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Curcumin is one of the promising herbal-based drugs. It has been shown to have antioxidant, antibacterial, anti-angiogenic and other biological activities. As curcumin's derivative, chalcone shares similar functions. Both of these two compounds have α,β-unsaturated carbonyl structures (enone), which is a typical 1,4-conjugate addition (Michael addition) acceptor. Glutathione is an endogenous tripeptide, whose sulfhydral group is a typical nucleophilic agent. In this case, the derivatives of curcumin and chalcone may be reduced by glutathione and their pharmacological functions would be changed.

The study herein focused on how to use quantum chemistry tools and transition state theory to access to the conjugate addition of α,β-unsaturated carbonyl compounds. In addition, the reductions of the derivatives of chalcone were studied. The characteristics of the reactions were obtained by analyzing geometries and energy profiles of the simplified reactions, as well as the influence of functional groups on derivatives in this type of reaction. This study may be generally useful for the scientific community for two fundamental reasons: (a) to provide general strategies to enhance or retard drugs from reacting with glutathione, and (b) to provide insight into computational methods that are able to help design potential lead candidates.

A STUDY OF CONJUGATE ADDITION OF CURCUMIN AND CHALCONE DERIVATIVES

By
Yingqiu Zhou

A Thesis Submitted to
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The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

Greensboro
2011

Approved by

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Committee Chair

APPROVAL PAGE

This thesis has been approved by the following committee of the Facultyof The Graduate School at The University of North Carolina at Greensboro.

Committee Chair _____

Committee Members _____

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Date of Final Oral Examination

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ABBREVIATION

ADME/Tox: Absorption, Distribution, Metabolism, Excretion and Toxicity

au: atomic unit

BAX: Bcl-2-associated X protein

BCL2: B-cell lymphoma 2

DMSO: Dimethyl sulfoxide

G2/M phases: Gap 2 phase and Mitosis phase in cell division

GSH: Glutathione

GST: Glutathione Transferase

HF: Hartree-Fock method

IKKβ: inhibitor of nuclear factor kappa-B kinase subunit beta

IR: Infrared

MM: Molecular Mechanics

PES: Potential Energy Surface

QM: Quantumn Mechanics

TNF: Tumor Necrosis Factors

TS: Transition State

CHAPTER I

INTRODUCTION

1.1 Medicinal Plant Drug Discovery

Plants have been used as medicines for thousands of years. By spoken or written word, plants are sorted and used to treat specific diseases. Since morphine was isolated from opium in the early 19th century, natural products have become important sources for new drugs. Some widely used medicines, such as Galantamine which treats Alzheimer's disease, were first discovered in plants. Generally, medicinal plant drug discovery includes lead identification, optimization, and development.[1] Except for lead identification, which mostly relies on different types of bioassays, both lead optimization and development have been assisted by the use of computational chemistry, including but not limited to ADME/Tox, docking, and scoring. These methods provide additional information helpful in determining whether or not a compound is a potential drug candidate.

1.2 Statement of the Problem

Unlike *in vitro* experiments, the effect of drugs may be influenced by many predicted or unpredicted factors *in vivo*. For example, glutathione interacts with the drugs having electrophilic structure, and the reactions are catalyzed by certain enzymes.

In our cases, the derivatives of curcumin and chalcone which contain α,β-unsaturated carbonyl moieties may be reduced by glutathione.[2] Then the reduced form of the derivatives may not have the same pharmacological functions. So the problem is whether the possibility of the reduction and prevention can be predicted using theoretical methods.

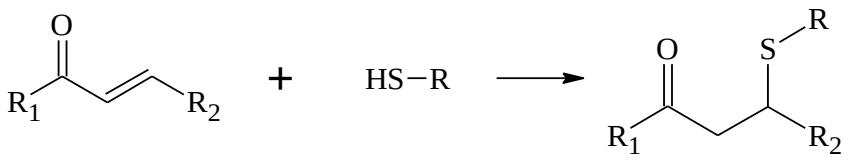


Figure 1. The general reaction between enones and thiols

1.3 Purpose of Study

The primary aim of this research is to study the reaction between glutathione and α,β-unsaturated carbonyl compounds with *ab initio* quantum chemistry methods. Based on the information, new compounds may be screened *in silico* for interactions with glutathione.

1.4 Research Questions

The study includes two parts. First, there is an interest in better understanding Michael additions by glutathione reduction. Glutathione has a sulfhydryl functional group, and thiols are good nucleophilic agents. The α,β-unsaturated carbonyl systems are good electron acceptors. Although the mechanism of Michael additions seems clear, when it comes to specific reactants, it is still necessary to characterize the whole

reaction. Second, there is an interest in the modifications of compounds that might be made to reduce their susceptibility for glutathione addition. By modifying the structure, the energy of reactants and products will be changed, as will the transition states and the activation barriers. Therefore, the dynamics of the reactions are subject to change. If one reaction has a much higher activation energy, it will be more difficult to form the Michael product. Understanding the electronic and steric interactions favoring and disfavoring this addition is the central goal.

1.5 Significance of the Study

Drug metabolism is an interesting area of pharmacology. In the past, studies were carried out to determine the chemical transformations drugs would undergo *in vivo*. Now, there are an increasing number of studies concerning the genetic diversity and its influence on drug metabolism. With this knowledge, combined with the use of computational methodologies, it is very likely that new drugs can be designed to be more effective and safer. Moreover, there are many reduction/oxidation agents within the human body. This study may be used as a reference for similar research which needs to enhance or retard drugs from reacting with those agents. These studies may provide insight into computational methods that are able to help find and modify potential lead candidates.

CHAPTER II

REVIEW OF LITERATURES

2.1 Curcumin as a Versatile Molecule

2.1.1 Chemical Structure

Natural products are experiencing a modest resurgence in popularity in medicinal chemistry. Many new drugs based on integrants of herbs from traditional medicine are available, while more compounds are being tested in labs and have great potential to treat diseases in the future.[3] Compared to synthetically derived drugs, herbal-based medicines may be safer and more effective. Curcumin is one of those compounds which may have a promising future. It is an active ingredient from a herb called *Curcuma longa*, which is widely used in traditional medicine in different countries.[4] The commercially available sources for curcumin contain curcuminoid complex. This complex is composed of 77% of curcumin I, 17% curcumin II (demethoxycurcumin) and 3% curcumin III (bisdemethoxycurcumin), as shown in Figure 2. Among these, compounds curcumin I shows better activity.[5, 6]

Curcumin was first discovered in 1815 and then identified as diferuloylmethane. The color of curcumin powder is yellow to slightly orange. It cannot dissolve in water but is soluble in ethanol, DMSO, and acetone.[5] Its chemical name is

1,6-heptadiene-3,5-dione-1,7-bis(4--hydroxyl-3-methoxy--phenyl)-(1E, 6E). Studies provide evidence suggesting the structural features that are responsible for its biological activity may be the bis-α,β-unsaturated β-diketone, the methoxy groups, the phenolic hydroxyl groups, and/or the double-conjugate bonds.[7-9] The collection of critical functional groups and their three-dimensional relationship is called the pharmacophore.

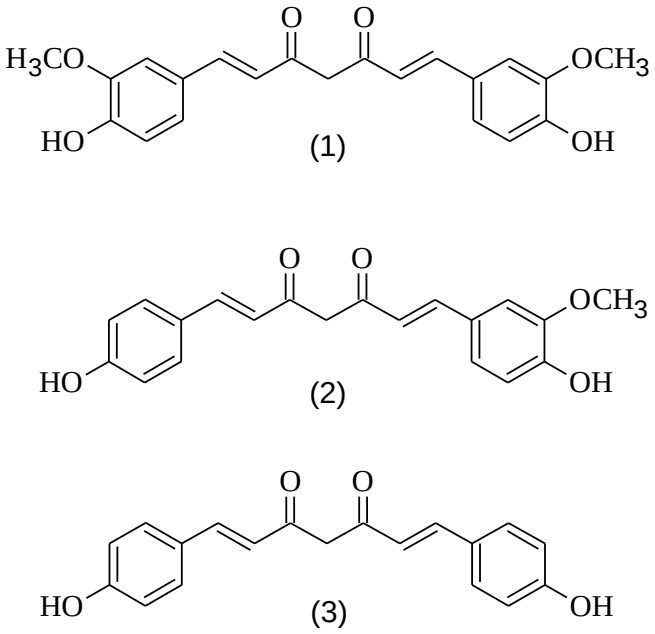


Figure 2. Chemical structures of curcumin I(1), II(2) and III(3) [10]

2.1.2 Functions

Curcumin is a versatile molecule. Numerous studies found curcumin to have antioxidant[11], antibacterial[12], antifungal[13], antiviral[14], anti-inflammatory[6], and anti-angiogenic[15] activities. It is also a potential agent to treat cancer and neurodegenerative diseases.[16, 17] On the molecular level, curcumin has the ability to

interact with many disease-related targets and involves some important signaling pathways. There is a relationship with several targets, including cycloxygenase (COX)-2, lipoxygenase (LOX), glutathione, protein kinase C, ATPase, multidrug resistance proteins 1 and 2 (MRP1 and MRP2).[5, 16, 18-21] Curcumin also regulates pathways by up-regulating or down-regulating some transcription factors. For example, p53 is involved in the pathways of apoptosis. Curcumin can activate p53 and induce p53-related cell death. It influences other important transcription factors like BAX, Caspase, TNF and BCL2 as well. Therefore, curcumin has great potential as a chemotherapeutical drug.[8, 15, 22]

The chemical structure of curcumin is symmetric. Assuming that the two are important for biological activity, the linking electrophilic portion may be modified to 1,3-diaryl-2-propen-1-ones, which has the common name chalcone. Studies show that it also has anti-inflammatory and anticancer activities.[15, 23] On the molecular level, it influences the formation of inmicrotubule and the cellular signaling pathways. [24]

2.2 Chalcone and Derivatives

Generating analogues of natural products with the help of high-throughput screening is an effective approach in drug discovery. Combinatorial biosynthesis and structure-activity relationships studies (SARs) are important parts of that type of research.[3] Compared to curcumin, chalcone derivatives are easier to prepare by aldol type condensations.[25] Many studies related to chalcone also include the generation of

novel chalcone derivatives. Most of the chalcone-based compounds have had the two aromatic rings modified. Those derivatives often have better activities and easier synthesis steps.[24-27]

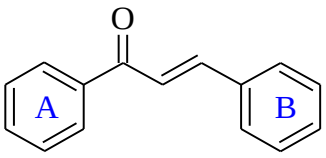


Figure 3. The chemical structure of chalcone

2.3 Conjugate Addition of Enones

Both curcumin and chalcone-based compounds include an α,β-unsaturated enone. It is a typical Michael addition acceptor. A Michael addition is a conjugate addition. It is a reaction where the nucleophile adds to the α,β-unsaturated carbonyl compound in a 1,4 fashion instead of a common 1,2-addition across the C=O bond.

The general mechanism involves electron donors attacking the unsaturated structure at the β-carbon. This mode of nucleophilic attack is due to the resonance stabilization of enones. The resonance results in the partial positive charge of the β-carbon, which will be attacked by nucleophilic agents as shown in Figure 4.

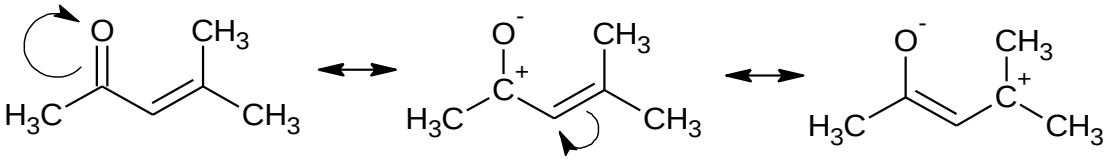


Figure 4. Resonance Stabilization of α,β-Unsaturated Ketones (Enones)

Below is the mechanism of a typical Michael addition reaction involving a thiol

and an α,β-unsaturated enone. In this reaction, electrons transfer from nitrogen to sulfur, and then to the β-carbon of enolate intermediate. This step also determines the rate of the reaction.[28]

Because this reaction is widely used in organic and bio-synthesis, there are a lot of studies about the catalysis and its application in polymer sciences. But actually, it is also very common inside the human body.

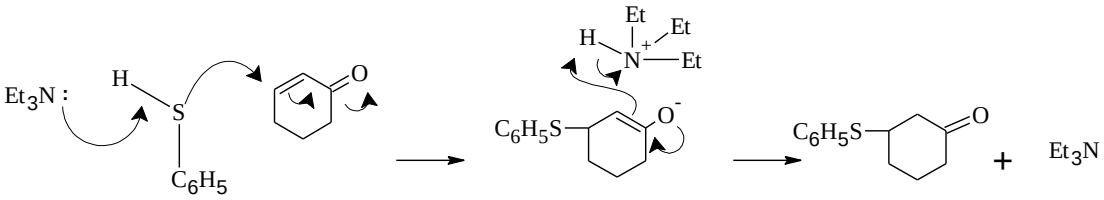


Figure 5. Mechanism of the Conjugate Addition of Benzenethiol to Enones in Nonpolar Solvents[28]

For example, some of the chalcone derivatives were reported to inhibit nuclear factor kappa B (NF-κB) pathway, and NF-κB is an important drug target for cancer.[25] The structure-activity relationship (SAR) study revealed the potential inhibition mechanism of those compounds reacting with IKKβ[25], as shown in Figure 6. This reaction involves reaction between the sulfhydryl and α,β-unsaturated carbonyl groups, which is a typical Michael addition reaction. It has been shown that the α,β-unsaturated carbonyl functional group is essential for the inhibition of NF-κB.[25]

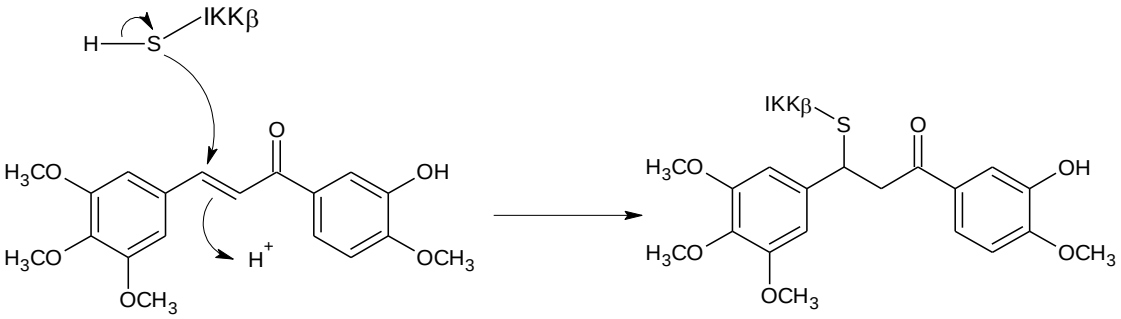


Figure 6. Possible mechanism of chalcone compounds to modify IKKβ[25]

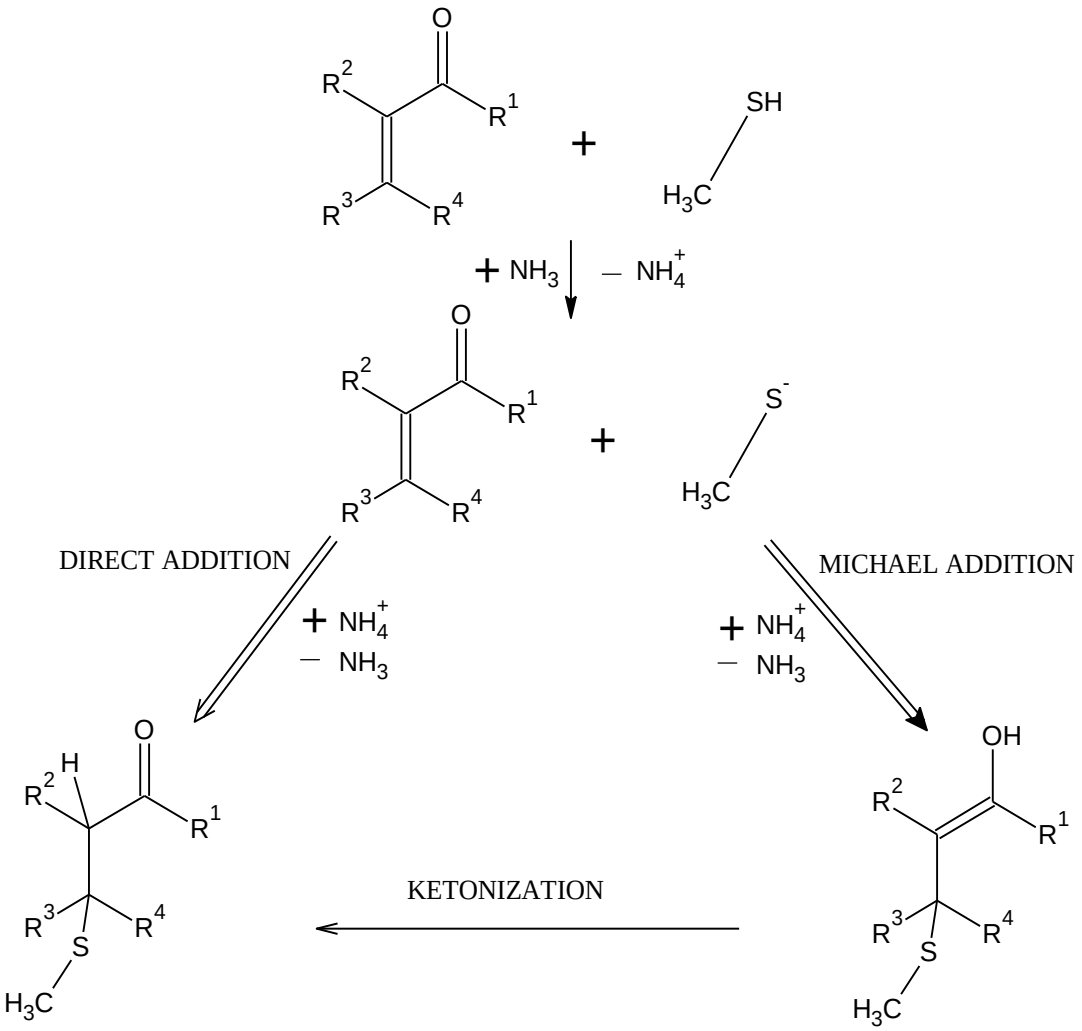


Figure 7. Possible reaction pathways for the Michael addition of thiols to α,β-unsaturated carbonyl compounds[2]

When thiols attack the α,β-unsaturated carbonyl structure, there are two possible

reaction pathways as shown in Figure 7. One mode is a 1,2-addition which is a direct nucleophilic attack on the carbonyl carbon, and the other mode is a 1,4-addition, which is also called Michael addition. The Michael addition involves a nucleophilic attack on the β-carbon. Previous computational work suggested that for some reactions, proton transfer is intramolecular in 1,2-additions while in 1,4-additions it is between molecules.[29] In this case, 1,4-addition is the more favorable one.[29] Some experimental data also agree with this statement. In some cases, even if the differences between their activation barriers are very close, 1,4-addition is still favored in those reactions.[2]

2.4 Glutathione and Glutathione Transferase

Compared to alcohols, thiols are more easily oxidized. Its deprotonated forms are called thiolates, and they are very potent nucleophiles. The pKa of thiols are ~8-10, which vary due to the changes of the environment. Under physiological conditions, thiols are usually in the form of protonated (R-SH) and deprotonated($R\text{-}S^-$), while the protonated state is the primary form.[2]

Glutathione is an endogenous tripeptide, which also has a sulfhydral group. It is composed of L-cysteine, L-glutamic acid, and glycine.[30] Glutathione is an antioxidant with a reducing sulfhydryl group. The reaction related to this functional group requires the presence of glutathione transferase (GST).[31] This enzyme has a quaternary structure. Glutathione transferase has different subtypes of enzymes. Based on the

studies of the characterization of site-specific mutated GSTs, the residues of active sites are in domain I and II, including Tyr6, Trp7, Val9, Leu12, Ile 111, Tyr115, Phe208, Ser209.[32]

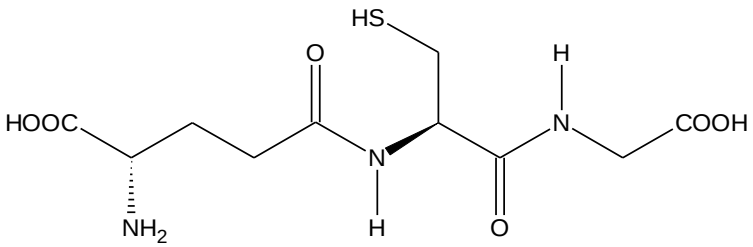


Figure 8. The chemical structure of glutathione

The studies on mutants of glutathione S-transferase described the special role of the hydroxyl group of tyrosine. As shown in Figure 9, both the hydroxyl groups of two tyrosines form hydrogen bonds and participate in the electron transfer chain. It is the rate-limit step of the reaction sequence.[32] Some other research also provided evidence that the hydroxyl groups of tyrosine may help to stablize the enol or enolate intermediate for the 1,4-addition reaction.[33] In addition to this, the protonation of tyrosine relates to the presence of GSH.[34] Therefore, this study provided information about how GSH reacts with enones.

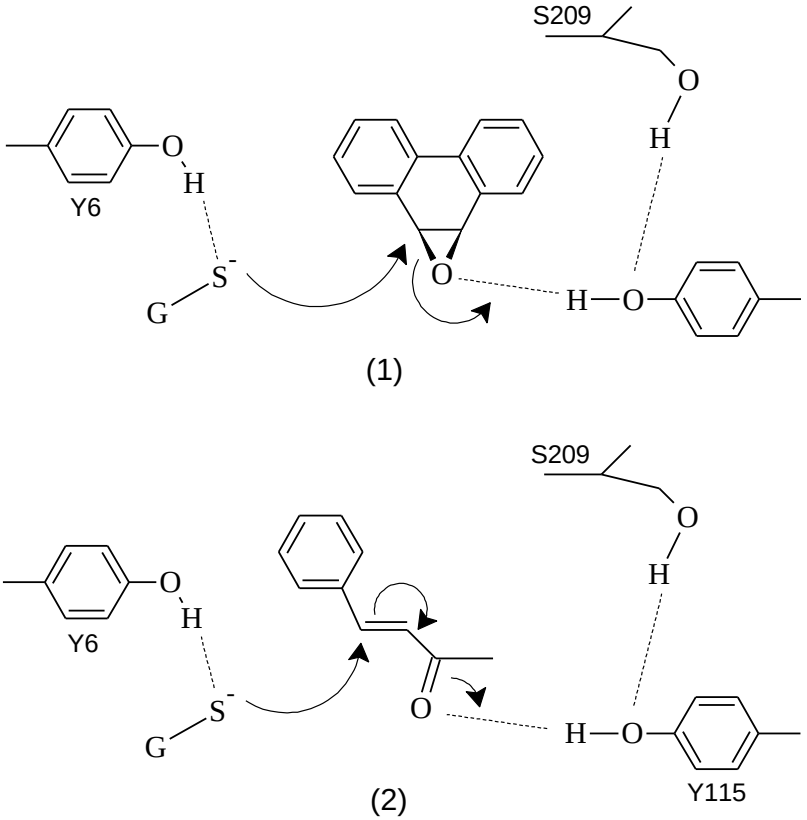


Figure 9. Y115(tyrosine) of GST participates the Michael addition of different substrates [32]

2.5 Potential Energy Surface

While experimental data provides clues to characterize the reaction, an increasing number of scientists are using computational tools to help their research. In computational chemistry, there is an extremely important concept called potential energy surfaces (PESs). PES satisfies the Schrödinger equation (Equation 1),

$$H=T_r+T_R+V(r,R) \qquad \text{(Eq. 1)}$$

where T_r and T_R are operators for kinetic energy of electron motion and nuclear motion respectively, and V(r,R) represents the potential energy from the electrostatic interactions between particles with charge. The Born-Oppenheimer approximation

neglects the kinetic energy of protons and separates vibrational, translational, and rotational motions due to the differences in mass of the proton and electron. Using this approximation, the Schrödinger equation can be converted and plotted by E(R) versus R, while E(R) as a parametric function of nuclear coordinates R. Plotting shows the potential energy surface. On a PES graph, the extreme is located. The energy minima are the local valleys, and the highest point of the pathway between the reactants and products is the optimized transition states. The pathway itself is the reaction path. Therefore, the first and second derivatives of the PES can be obtained to predict the transition state and the reaction pathway. For example, the Hessian matrix is used to do the minimization. By the hybrid of QM/MM, *ab initio* PES calculation can be conducted.[35]

2.6 Transition State Theory

Transition State Theory is very important for calculating the reaction pathway. In this theory, with the formation of new bonds and/or interactions, as well as the change of energies, the reactants are converted to the intermediate transition state initially. Then proceeding along the reaction pathway, the products will be formed. Because the transition state is not stable and short-lived, it is difficult to prove its existence experimentally. The transition states of the reactions, however, can be approximately predicted using computational PES methods. As discussed above, they should be the highest point of the reaction pathways. The activation energy also determines the

reaction. It is the amount of energy needed for a reaction to occur. The activation energy is the differences between the energy of the transition state and the reactants. The lower the activation energy is, the faster the reaction will occur. For instance, a catalyst is able to lower the energy of transition state. This will result in even faster reactions.

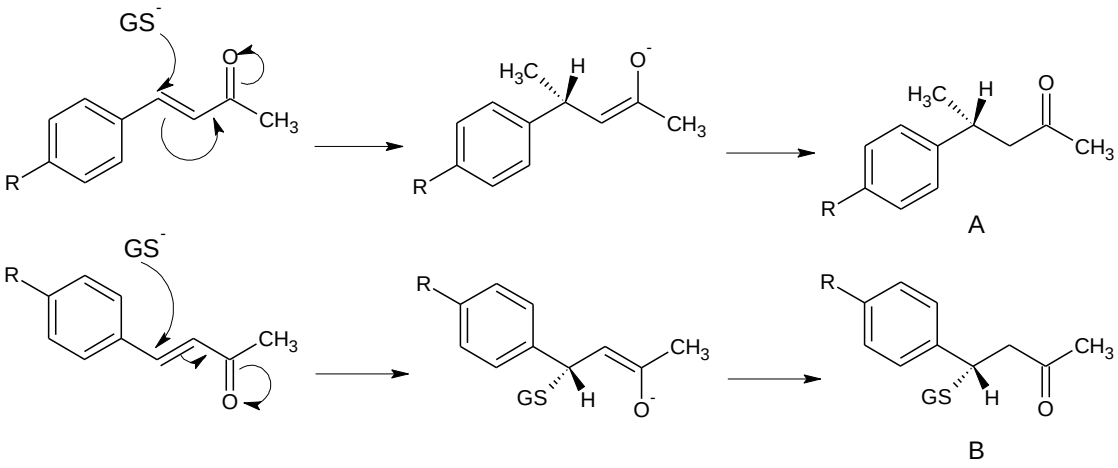


Figure 10. Two possible transition states in the Michael addition of phenylbutenone[36]

For example, because of the chiral center, the Michael addition products have two possible configurations. In Figure 10, there should be two different stereochemical products A and B. The experimental data showed that the ratio of these two products is not 1:1 but 9:1.[36] With the stabilization effect by the enzyme, the activation barrier of transition state A was lower than transition state B. Therefore, the reaction generating product A then became the favorable reaction pathway.

In recent years, some studies applied the transition state theory by calculating the energies and geometries to help new drug design and modification. One example is that Xiong and his colleagues used QM/MM calculation to reveal the enzyme-catalyzed

reaction pathways and activation barriers.[37] The calculation provided the details of the reactions and geometries were very useful for further rational drug design.[37] Clearly, the study of transition state structure, combined with computational studies and structure-activity relationship studies, will help to design transition state analogues, which can be used to find new drugs or improve the high-affinity features.[38]

CHAPTER III

METHODOLOGY

3.1 Environment and Settings of *Ab initio* Calculation

3.1.1 Quantum Chemistry Methods

Quantum Chemistry calculation methods were used in this study. Compared to molecular mechanics, quantum mechanics describes the electronic structure of molecules from fundamental theoretical principles. Thus, it is more accurate while having a relatively higher computational cost. The computational cost increases with the complexity of model systems and basis sets used in the calculation. In addition, *ab initio* methods are more expensive than semi-empirical methods with the important advantage of being accurate.

In this study, calculations usually started from semi-empirical methods to get roughly optimized results and then applied Hartree-Fock method, which should save computing time. However, semi-empirical methods sometimes resulted in the wrong transition states conformations which will be described later.

3.1.2 Calculation Environment and Settings

In this study, all the calculations were carried out using SPARTAN 08' (Wavefunction Inc.) with CentOS(Linux) operation system. The software provides

tools to calculate the geometries, energies, and other properties of the reactions.

All model systems were calculated by Hartree-Fock *ab initio* methods with the 3-21G basis set.[39] Frequencies in the IR spectrum were also calculated to verify the validity of transition states. Because the reactions occurred under physiological condition, it is necessary to consider solvent effect. In this study, all the calculations were computed with water as solvent.

3.1.3 General Procedure of Calculations

Both the equilibrium geometries and transition states of reactants and products in each model system were calculated by SPARTAN. The initial optimizations of equilibrium geometries were conducted with a semi-empirical method. Then the Hartree-Fock model with the 3-21G basis set were applied to get a more reliable result. In order to obtain better accuracy without significantly increasing the calculation cost, the 6-31G* basis set was used to study the conjugate addition with some simpler model systems.[39]

The SPARTAN software provides a build-in tool to predict the transition states of reactions. By indicating the electron transfer of the reaction, the software can give a roughly estimated transition state. However, it could go wrong in some cases and require manually adjusted to get appropriate results. Afterwards, the detailed profiles of transition states could be obtained by setting the calculation with “transition state” task. The other calculation settings such as calculation models, basis sets, solvent, etc.

were similar with the calculation of equilibrium geometries.

After the completion of the calculations, geometric parameters (distance, angle, and dihedral angles), energy profiles, IR spectrum, molecular orbitals and other properties were viewed in SPARTAN for analysis.

3.2 Research Design

3.2.1 Simplify the Reaction

The proposal research plan was designed to study the reaction pathways of the 1,4-conjugate addition between glutathione and α,β-unsaturated carbonyl compounds, as well as the influences of different functional groups on the activation barriers and geometries. The more complex a system, however, the more time-consuming the calculation. Since glutathione is a tripeptide which is not suitable for quantum chemistry calculation, without access to a cluster system, it is a wise choice to build initially the simplest model to mimic the reaction.

In this study, glutathione was simplified to methanol or methanethiol; all α,β-unsaturated carbonyl compounds had no more than four additional functional groups besides the backbone structure. Because the reaction sites are the same, the results and conclusions ought to have similar characters of the conjugate addition between glutathione and enones.

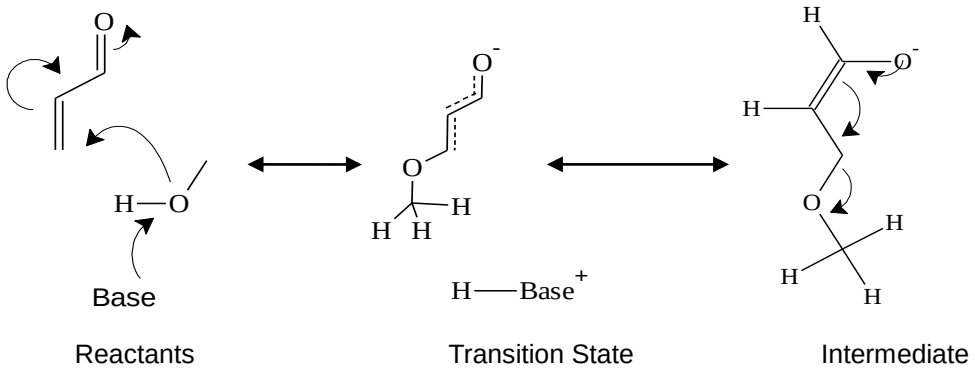


Figure 11. The transition state is reversible

In this reaction, methanol reacts with propenal and form the intermediate, and then form the final ketone product. This study only focuses on the first part. Because this reaction is an intermolecular reaction which means the relative positions of these reactants may affect the energies. In addition, an extra base is involved in the reaction which brings more components to computation. All these increase the potential inaccuracy of the results. As shown in Figure 11, since the transition states can be obtained starting from either reactants or intermediates/products, it is more convenient to calculate an intramolecular reaction if in a reverse direction.

Methanol or methanethiol were used to replace the large molecule of glutathione. Oxygen and sulfur atoms have similar properties, but since the oxygen atom has a smaller size, it is easier for computation of complex systems. In this study, the results and conclusions of derivatives were based on methanol model systems. Both methanol and methanethiol model systems were studied for the conjugate addition reactions.

3.2.2 Model Systems

As mentioned, the conjugate addition between glutathione and enones were simplified for calculation convenience. Thus, the first step is to build up a simplest model system. In this case, the simplest enone and methanol were chosen as shown in Figure 12. The intermediate of these two reactants contains only the backbones of the reaction with a negative charge by deprotonation. Then the absence of the catalytic base in the model system could be neglected. The model system with methanethiol was also studied. By obtaining the transition state, the information obtained from this basic reaction will give clues for further study.

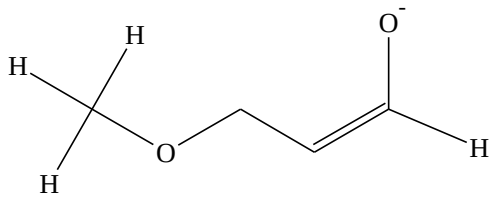


Figure 12. Structure of intermediate from methanol and propenal

Because the molecule of chalcone has two phenyl groups, the reaction from the previous model system is insufficient. Therefore, the hydrogen connected to the carbonyl carbon was replaced by a phenyl group. Since the intermediate contains a double bond, it is necessary to consider the *trans*- and *cis*- isomers as shown in Figure 13. The geometries and energies of isomers are different, as well as the transition states. Similarly, model systems with two phenyl groups in Figure 14 and model system with methanethiol were also studied.

Based on the data obtained from simple, with one phenyl group and with two phenyl groups model systems, the characteristics of this type of conjugate addition could be concluded.

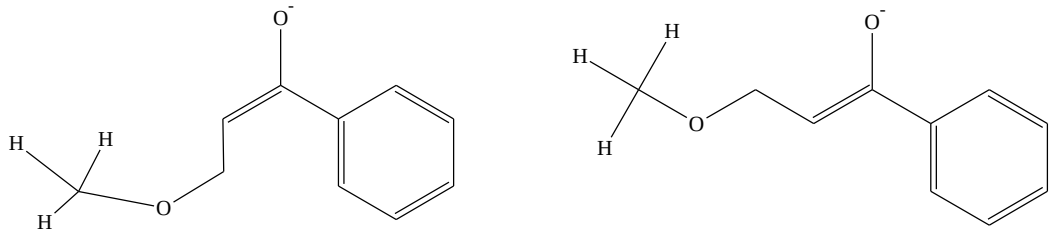


Figure 13. *Trans*- and *Cis*- Isomers of one phenyl group system

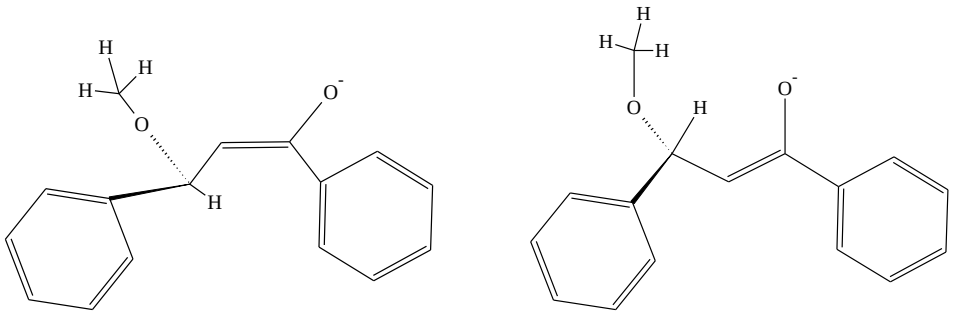


Figure 14. *Trans*- and *Cis*- Isomers of two phenyl groups' system

3.2.3 Derivatives of Chalcones

The information of the 1,4-addition provides references for further investigations. In this study, it is about the derivatives of chalcones. Some common modifications of curcumin or chalcone might include the additional of R-groups on either or both phenyl rings, or substituting phenyl groups with other similar aromatic rings. The aim of this study was to determine whether those functional groups will affect the energies and

geometries of the reaction. Because this is a preliminary research, most derivatives were selected from those with existing experimental data.[8] The modification took isomers under consideration as shown in Figure 15, since isomers have different energies and geometries. Follow the same steps, geometries and energies of the reactants, intermediates, and transition states were examined. The information obtained *in silico* may provide clues for more effective modifications of curcumin analogues.

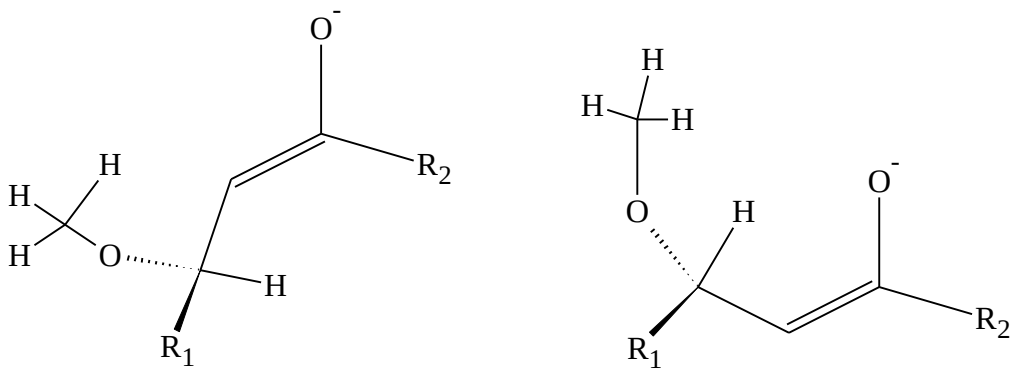


Figure 15. General forms of *trans/cis* intermediates of the derivatives

3.3 Data Analysis

3.3.1 Data Collection

SPARTAN generates profiles of reactants, transition states, and products. Energies and IR Spectrum were computed as well. Geometries such as dihedral angles, angles and distances of atoms near the reaction sites are also recorded and compared. The unit of energy in *ab initio* calculation (au) was converted into kcal/mol.

3.3.2 Data Analysis

The calculations provide information on the geometries and energies of the reactants and products, as well as the transition states. Potential energy surface theory can help to find out the transition states and the reaction pathways by quantum chemistry computation. They also relate to the activation barriers and the energies of the products. Besides, the transition states geometry provides information about how the conjugate addition happens.

The data collected from derivatives will be more useful for future studies. For example, it may help to design new derivatives. After adding different functional groups to several sites of the reactants, both the geometries and energies are changed.

The data about energies of reactants, products, and transition states were collected and compared as shown in Figure 16. Activation energies or activation barriers were defined as ΔE1, which is the difference in energies between the transition state and the reactant, while ΔE2 was defined as the difference in energies between the transition state and the intermediate. After obtaining the activation energies, the differences between them and the original reaction were compared. Moreover, the corresponding geometries of transition states are checked to see if they are reasonable.

It is believed that the activation barrier is relevant to whether the reaction happens and the reaction rate. This study was concerned with uncovering ways to prevent the conjugate addition from occuring; in other words, the reactions with

higher relative activation barriers and appropriate geometries will be good candidates for further experimental studies as potential drug candidates. By repeating the calculation, those with larger activation barriers may be the potential drug candidates we are seeking.

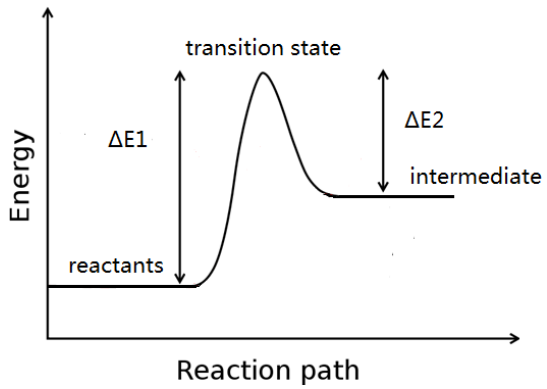


Figure 16. Reaction coordinate and energies

3.3.3 Data Verification

It is possible that the wrong transition states are obtained after calculation. There are several ways to rule out fake transition states. The most reliable method is based on the idea that the reaction sites will share similar geometries in the same type of reactions. Therefore, the data obtained from the previous calculation could be considered as criteria. If the geometries of the derivative models are far from the chalcone model, it is very likely that the calculation gives an invalid result. The more accurate the calculation model is, the less chance it will provide a wrong transition state. However, the computational time prevent the use of larger basis set or more accurate methods such as density function theory with the limited compute resources

available.

Another way is to examine the validity of the transition state by computing the vibrational frequencies. The transition state should have only one negative frequency in the calculated IR spectrum. In SPARTAN, it will appear at the top of the chart and is very easy to identify the transition state. This negative value represents the vibrational motion which goes towards reactants in one direction and products in the other direction.

CHAPTER IV

RESULTS AND DISCUSSION

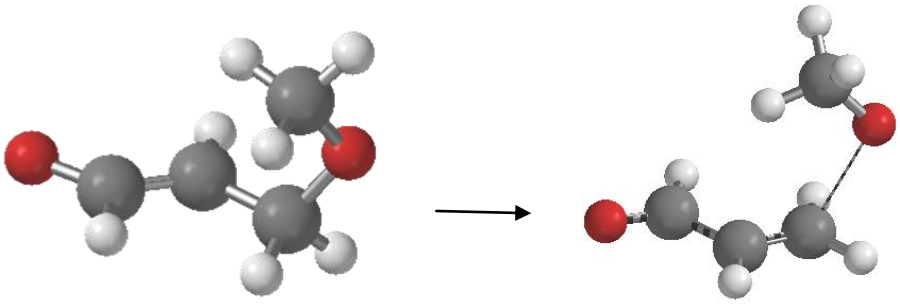
4.1 Model Systems with Methanol

4.1.1 Simple Model

For computational convenience, the study started from a very simple model system which consisted of the intermediate from one methanol molecule and one acrolein/propane molecule as shown in Figure 12.

Table 1. Calculation results of simple model

Calculation Settings	HF with 3-21G	HF with 6-31G*
Reactant (kcal/mol)	-1.9051E+05	-1.9160E+05
Intermediate (kcal/mol)	-1.9052E+05	-1.9159E+05
Reaction (kcal/mol)	-1.9048E+05	-1.9156E+05
C-O (Å)	2.30	2.06
C-C-O (degrees)	118.31	116.07
C-C-C-O (degrees)	85.93	92.53
ΔE1 (kcal/mol)	29.23	40.09
ΔE2 (kcal/mol)	33.42	27.27



Intermediate Transition State

Figure 17. Structures of simple model with methanol

The calculation methods presented use Hartree-Fock methods with 3-21G basis set, as well as Hartree-Fock method with 6-31G* basis set. Theoretically, 6-31G* basis set ought to provide better accuracy for the calculations. In this case, all the computed energies were around 100 kcal/mol lower than calculations with 3-21G basis set. The calculated data of geometries were close, and the energies were slightly different. If compared with the actual structures of the transition states visually, there were not much difference between the two results. One difference in geometries was with the methyl group of methanol, which might be the major contributor to the differences in energies of the transition states. Figure 17 shows the structures of this model system. All the numbers would be compared with the other models.

4.1.2 Model with one Phenyl group

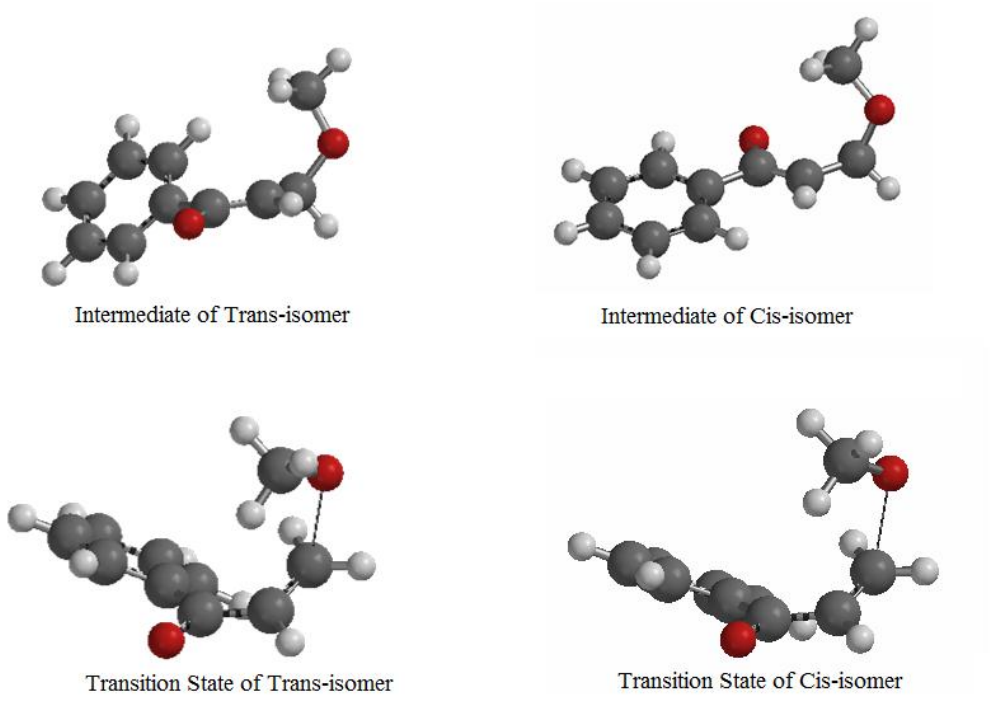


Figure 18. Structures of isomers with one phenyl group

Table 2. Calculation results with different basis sets for *trans*-isomer with one phenyl group

Basis Set	3-21G	6-31G*
Intermediate (kcal/mol)	-3.3376E+05	-3.3563E+05
Transition State (kcal/mol)	-3.3373E+05	-3.3561E+05
C-O (Å)	2.24	2.03
C-C-O (degrees)	117.32	115.98
C-C-C-O (degrees)	86.41	88.04

Table 3. Calculation results with different basis sets for *cis*-isomer with one phenyl group

Basis Set	3-21G	6-31G*
Intermediate (kcal/mol)	-3.3377E+05	-3.3564E+05
Transition State (kcal/mol)	-3.3373E+05	-3.3561E+05
C-O (Å)	2.24	2.02
C-C-O (degrees)	117.80	116.35
C-C-C-O (degrees)	86.88	83.81

Table 4. Comparison of calculation results with two isomers

Conformation	*Trans*-	*Cis*-
Reactant(kcal/mol)	-3.3376E+05	-3.3376E+05
Intermediate (kcal/mol)	-3.3376E+05	-3.3377E+05
Transition State (kcal/mol)	-3.3373E+05	-3.3373E+05
C-O (Å)	2.24	2.24
C-C-O (degrees)	117.32	117.80
C-C-C-O (degrees)	86.41	86.88
ΔE1 (kcal/mol)	27.41	36.07
ΔE2 (kcal/mol)	30.22	38.84

Both curcumin and chalcone compounds have phenyl groups. Due to the size and chemical properties, it is necessary to consider the influence of phenyl groups to this conjugate addition. The first step is to replace the aldhyde hydrogen with a phenyl group. In this model system, isomers were studied. Both isomers were calculated with two basis sets. The energy and geometric parameters were similar, which supported the validity of each transition state as shown in Table 2 and Table 3. The structures of

the isomer intermediates and the corresponding transition states are shown in Figure 18. Obviously, the intermediates of two isomers are quite different while the transition states are quite close. The numbers in Table 4 are also consistent with it.

4.1.3 Model with two Phenyl groups

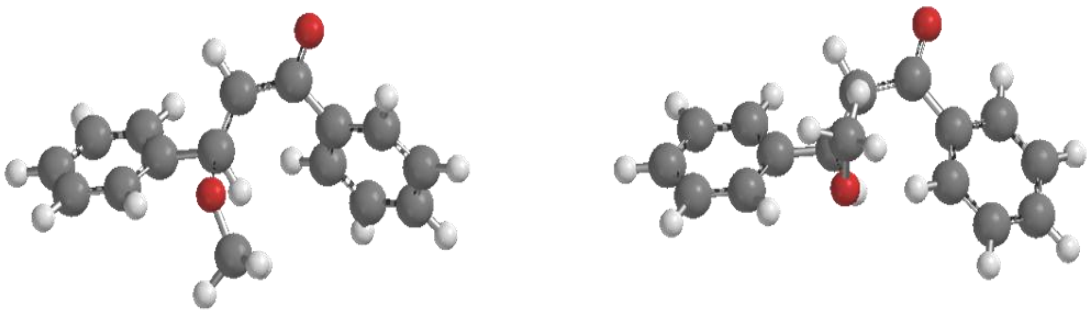


Figure 19. Structures of transition states of *cis* (L) and *trans* (R) isomers

Two phenyl groups were added to the simple model to generate chalcone. The two additional benezene rings significantly increased the calculation cost and influenced the energies and geometries of the transition states. Therefore, only Hartree-Fock method with 3-21G basis set was applied for this model system.

Table 5. Computation results of isomers with two phenyl groups

Conformation	*Trans-*	*Cis-*
Reactant(kcal/mol)	-4.7700E+05	-4.7701E+05
Intermediate (kcal/mol)	-4.7700E+05	-4.7701E+05
Transition States (kcal/mol)	-4.7698E+05	-4.7697E+05
C-O (Å)	2.17	2.14
C-C-O (degrees)	111.64	112.20
C-C-C-O (degrees)	79.13	73.87
ΔE1(kcal/mol)	17.67	40.55
ΔE2(kcal/mol)	18.84	41.33

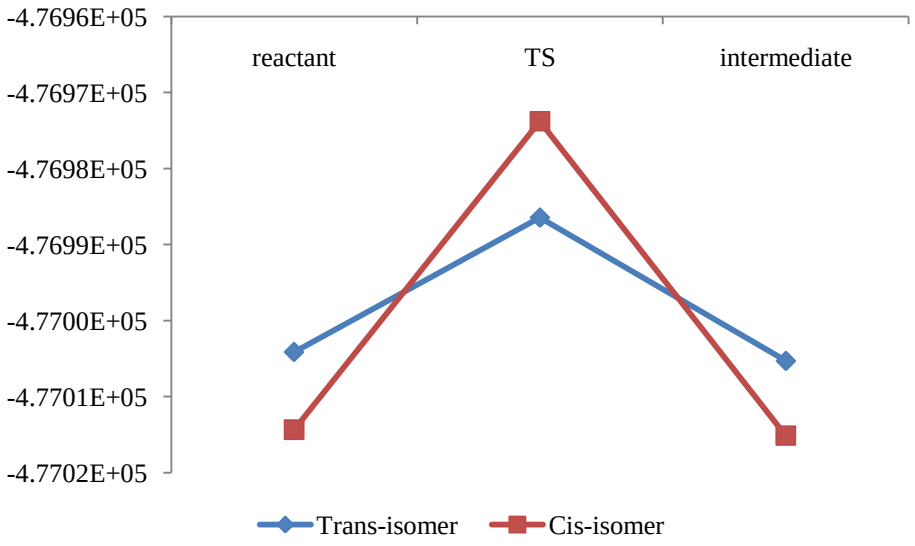


Figure 18. Graphic comparison with reactions of *trans-/cis*-isomers

Again, the geometries of two isomers are close except the methyl groups and the torsion of the phenyl groups, as shown in Figure 19. And data in Table 5 confirms it. However, since the intermediate of *cis*-isomer has a lower energy for reactants and

intermediates, as well as a higher energy for transition states, both ΔE1 (activation energy) and ΔE2 are greater than *trans*-isomer model as shown in Figure 20. Considering that the lower energies the more stable compounds are, and larger activation energies increase the difficulties for reactions, the following studies on derivatives were studied mainly based on *cis*-isomer models.

4.1.4 Models with Methanol

Table 6 compares three models with methanol as the reactant. The geometries of the transitions states of three models are very close. It implies that this type of conjugate addition follows similar patterns. From the information of the calculated transition states' geometries, the reaction details may be speculated. The dihedral angle of model with two phenyl groups is slightly different from the other two. It may be due to the angles between phenyl groups with the plane of the reaction site. Therefore, the existence of phenyl groups affects the geometries of the transition states, especially the dihedral angles.

The two model systems with phenyl groups have another feature in common, that is the transition states from *cis*-isomers have larger activation barrier and lower energy for intermediate. Therefore, *cis*-isomer is very likely to be the actual form in reactions. In addition that based on the calculated energies, the activation barrier increases with more phenyl groups. It is probably due to the more crowded space which makes the nucleophilic agents difficult to attack β-carbon.

Table 6. Comparison of transition states of *cis*-isomer models with methanol

	C-O(Å)	C-C-O(°)	C-C-C-O(°)	ΔE1(kcal/mol)	ΔE2(kcal/mol)
1	2.302	118.31	85.93	29.2332	33.4180
2	2.238	117.80	86.88	36.0730	38.8448
3	2.143	112.20	73.87	40.5472	41.3272

(1-simple model; 2-model with one phenyl group; 3-model with two phenyl groups)

4.1.5 Incorrect Transition State

Sometimes, the calculations gave incorrect transition states with unrealistic geometries and more than one negative frequency in IR spectrum. Most of them shared some common characteristics. Firstly, the energies of transition states were lower; secondly, the distance between carbon atom and oxygen atom is greater; the dihedral angles are totally different. Usually these wrong transition states were obtained by semi-empirical methods. But if starting from Hartree-Fock methods, only the correct transition states were achieved. This no doubt is due to the inaccuracy of semi-empirical methods.

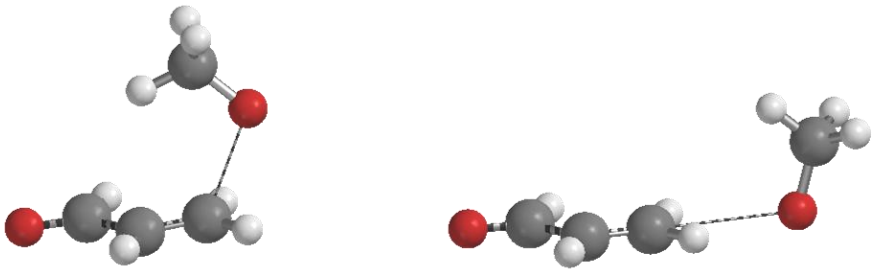


Figure 19. Structures of correct(L) and incorrect(R) transition states

Table 7. Comparison the correct transition state with the wrong one

Conformation	Correct	Wrong
Reactant(kcal/mol)	-1.9051E+05	-1.9051E+05
Intermediate (kcal/mol)	-1.9052E+05	-1.9052E+05
Transition State (kcal/mol)	-1.9048E+05	-1.9049E+05
C-O (Å)	2.30	2.93
C-C-O (degrees)	118.31	177.55
C-C-C-O (degrees)	85.93	140.18
ΔE1(kcal/mol)	29.2332	23.4990
ΔE2(kcal/mol)	33.4180	27.6839

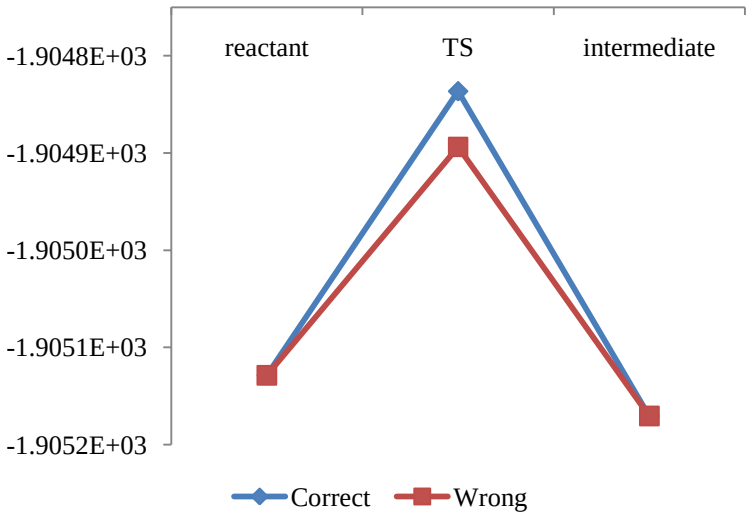


Figure 20. Graphic comparison of two transition states

Ab initio methods use a much more sophisticated Hamiltonian, only the wavefunction is being approximated to solve the Schrödinger equation. In contrast, semi-empirical methods approximate the Hamiltonian and then solve the Schrödinger equation accurately.[40] Due to this reason, in some cases, the semi-empirical methods may differ from those obtained with *ab initio* methods. In this case, the

semi-empirical methods provided pseudo transition states with lower energies. By data verification, these incorrect results were ruled out.

4.2 Model Systems with Methanethiol

4.2.1 Models with Methanethiol

Because the glutathione reacts with enones by thiol functional group, methanol needs to be replaced by methanethiol. Then the results will be more helpful for future research. Similarly to model systems with methanol, three model systems with 0, 1, and 2 phenyl groups were used in the calculations, analyzed and compared.

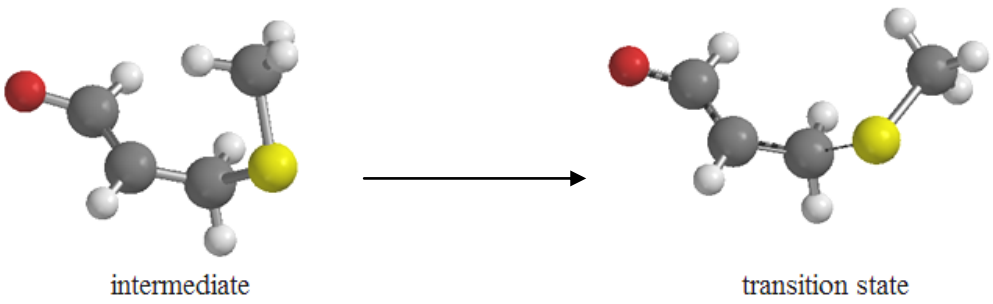


Figure 21. Structures of simple model with methanethiol

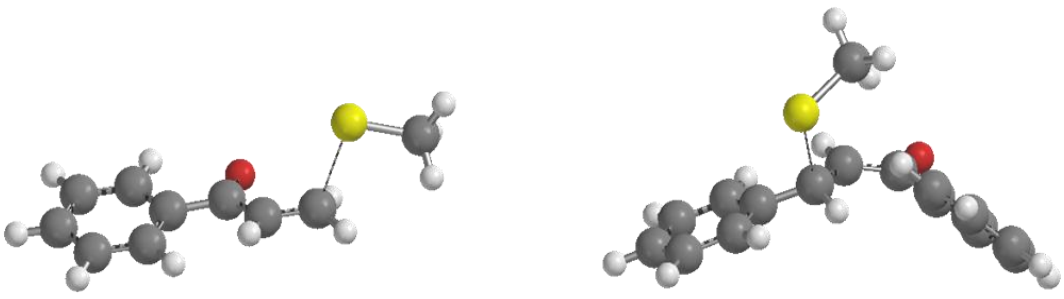


Figure 22. Structures of transition states of one phenyl(L) and two phenyl(R) groups

Figure 23 and Figure 24 show the structures of the three model systems. In Table

8, the results from two basis sets were very close, which increases our confidence in the reliability of the calculations. The *cis*-isomers in reactants and intermediates still have lower energies, with higher energies for the transition states. The geometries for all three models were similar and the activation barrier increases with the numbers of phenyl groups.

Table 8. Comparison of simple model with methanethiol

Basis Set	3-21G	6-31G*
Reactant (kcal/mol)	-3.9210E+05	-3.9407E+05
Intermediate (kcal/mol)	-3.9210E+05	-3.9407E+05
Transition State (kcal/mol)	-3.9209E+05	-3.9406E+05
C-O (Å)	1.86	1.85
C-C-O (degrees)	114.93	115.57
C-C-C-O (degrees)	108.07	111.28
ΔE1(kcal/mol)	11.37	10.81
ΔE2(kcal/mol)	4.91	4.48

Table 9. Comparison of models with one phenyl group and methanethiol

Conformation	*Trans*-	*Cis*-
Reactant(kcal/mol)	-5.3535E+05	-5.3535E+05
Intermediate (kcal/mol)	-5.3534E+05	-5.3535E+05
Transition State (kcal/mol)	-5.3533E+05	-5.3533E+05
C-O (Å)	2.30	2.34
C-C-O (degrees)	115.55	114.59
C-C-C-O (degrees)	98.78	91.98
ΔE1(kcal/mol)	14.86	19.77
ΔE2(kcal/mol)	7.56	13.09

Table 10. Comparison of models with two phenyl groups and methanethiol

Conformation	*Trans-*	*Cis-*
Reactant(kcal/mol)	-6.7859E+05	-6.7860E+05
Intermediate (kcal/mol)	-6.7858E+05	-6.7859E+05
Transition State (kcal/mol)	-6.7857E+05	-6.7857E+05
C-O (Å)	2.29	2.38
C-C-O (degrees)	111.40	108.69
C-C-C-O (degrees)	98.62	87.32
ΔE1(kcal/mol)	19.37	25.73
ΔE2(kcal/mol)	6.24	12.39

Table 11. Comparison of transition states of *cis*-isomer models with methanethiol

	C-O(Å)	C-C-O(°)	C-C-C-O(°)	ΔE1(kcal/mol)	ΔE2(kcal/mol)
1	1.86	114.93	108.07	11.3679	4.9089
2	2.298	115.55	98.78	14.8607	7.5584
3	2.285	111.40	98.62	19.3731	6.2374

(1-simple model; 2-model with one phenyl group; 3-model with two phenyl groups)

4.2.2 Comparison of Models with Methanol and Methanethiol

In comparing Tables 6 and 11, it is easy to see the trends for the methanethiol models compared to the methanol models. This implies that the computational results are consistent, and the geometries data may be used as references for the following studies. Due to the size of the atom and less electro negativity, it is more difficult to run calculations on models with sulfur atom. There was also a higher chance however,

of getting incorrect transition states. Therefore, it is necessary to calculate the model systems with methanol first, and to use these results as the starting part for the methanethiol calculations.

4.3 Models with the Derivatives of Chalcones

4.3.1 Computation with Derivatives of Chalcones

As mentioned in 4.1.2, in model systems, *cis*-isomers have lower energies of reactants and intermediates. For derivatives, the calculations showed similar results, and the transition states from *trans*- and *cis*-isomers are of little differences. Therefore, only the results computed from *cis*-isomer of derivatives, as shown in Figure 25, are discussed here.

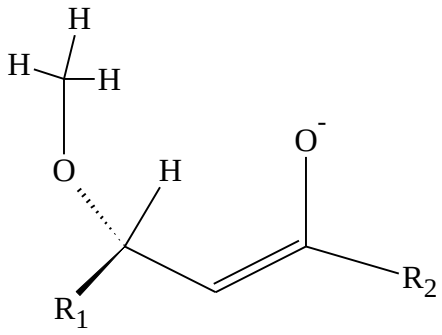
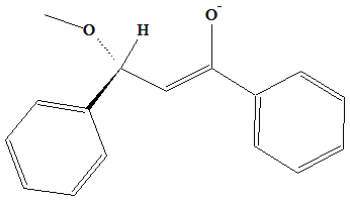
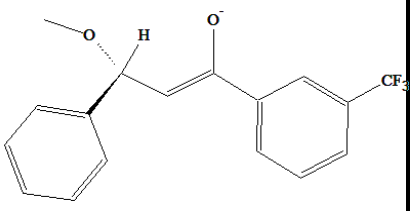
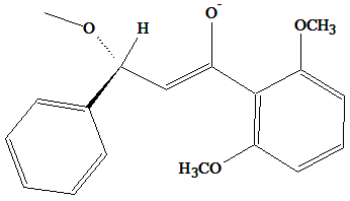
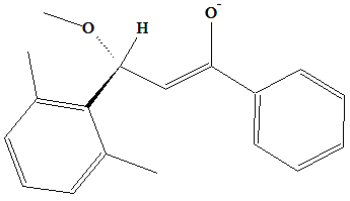
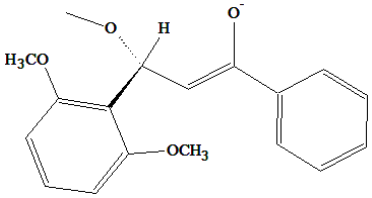
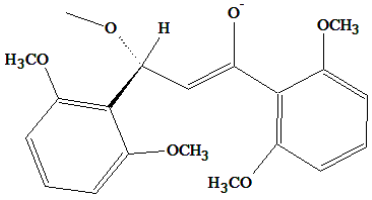


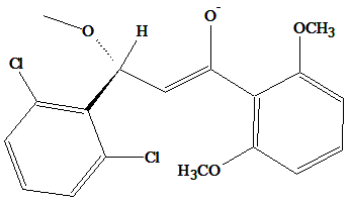
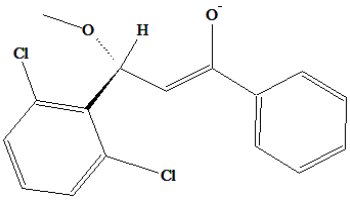
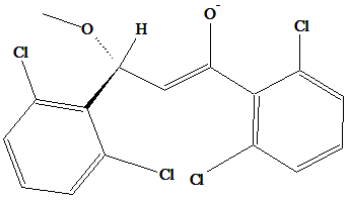
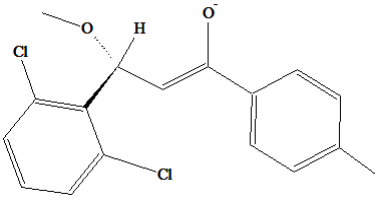
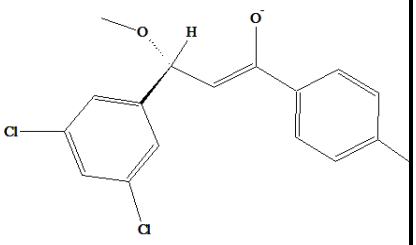
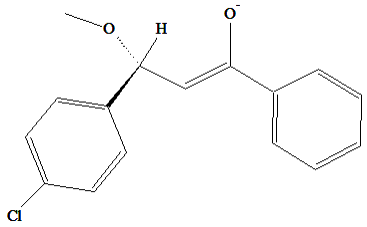
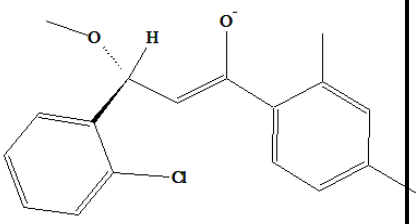
Figure 23. General structure of derivatives

Most of the derivatives of chalcones were selected from earlier research in Bowen group.[8] There were experimental data available for these compounds. All substitutions are based on two phenyl groups. Methyl, methoxy, chloro, trifluoromethyl, and propyl groups were added to the chalcone structures as noted in

Table 12.

Table 12. List of chalcone derivatives with structures

No.	R1	R2	Structure
1	phenyl	phenyl	
2	phenyl	2-trifluoromethylphenyl	
3	phenyl	1,5-dimethoxyphenyl	
4	1,5-dimethylphenyl	phenyl	
5	1,5-dimethoxyphenyl	phenyl	
6	1,5-dimethoxyphenyl	1,5-dimethoxyphenyl	

7	1,5-dichlorophenyl	1,5-dimethoxyphenyl	
8	1,5-dichlorophenyl	phenyl	
9	1,5-dichlorophenyl	1,5-dichlorophenyl	
10	1,5-dichlorophenyl	3-methylphenyl	
11	2,4-dichlorophenyl	3-methylphenyl	
12	3-chlorophenyl	phenyl	
13	1-chlorophenyl	1,3-dimethylphenyl	

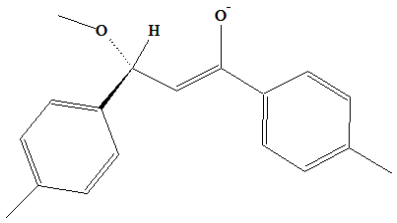
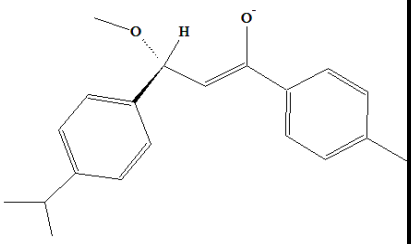
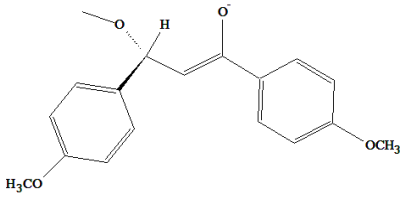
14	3-methylphenyl	3-methylphenyl	
15	3-isopropylphenyl	3-methylphenyl	
16	3-methoxyphenyl	3-methoxyphenyl	

Table 13 contains all the molecular geometry and energy profiles of 16 derivatives of chalcone used in this study. The molecular geometries of these derivatives were close according to the statistical data in Table 14. Among them, the dihedral angles have the greatest standard deviation, which indicates the dihedral angles are most influenced by the modifications on phenyl groups. The dihedral angles describe the planes and torsions at the reaction sites, which can be considered as the space for nucleophilic agents attacking the β-carbon. Therefore, it relates to the activation energies. However, according to the data, it seems there are no direct relationships between dihedral angles and activation energies. But if the dihedral angles differ significantly from chalcone, which has a relative higher activation barrier,

it is very likely that ΔE1 also will have a lower number. Chalcone itself has a relative higher activation barrier in reaction compared to most of its derivatives. Thus, chalcone may not easily react with glutathione and have a longer half-life. Since the dihedral angles change, modifications on both benzene rings are able to change the activation energies. Then it is possible to design molecules with higher activation energies. Figure 26 is the graph compared energies of different derivatives.

Table 13 Calculation results of derivaties with geometries and energies

No.	C-O(Å)	C-C-O(°)	C-C-C-O(°)	ΔE1(kcal/mol)	ΔE2(kcal/mol)
1	2.143	112.20	73.87	40.5472	41.3272
2	2.205	111.40	71.17	33.5247	39.2821
3	2.277	113.03	78.33	34.5476	35.2824
4	2.143	110.35	65.41	31.7169	39.9285
5	2.175	112.27	79.79	41.3360	39.8249
6	2.175	111.75	86.95	30.0132	30.4782
7	2.178	118.67	84.71	34.9893	35.9312
8	2.165	115.75	81.95	33.2323	35.3100
9	2.335	119.69	82.31	25.5516	42.8338
10	2.158	115.95	82.11	33.3829	34.9460
11	2.103	113.89	83.20	29.9190	34.2118
12	2.111	113.63	82.59	31.5757	34.9021
13	2.131	110.50	83.22	28.2750	31.7834
14	2.191	111.47	72.46	36.4709	36.3347
15	2.112	113.69	82.10	33.4676	33.2053
16	2.187	112.09	72.71	36.1891	35.0307

Table 14. Statistics of geometries of derivatives

Geometry Parameter	C-O(Å)	C-C-O(°)	C-C-C-O(°)
Average	2.174	113.52	78.93
Standard Deviation	0.0605	2.7354	5.9929
Confidence Interval	0.0249	1.1248	2.4644

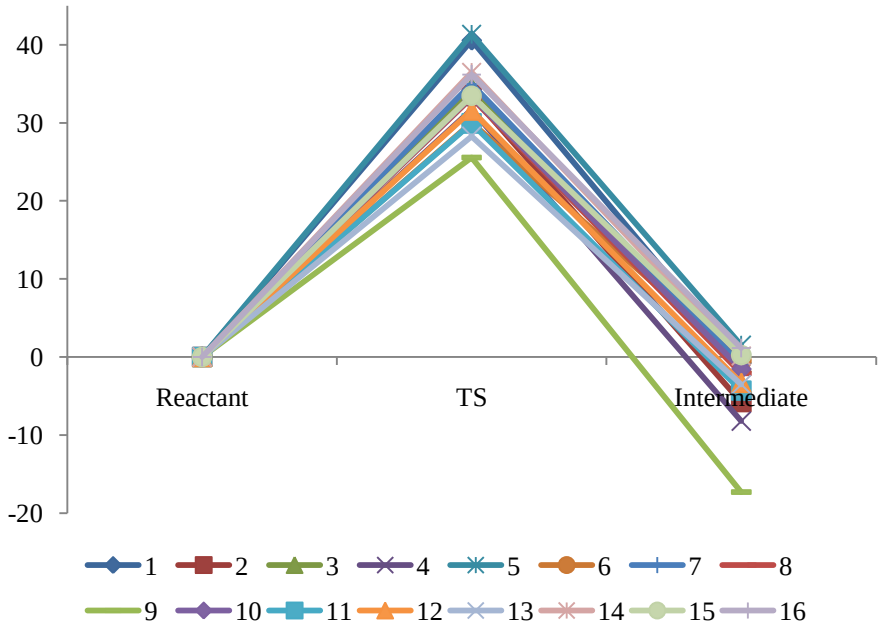


Figure 24. Graphic comparison of derivatives

The analysis suggests that adding functional groups near the reaction site does not necessarily increase the difficulty of the reactions. There is no functional group that can significantly increase the activation barrier. If the analysis is combined with the actual structures of the transition states, the torsion angle of phenyl groups and the reaction sites may play more important roles. Take derivatives **6**, **7** and **9** as examples, all of them have additional functional groups on 1,5-position of both benzene rings. Derivatives **7** and **9** have the largest and smallest activation energy respectively. The

structures of the reactants may give some clues as shown in Figure 27. Compared to methoxy groups, the halo groups were almost on the same plane. Then there will be fewer obstacles for nucleophilic agents to attack the enones. Therefore, derivative **9** has the smallest ΔE1 value. For derivatives **14**, **15** and **16**, they have the similar modifications which are all far from the reaction sites. Derivatives **14** and **16** indeed have close activation energies while **15** is less than them. This suggests that besides the spacial structure of the reaction site, the characteristics of different functional groups also contribute to the change of the activation barrier. However, there is no clear pattern about it.

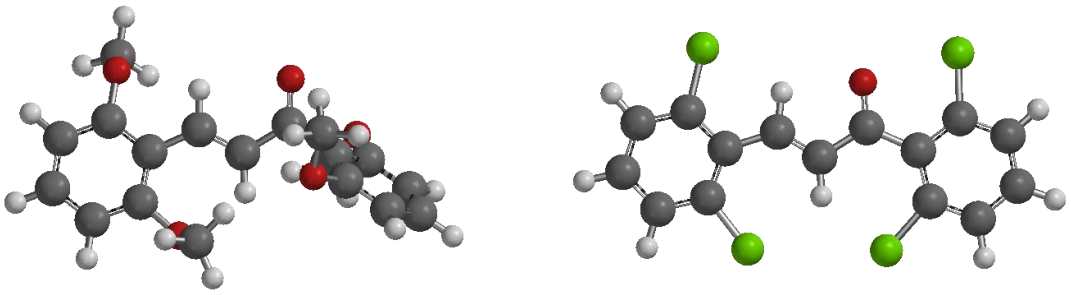


Figure 25. Reactants of derivative 7(L) and 9(R), methanol is not included

4.3.2 Comparison with Calculation and Experimental Results

For earlier work in the Bowen group, most of the derivatives in Table 12 were studied experimentally. The activities of the compounds were evaluated by the percent inhibition of *in vitro* endothelial cell proliferation.[8] Table 15 contains the calculation results and the experimental results for comparison.

As the calculations imply, chalcone itself has a larger activation barrier; it showed

a better inhibition percentage *in vitro* experiment. ΔE1 is not in proportion with the experimental activity. But if the activation barrier is smaller, it is very likely that this derivative showed less activity in cells. One exception is those with chloro groups. As mentioned in 4.3.1, chloro groups were not helpful in increasing the activation energies due to the spacious reaction site. However, in the experiment, they showed better activity compared to those with methoxy groups. In this case, the experimental data does not have the same pattern with computations. In other words, the calculation result is not consistent with experiment. Thus, it is very likely that there are other mechanisms playing important roles for the activities of compounds

Table 15. Comparison of calculation and experimental data

No.	ΔE1(kcal/mol)	ΔE2(kcal/mol)	SVR growth inhibition		
			1μg/ml	3μg/ml	6μg/ml
1	40.5472	41.3272	71.6	92.8	94.4
2	33.5247	39.2821	42.3	87.4	96.9
3	34.5476	35.2824	25.8	39.8	63.5
4	31.7169	39.9285	47.7	57.9	89.6
5	41.3360	39.8249	31.8	56.4	60.8
6	30.0132	30.4782	36.2	49.2	39.2
7	34.9893	35.9312	23.2	43.7	52.3
8	33.2323	35.3100	4.6	61.0	94.0
9	25.5516	42.8338	48.3	75.3	93.7
10	33.3829	34.9460	19.9	10.4	84.7
11	29.9190	34.2118	-	-	-
12	31.5757	34.9021	-	-	-
13	28.2750	31.7834	29.6	25.3	73.4
14	36.4709	36.3347	36.3	67.3	89.5

15	33.4676	33.2053	19.5	14.2	59.2
16	36.1891	35.0307	29.1	63.4	85.2

There are many reasons for the inconsistency. Firstly, the model system uses methanol. Although oxygen atom is similar to sulfur atom, there are still many differences such as the size and electronegativity. The simplified model systems affect the generality of the calculations. The glutathione and methanethiol are very different structurally, except for the thiol group. If this reaction is catalyzed by enzymes, the shape of the substrates and other properties should also be taken under consideration.

The suggested glutathione reduction may be only part of the reaction mechanism There are other factors influencing the activity of the derivatives. For example, Boumendjel and his colleagues suggested the lipophilicity of the chalcone derivatives affect the activities by testing the percentage of G2/M phases.[41] Therefore, the study can only be as reference to study the conjugate addition. The information obtained by computational methods may be helpful but it cannot replace the roles of experimental studies to predict the activities of the compounds.

CHAPTER V

CONCLUSION AND FUTURE WORKS

5.1 Conclusion

This study was done by quantum chemistry methods. It covers the conjugate addition of enones and the derivatives of chalcones. Reactants, transition states, and intermediates were computed by *ab initio* methods in SPARTAN, a computational chemistry software program. The molecular geometries and energies have been calculated, analyzed, and summarized. The derivatives of chalcone were also studied in the same way. In additional, the calculation results of the derivatives were compared with experimental data.

With different model systems, the geometries of reaction site of the transition states still share similarities. The calculations suggest that the nucleophilic addition maintains essentially the same pattern. Then the information of the reaction from the simplified model systems can be helpful for the actual reactions between glutathione and enone compounds.

The modifications on phenyl groups have been shown to have more influence on the activation energies rather than geometries; thus, it is possible to alter the activation barrier by modifications in order to make it difficult for conjugate addition. However,

it is not reliable to predict the activity of the compounds by calculating energies of the conjugate reactions. There are more factors contribute to the reactions. Therefore, the experiments are necessary to screen novel chalcone and curcumin derivatives.

5.2 Future Study

Firstly, the derivatives with methanethiol need to be studied and compared to those with methanol. In this study, although two model systems share similarities in geometries of the reaction sites, the differences in energies will be more interesting. It is possible that with methanethiol, the derivatives may display a more clear pattern in the relationship with the activation energies.

Secondly, more derivatives will be tested. There are much more derivatives of chalcone reported in different articles. Various modifications should be considered to validate the method and enrich the data. For example, replace phenyl groups with other aromatic rings. Also more complex compounds need to be studied as well.

The next step is to replace methanol and methanethiol with other structures which are closer to glutathione. The robustness of the model systems will be tested, and the results will be more reliable than those simplified model systems.

Furthermore, other reactions may be studied in a similar way. Although computational methods may not be very accurate to predict independently reactions and activities, it may save time and cost before starting the synthesis and bioassay tests.

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