unsaturated carbonyls. Each category of electrophiles should be examined on a case-by-case basis to establish its own reactivity threshold for mutagenicity. This approach may also be applied to further examples of covalent-bond-forming MIEs, such as skin sensitization,[29,30] in which the MIE is considered to be the covalent modification of epidermal proteins by electrophilic toxicants. This is analogous to the reaction we have modeled with DNA. The cysteine residue of a skin protein could be modeled using methanethiol, and activation energy barriers should be able to differentiate between strong, moderate, and weak skin sensitizers and nonsensitizers.[31] In this way, our approach based on transition state modeling would complement other quantum-mechanical quantitative structure−activity relationships (QSARs). These include linking the energy of intermediate species to local lymph node assay data[32] and thiol reactivity data[33,34] and the calculation of reaction kinetics for the reaction of thiols with *α,β*-unsaturated carbonyls.[35]

In summary, the MIE for covalent binding of DNA to an *α,β*-unsaturated carbonyl electrophile has been modeled using DFT transition state modeling, and the activation energies obtained indicate a clear link between these chemical properties and the mutagenicity potential.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jcim.8b00130.

Cartesian coordinates, energies, free energies, and numbers of imaginary frequencies for all stationary points and values of imaginary frequencies for all transition structures (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AOP, adverse outcome pathway; DFT, density functional theory; MIE, molecular initiating event; QSAR, quantitative structure−activity relationship

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