UNDERSTANDING THE METABOLISM OF DRUG LIKE MOLECULES BY HEME & NON-HEME MODELLED ENZYMES USING QUANTUM MECHANICAL TOOLS

SUMMARY

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Nidhi Awasthi

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Under the Supervision of

Prof. Devesh Kumar

DEPARTMENT OF APPLIED PHYSICS

SCHOOL FOR PHYSICAL SCIENCES

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A CENTRAL UNIVERSITY)

LUCKNOW (U.P.), INDIA – 226025

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UNDERSTANDING THE METABOLISM OF DRUG LIKE MOLECULES BY HEME & NON-HEME MODELLED ENZYMES USING QUANTUM MECHANICAL TOOLS

Chapter-1 Introduction

Enzymes are typically proteins that increase the rate of biochemical reactions which continuously occur in human cells. These are very important for functions of the body, such as digestion, respiration, muscle nervous system, and metabolism. Many enzymes are used for breaking large compounds into small parts, so that it is more easily absorbed by the body. Some enzymes bind the two different molecules and form a new molecule with different properties. Each enzyme increases only the rate of a specific reaction, so these are the selective catalyst. Enzymes are the catalyst of life. All enzymes are originated from living organisms. The general way of searching enzymes is growing the organism and isolating it from animal organs, parts of plants, inside microorganisms. First, enzymes were found from animal and plant parts. These enzymes are protease papain from papaya fruit or rennet from calve's stomach. Nowadays, enzymes originated from microorganisms. It is the most technical enzymes. The microorganism is omnipresent. They are found in freezing Antarctica water, in hot water, in acidic spring water on the volcanoes, and in alkaline lakes.

Protein enzymes are made of one or more amino acid chains. This chain is usually called polypeptide chains. These chains determine the characteristic of the protein's structure. If the temperature or pH of enzymes is changed, the protein may change its denature and enzymatic ability. An enzyme interacts with a specific type of substance

or a group of substances usually called substrate, for catalyzing the chemical reactions. For initiating the reaction, an energy barrier should be overcome, which is present in most of the reactions. These barriers protect the unrespectable degradations of complex molecules. So, it is useful for the preservations of life. During the metabolic process, these energy barriers are surmounted and break down the complex molecules. For completing the reaction some additional heat is needed calling "activation energy". But high activation energy increases the temperature that would kill the cell. Active site of Cytochrome P450 enzyme is shown in Figure 1.1 taken from PDB file 1W0E.

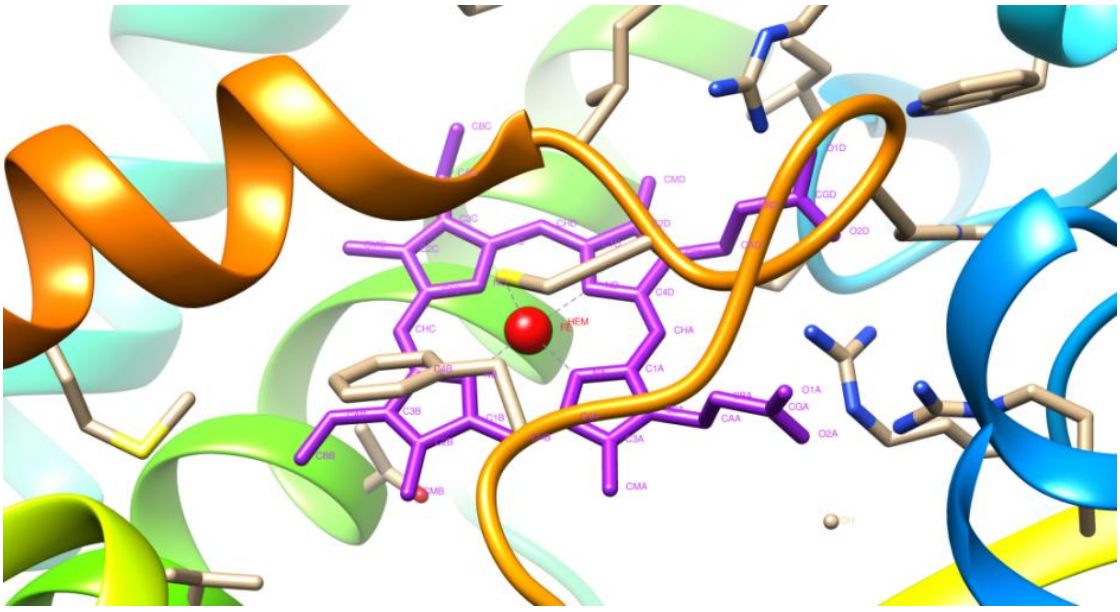

Figure 1.1: Active site of Cytochrome P450 as taken from PDB file 1W0E.

Here, enzyme plays an important role in lowering the activation energy. They react with some specific substrate and form an intermediate complex, a "transition state", that requires less energy for further preceding the reaction. This intermediate complex is rapidly broken down and forms a product. The unchanged enzyme is then ready to react with other substrates. The region, which binds to the substrate, is called the

active site. It forms one type of cavity, where the substrate binds. It permits only some particular substrate; hence, it is also used for determining the specificity of enzymes.

Cytochrome P450 enzymes are the superfamily of enzymes containing heme cofactor present in all kingdoms of life, like in animals, plants, fungi, protists, bacteria, and also in viruses. They have been not found in Escherichia coli. More than 50,000 enzymes are found. In Cytochrome P450, the term P450 has derived from the spectrometric peak at wavelength 450 nm of the absorption maximum of the enzyme when it is in a reduced state and complexed with carbon monoxide. These enzymes are responsible for many chemical reactions like detoxification and synthesis etc. Many of the Cytochrome enzymes are required a protein to transfer one or more electrons to reduce the iron. The P450 enzyme is metabolized various compounds using high-valent iron (IV) oxo species complex, generally known as Cpd1. This complex is formed during the catalytic cycle of cytochrome P450. Initially, in the catalytic cycle, P450 is resting and a water molecule is ligated with it. Whenever substrate enters, it tightly binds with Porphyrin by expelling the water molecule.

Heme enzyme is one of the most useful enzyme biochemical reactions, especially in catalytic reactions. It contains heme cofactor. The main function of the heme enzyme is- to transfer the electron, to store and transfer the oxygen molecules. In the structure of the heme enzyme, an iron ion present at the center of the Porphyrin ring, and this iron is ready to react with specific substrates. Cytochrome P450 is a superfamily of many enzymes containing heme cofactor. In Cytochrome P450, the heme group is the active site of the enzyme. Like Cytochrome P450, heme-copper oxidase is also a superfamily of another enzyme. It also contains the heme factor and useful in the catalysis of various biochemical reactions. These are membrane enzyme which catalyzes the oxidation, aggregate the formation of many proteins and degradation of

proteins in small peptides. Active site of HEME enzyme is shown in Figure 1.2 taken from PDB file 5B4Z.

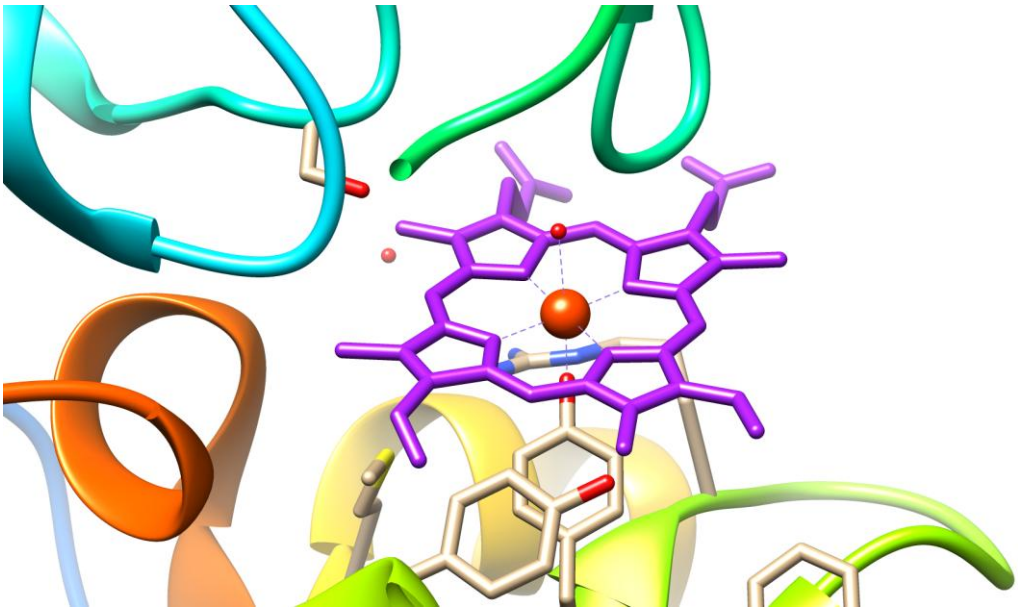

Figure 1.2: Active site of HEME enzyme as taken from the PDB file 5B4Z.

This enzyme is the same as the heme enzyme, but it does not contain the Porphyrin prosthetic group. These enzymes are also participating in biochemical processes. Non-heme enzymes are divided into two main categories:

1. Mononuclear Non-enzyme iron enzyme
2. Dinuclear Non-enzyme iron enzyme

It is one of the main reaction which operates the all biochemical reaction processes. The catalytic cycle of Cytochrome P450 is like an automatic nano-machine that once it operates, continuously run. This cyclic reaction is completing in seven steps, as shown in Figure 1.3. The cyclic reaction starts with the resting state (1), in which a distal water molecule is bound to the six ligated positions of the low spin state of hexa-coordinated ferric ion. The entry of the substrate, like alkane AlkH, displace the water

molecule remaining the Penta-coordinated ferric-Porphyrin (2), this ferric complex has a slightly better electron accepter tendency than the resting state.

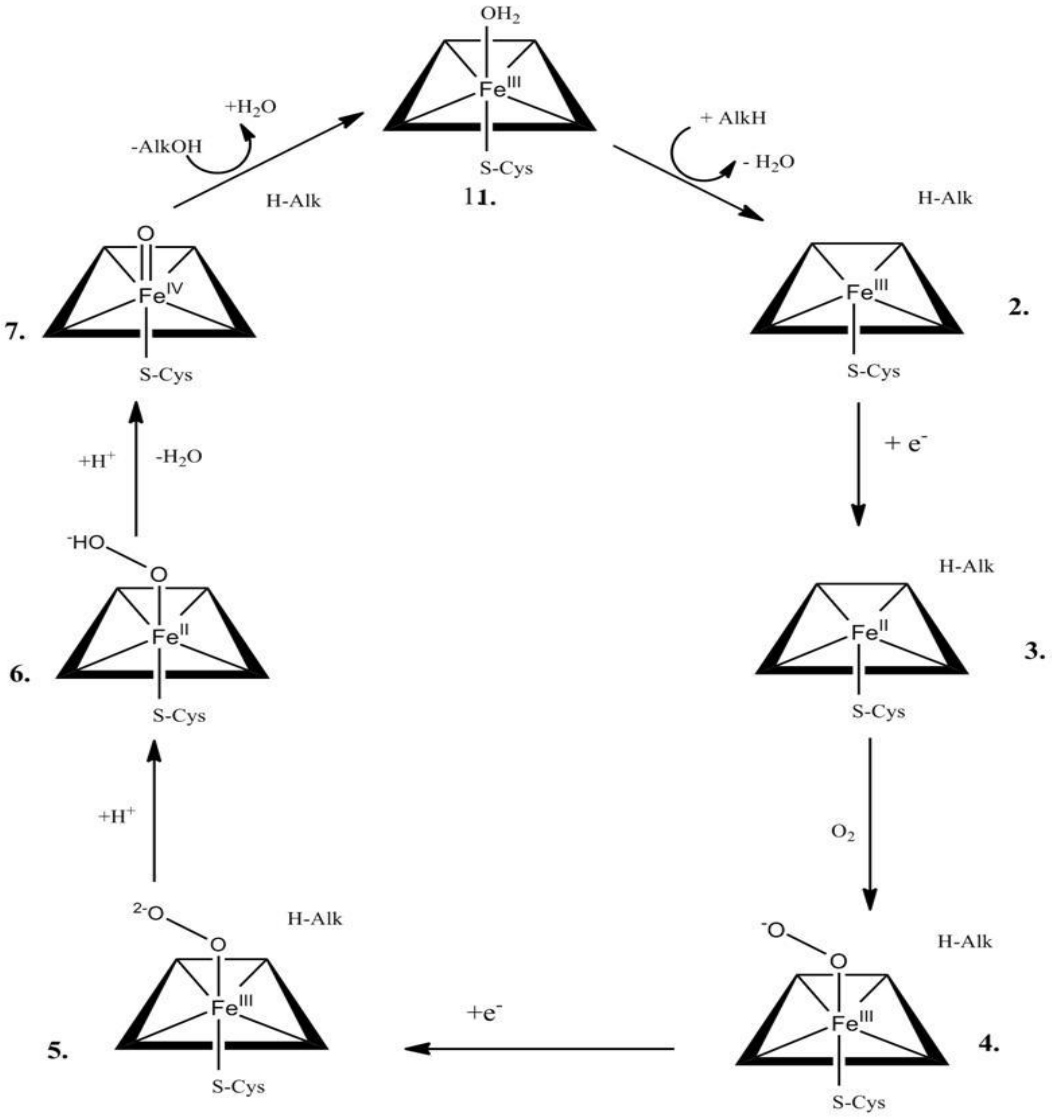

Figure 1.3: Catalytic Cycle of Cytochrome P450.

Therefore it take one electron from the reductase protein and trigger from low spin state to high spin state and form five coordinated high spin state of ferrous complex (3), this ferrous complex further bound with O_2 and formed ferrous dioxygen complex (4), it has a good electron accepter tendency, which accepts one electron from reductase protein, resulting in ferric-peroxo anion species (5), this is rate determining step in catalytic cycle.

Porphyrin is a normal end product of hemoglobin metabolism and is the substance responsible for the reddish staining that is seen around the eyes of some breeds of dogs. A Porphyrin is a group of macrocyclic aromatic compounds made of four pyrrole rings which are internally connected by a methane bridge (=CH-). It is a square planar molecule and can extend its structure due to its aromatic character. Pyrrole rings are attached in such a manner, that nitrogen atoms facing at the center and form Porphyrin. A metal ion is captured by the center of Porphyrin, which naturally forms a stable metallic complex. Metal ions of these complexes, accept six coordinating ligands form an octahedral structure. So, these complexes are widely used in chemical reactions of a biological system. Hence, the study of Porphyrin is essential for the investigations of drug metabolism through Cytochrome P450 enzymes.Optimized structure of Porphyring is shown in Figure 1.4.

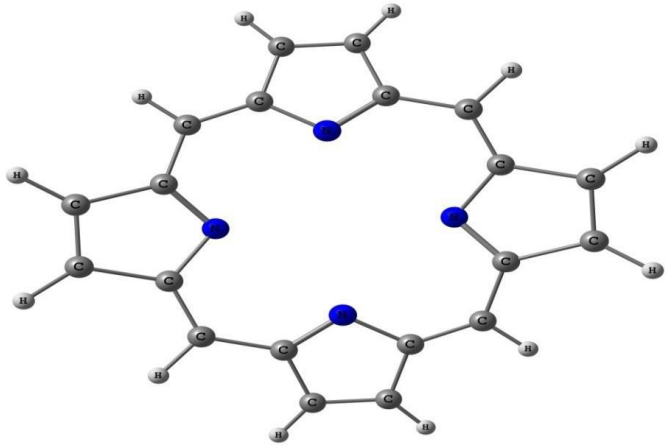

Figure 1.4: Optimized geometry of Porphyrin ring.

Chapter-2 Computational Methodology

According to quantum mechanics (QM), wave function (ψ) of any molecular system held all information related to that molecular system and it is obtained by solving the Schrödinger wave equation [2.1]:

$$H\Psi = E\Psi \qquad (2.1)$$

This is well known time-independent Schrödinger wave equation, where H is the Hamiltonian Operator of the given molecular system and E is the energy eigenvalue of the corresponding Hamiltonian Operator H. Ψ is a well behaved mathematical function also known as the wave function, whose square represents the probability density.

The wave function of the molecule represents its nuclear as well as electronic motions together, by using Born Oppenheimer approximation, the electronic wave function is separated from the total wave function.

When the nuclei of two or more atoms are fixed at a particular distance, then their electronic wave function sufficiently provides all the physical and chemical properties of the molecule. But for a multi-electron system, the electronic part of the Hamiltonian operator of the Schrödinger equation is given by:

$$H_e = -\sum_P \frac{1}{2}\nabla_P^2 - \sum_A \sum_P Z_A r_{AP}^{-1} + \sum_{p<q} r_{pq}^{-1} \qquad (2.2)$$

In the above equation, the first term represents the kinetic energy; the second term is the potential energy, whereas the third term is potential energy, which is raised due to inter-electron interactions. Thus the modified Schrödinger wave equation for the 'n' electron system is given by:

$$H_e(1,2,....,n)\Psi_e(1,2,....,n) = E_e\Psi_e(1,2,....,n) \qquad (2.3)$$

In the above equation, Ψ_e is an electronic part of the total wave function. The detailed treatment of quantum mechanical problems involving the electronic structure of the molecule is equivalent to the complete solution of the appropriate Schrödinger equation.

The equation of energy of the system given below is written in the form of Dirac notation, the equation of the energy of the system is given by-

$$E_e = \frac{\langle \Psi'_i \mid H_e \mid \Psi'_i \rangle}{\langle \Psi'_i \mid \Psi'_j \rangle}$$

The Hamiltonian is given as:

$$H_e = \sum_{p}^{N} h + \sum_{i=1}^{N} \sum_{j>i}^{N} g_{ij} + V_{nn} \tag{2.7}$$

where,

$$h_p = -\frac{1}{2}\nabla^2 - \sum_{a} \frac{Z_a}{|R_a - r_p|} \tag{2.8}$$

and

$$g_{ij} = \frac{1}{|r_i - r_j|} \tag{2.9}$$

Where one-electron operator (h_i) describes the motion of an ith electron in the field of all nuclei, (g_{ij}) is two-electron operator giving the repulsion between two electrons while V_{nn} is the nuclear-nuclear interaction energy. The energy can be expressed as:

$$E = \sum_{i}^{N} \langle \varphi_i \mid h_i \mid \varphi_i \rangle + \frac{1}{2} \sum_{ij}^{N} \left(\langle \varphi_j \mid J_i \mid \varphi_j \rangle - \langle \varphi_j \mid K_i \mid \varphi_j \rangle \right) + V_{nn} \tag{2.10}$$

$$J_{12} = \langle \varphi_1^{(1)} \varphi_2^{(2)} \mid g_{12} \mid \varphi_1^{(1)} \varphi_2^{(2)} \rangle \tag{2.11}$$

Where, J operator represents the classical repulsion between the two charge distributions described by $\varphi_{12}(1)$ and $\varphi_{22}(2)$:

$$K_{12} = \langle \varphi_1^2 \varphi_2^{(2)} \mid g_{12} \mid \varphi_2^{(1)} \varphi_1^{(2)} \rangle \tag{2.12}$$

The K operator represents the exchange integral that has no classical analog.

Density functional theory (DFT), in contrast to wave function in quantum mechanics, describes the energy as a functional of the electron cloud density [ρ (Ψr)]. Kohn et al. derived a set of single-electron equations that enables one to calculate the electron density and consequently the total energy of the system.

The first three terms resemble the Hartree-Fock Hamiltonian shown in equation.2.7, 2.8, and 2.9 above as a function of the nuclear coordinates (R), and the coordinates of the electrons (r), and is shown below:

$$E_{el} = -\frac{1}{2}\sum_i \int \varphi_i(r_1)\nabla^2\varphi_i(r_1)dr_1 + \sum_A \int \frac{Z_A}{|R_A - r_1|}\rho(r_1)dr_1 + \frac{1}{2}\frac{\rho(r_1)\rho(r_2)}{|r_1 - r_2|}dr_1 dr_2 + E_{XC} \quad (2.18)$$

The exchange energy is generally estimated from Slater exchange function given by:

$$E_X^{Slater} = -\frac{9}{4\alpha_{ex}}\left(\frac{3}{4\pi}\right)^{1/3}\sum_\gamma \int \left[\rho_1^\gamma(r_1)\right]^{4/3} dr_1 \quad (2.19)$$

In the above equation, α_{ex} is an exchange scale factor, which has the value 2/3 for an electron gas system. The commonly used correlation energy functional (E_C^{VWN}) was named after Vosko, Wilk,and Nusair[25] and represents the correlation energy per electron in an electron gas system $\varepsilon_C\left[\rho_1^\alpha \rho_1^\beta\right]$ with spin densities ρ_1^α and ρ_1^β, and it is given as:

$$E_C^{VWN} = \int \rho_1(r_1)\varepsilon_c\left[\rho_1^\alpha(r_1), \rho_1^\beta(r_1)\right]dr_1 \quad (2.20)$$

A basis set represents a group of mathematical functions which describe the shape of the orbitals of an atom. To solve the Schrödinger equation, one has to optimize the wave function with the help of perturbations from the Hamilton operator to find the energy eigenvalues. In this approach, occupied atomic orbitals (□) of all atoms in the

molecule are taken to create a set of molecular orbitals (φ_i) through fractions (c_{ri}) of all atomic orbital components as shown in the equation below:

$$\varphi_i = \sum_r c_{ri}\chi_r \qquad (2.26)$$

The number of active orbitals and virtual orbitals are taken into consideration decides the quality of molecular orbitals thus obtained. Hence, to achieve a good quality of molecular orbital wave function calculations, large number of such orbitals are required to be considered in the calculations so that the energy gets converge within the basis set limit.

Energies calculated by using computational quantum methods refer to only gas-phase data. To make computational results on par with experimental results for comparison, the effect, solvent needs to be incorporated into the calculations. To add solvent molecules in the model system and re-optimize the resultant structure is the simplest way to achieve this effect. But these types of calculations will increase computational time and also it is not always practical, because the solvent molecules can exist in several conformation. Hence, instead of adding the solvent molecule, a more common approach is to add solvent corrections to the energetics using an implicit solvent model. This can either be done at a single point level, whereby the energy is recalculated with a solvent model of the gas phase optimized geometry, or by re-optimizing the structure using a solvent model. The popular software packages, such as Gaussian and Jaguar, include solvent models like the Polarizable Continuum Model (PCM).

A chemical reaction can be studied using quantum chemical ab-initio and density functional theoretical (DFT) methods, employing the concept of transition state theory

(TST). The theory assumes a special type of chemical equilibrium (quasi-equilibrium) between reactants and the transition state. TST assumes that even when the reactants and products are not in equilibrium with each other, the activated complexes are in quasi-equilibrium with the reactants. Transition state theory fails for some reactions at high temperatures. The theory assumes that the reaction system would pass over the lowest energy saddle point on the potential energy surface. Other forms of TST, such as microcanonical variational TST, canonical variational TST, and improved canonical variational TST, where the transition state is not necessarily located at the saddle point, are referred to as a generalized transition state theory. According to TST, a simple one-step chemical reaction starts from a reactant complex (RC) and proceeds through a transition state called (TS) from where the product complex (PC) is formed.

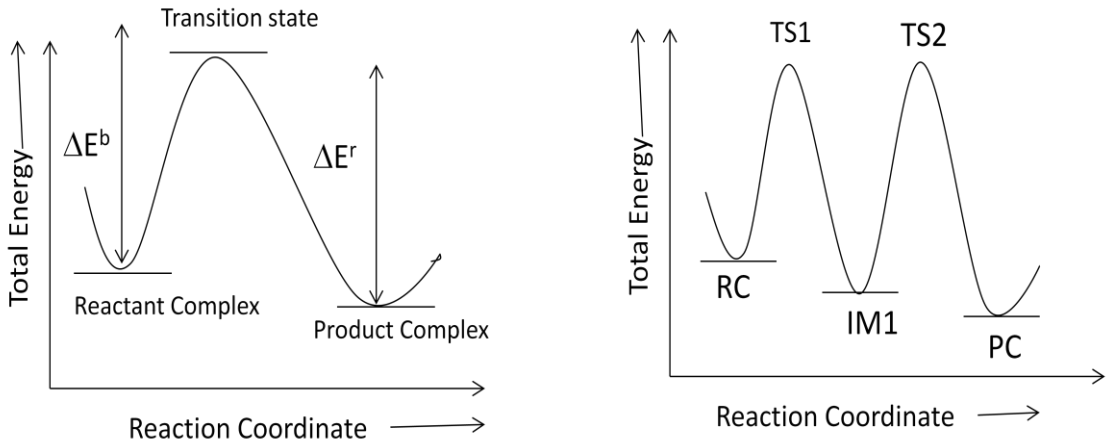

Figure 2.1 Potential energy surfaces for a single-step reaction (a) and three-step reaction (b).

On the PES, the energy difference between the total energies, of TS and RC is termed as the barrier energy (□ E^{b}), and the difference in total energies of the TS and PC is termed as released energy (□ E^{r}) (Figure. 2.1(a)). The barrier energy of a reaction can be obtained using the concept of potential energy surface (PES).

Chapter-3 Metabolism of 8-aminoquinoline (8AQ) Primaquine via aromatic hydroxylation step mediated by Cytochrome P450 enzyme using Density Functional Theory

Fundamentally, hydroxylation is of two types – one is (a) aliphatic, another is (b) aromatic. Both hydroxylations are important and their reaction pattern is different. Present research is focused on the study of aromatic hydroxylation. In the modern synthesis chemistry, direct insertion of hydroxyl group into the aromatic compound is one of the most challenging fields, because of the strong bond of hydrogen and carbon (C-H) atom of the benzene ring. But, despite this ambivalence, Cytochrome P450 catalyzes aromatic compound in relatively easy way. Hydroxylations of aromatic rings are important chemical reactions and are catalyzed by several metalloenzymes. The 8-aminoquinoline (8AQ) drug primaquine (PQ) is a prime anti-malarial drug, used in the treatment of malaria due to plasmodium vivax and plasmodium ovale. It is also used in the treatment of pneumocystis pneumonia together with clindamycin, as an alternate treatment. It is one of the safest and most effective drugs and is placed in the list of essential drugs of World Health Organization (WHO). It was developed over 70 years ago. But, unfortunately, the reaction pathway was still blurred. Density functional theory (DFT) was involved using Gaussian 09 software. In this work, all calculations were performed for Porphyrin cation radical iron (IV) oxo species i.e $Por^{+\cdot}Fe^{iv}=O$, which is commonly referred as (Cpd I), in two different spin states, doublet as well as quartet with primaquine as a substrate. Optimization geometries of reactant, intermediate, product is performed and a genuine pathway for the reaction mechanism is found on potential energy surface (PES) with subsequent transition states.

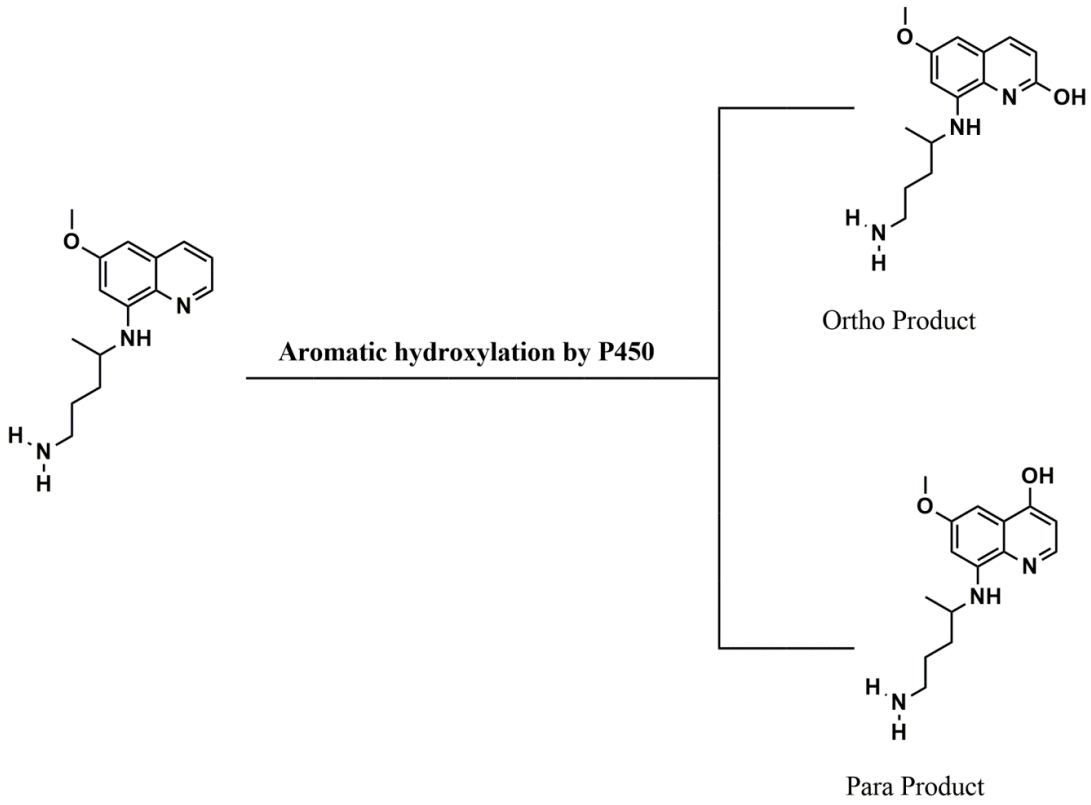

Figure 3.1: Aromatic hydroxylation of Primaquine at ortho (2PQ) and para (4PQ) position by Cytochrome P450.

Initially, optimization of geometry was calculated by hybrid density functional B3LYP using LANL2DZ basis set on iron atom and 6-31G basis set on the rest of the atom (BS1) abbreviated as B3LYP/BS1. The proposed work tried to explain aromatic hydroxylation of Primaquine at ortho position of the aromatic ring (C-2), to a complex intermediate (IM1) by attaching at the ortho position of carbon (C-2) with oxygen atom of Cpd I. The energy profile for ortho position of Primaquine is shown in Figure 3.2. Moreover, the transfer of electron and spins of electron is also investigated by charges and spin densities (Mulliken and NBO atomic charges) using BS1 basis set and is reported in Table 3. 1.

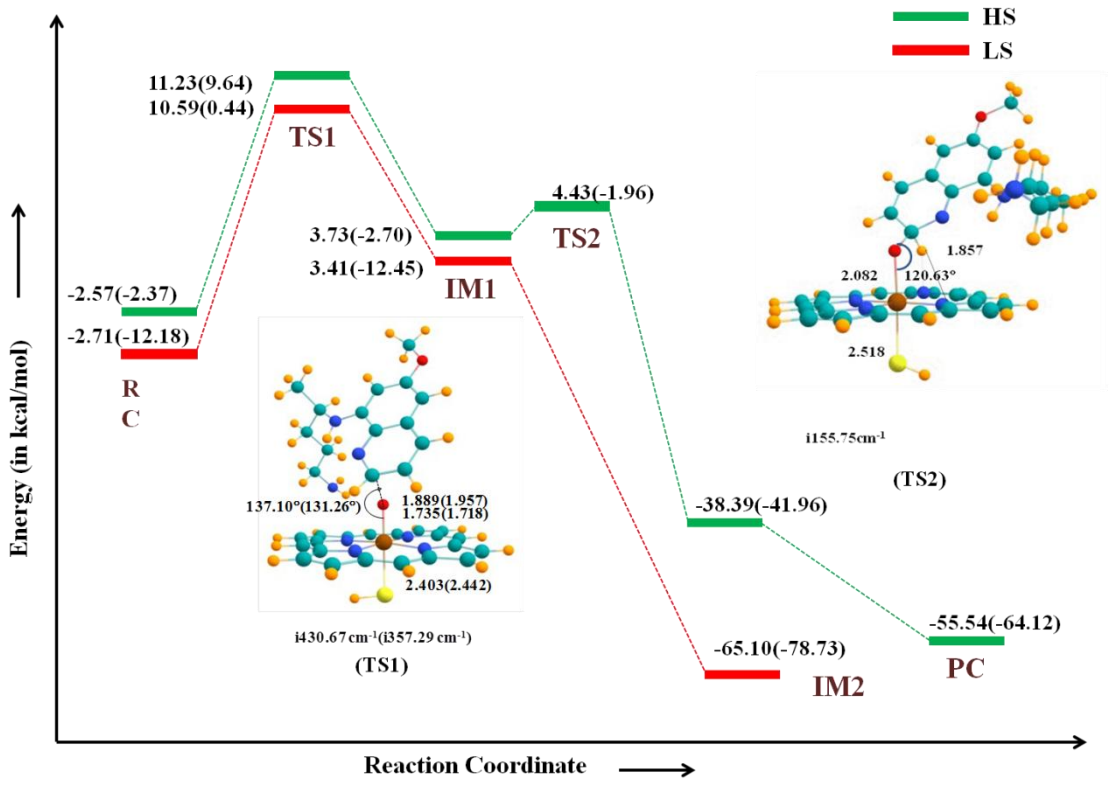

Figure 3.2: Potential energy surface of hydroxylation of Primaquine (2PQ) by Cytochrome P450 with energies in kcal/mol. Bond length, bond angle and imaginary frequencies (in cm^{-1}) of quartet as well as doublet (in bracket) spin state is shown. All energies are calculated at B3LYP/BS1//B3LYP/BS2 level of theory.

Table 3.1: Spin densities and charges of Cpd1 & 2PQ position of substrate (Primaquine) using BS1 basis set.

Reactant Complex										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	1.07	0.94	0.43	-0.00	0.54	0.50	-0.34	0.10	0.00	-0.04
M_2	1.20	0.89	-0.50	-0.00	-0.58	0.51	-0.34	-0.11	0.00	-0.05

TS 1										
	Spin Density					Charge				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	1.35	0.74	0.02	0.60	0.27	0.45	-0.41	-0.37	0.35	-0.02
M_2	1.59	0.33	-0.22	-0.49	0.21	0.45	-0.42	-0.34	0.37	-0.07
Intermediate 1										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	1.89	0.35	-0.13	0.91	-0.03	0.49	-0.50	-0.35	0.35	0.01
M_2	1.77	0.28	-0.13	-0.85	-0.08	0.47	-0.50	-0.35	0.35	0.02
TS 2										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	2.62	0.09	-0.10	0.22	0.26	0.53	-0.54	-0.44	0.15	-0.23
Intermediate 2										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	2.83	0.09	0.04	-0.11	0.00	0.51	-0.62	0.02	-0.41	-0.12

M_2	1.10	0.00	-0.08	0.00	-0.28	0.35	-0.51	-0.56	0.20	-0.00
Product										
Spin Density						**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	2.5	0.00	0.02	0.00	0.46	0.53	-0.60	-0.41	0.04	-0.16

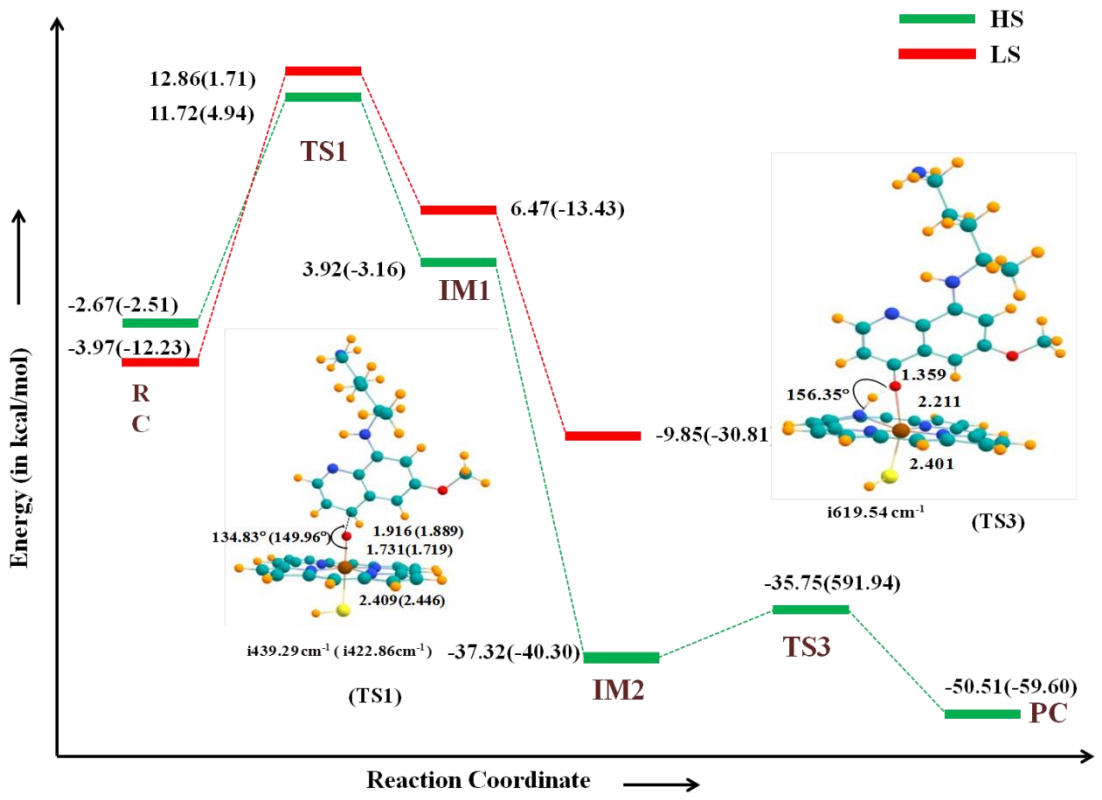

Figure 3.3: Potential energy surface of hydroxylation of Primaquine (4PQ) by Cytochrome P450 4 with energies in kcal/mol. Bond length, bond angle and imaginary frequencies (in cm^{-1}) of quartet (in green color) as well as doublet (in bracket in red color) spin state is shown. All energies are calculated at B3LYP/BS1//B3LYP/BS2 level of theory.

Abstraction of hydrogen atom at the para position of primaquine is identified as 4PQ. All properties are also investigated for 4PQ by quantum mechanical (QM) methods. Quantum mechanical calculations clearly depicted above hydroxylation reaction is rebound free process having more than one transition state with short lived intermediate state and C-O bond formation step to be rate determining step of the reaction. It can been seen from the energy profile for ortho (2PQ) and para (4PQ) position of Primaquine that quartet surface (HS) is responsible for the hydroxylated product whereas doublet spin surface profile is resulted in the formation of a *suicidal complex*.

Chapter-4 Interplay between two degenerate spin state determines the hydroxylation of 4-nitrophenol catalyzed via Cytochrome P450

4-nitrophenol is formed during the synthesis of paracetamol. Initially, it reduced to 4-aminophenol, after that acetylated via acetic anhydride. It is also product of essential enzymatic reactions of several substrate like- 4-nitrophenyl phosphate, 4-nitrophenyl acetate, 4-nitrophenyl-β-D-glucopyranoside and more other derivatives. It plays a crucial role in xenobiotic metabolism process in both, human and mouse. In field of medicinal chemistry, it is used in manufacturing of drugs, insecticides, fungicides. Its structure has phenolic compound, in which nitro group is attached at the opposite of the hydroxyl group on benzene ring. It is also known as *p*-nitrophenol or 4-hydroxynitrobenzene due to presence phenol group. It is mostly used in detection of the presence of alkaline phosphatase activity. The aromatic hydroxylation of 4-nitrophenol is shown in Figure 4.1.

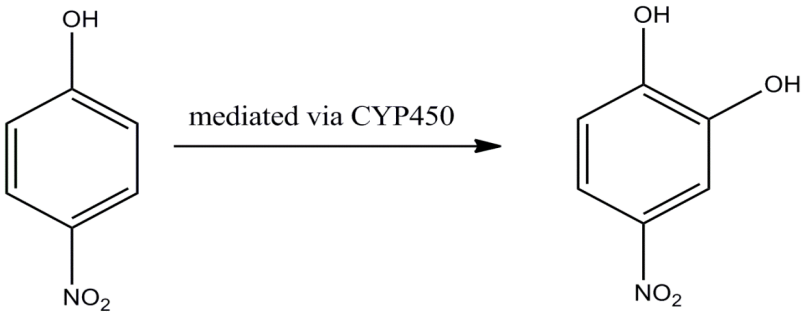

Figure 4.1: Aromatic hydroxylation of 4-nitrophenol via Cytochrome P450.

Present study comprises an investigation of reaction energy profile by Quantum Mechanical (QM) method. All calculations have been performed by Gaussian 09 package [21-22]. Density functional theory (DFT) with hybrid functional B3LYP [3,17,23-28] using LAN2DZ basis set on iron atom and 6-31G basis set on rest of the atom (BS1). Initially, substrate bind with oxygen atom (O38) of Cpd1 at interacting distance of 3 Å to form a stable reactant complex (RC) with energies -10.28 eV & -18.30 eV at high as well as low spin surfaces respectively. After this, charge of O38 of CpdI is transfer to the carbon atom (C49) of substrate, gives first transition state (TS1) with comparatively less energy 10.66 eV & 10.25 eV at high as well as low spin surfaces respectively. This step is the slowest step of reaction and commonly known as *rate determining step*, from this we can predict the rate of reaction. We also reported the barrier energy of both surfaces is close to 10 kcal/mol, indicating requirement of less energy for crossing the both surfaces of reactant complex. Further, first intermediate complex (IM1) with energy 4.66 kcal/mol is formed only at the high spin surface. In the next step, hydrogen atom (H53) get attached with nitrogen atom (N2) of Porphyrin ring forming a second intermediate state (IM2) with -46.47 kcal/mol & -48.76 kcal/mol energy at high as well as low spin surfaces respectively. Further, this hydrogen atom (H53) attached to the abstracted oxygen atom (O38) of Cpd I, and form the most stable product complex (PC) having energy -56.62 kcal/mol with third

transition state (TS3) having energy -44.08 kcal/mol at high spin surface. Negative value of third transition state shows less energy required to form product complex from second intermediate step. The energy for aromatic hydroxylation is shown in Figure 4.2. The present work offered a complete reaction pathway of aromatic hydroxylation of 4-nitrophenol with Cpd I by DFT at B3LYP level of theory. Quantum mechanical calculations above clearly depicted the hydroxylation reaction has more than one

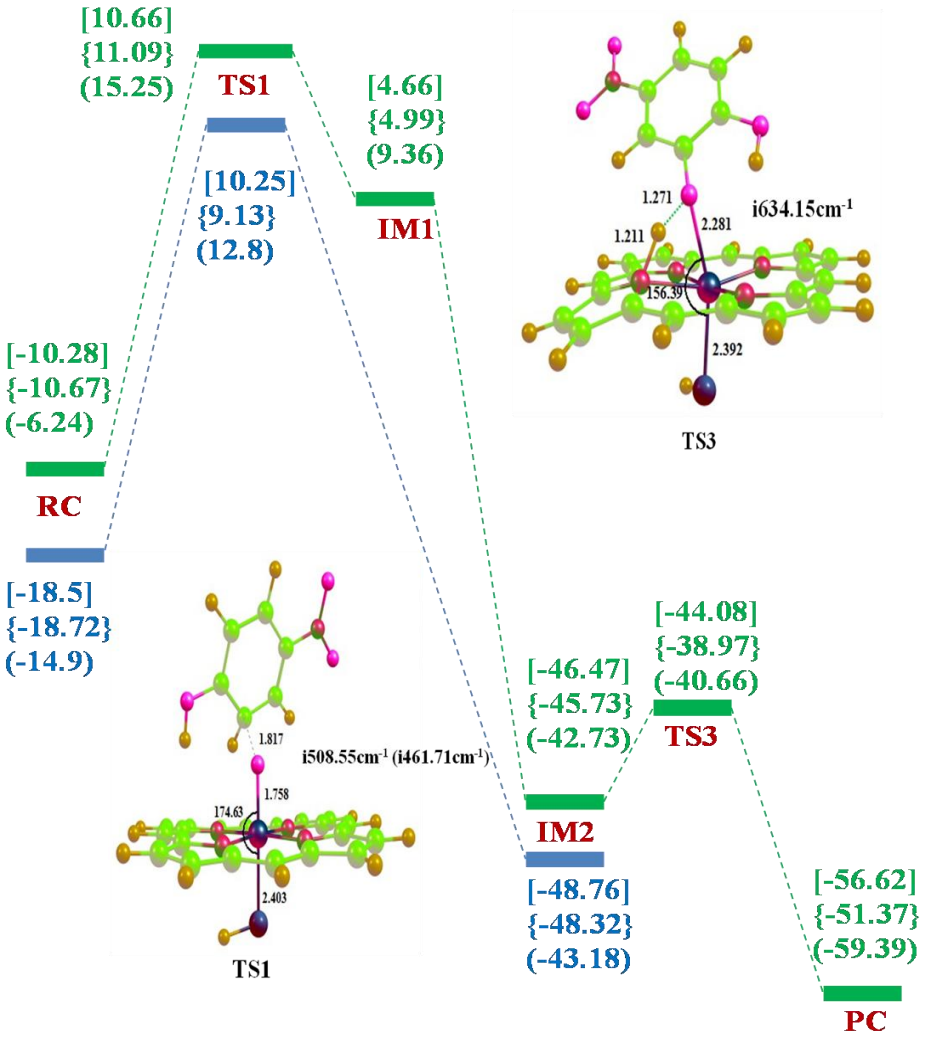

Figure 4.2: Potential energy surface of hydroxylation of 4-nitrophenol by Cytochrome P450 with energies in kcal/mol, Bond length (in Å), bond angle (in °) and imaginary frequencies (in cm^{-1}) of quartet (in green color) as well as doublet (in blue color) spin state is shown. Energies are calculated by 6-31G basis set reported in large bracket, by "LACVP" basis set reported in curly bracket and by solvent effect is in small bracket.

transition state with short lived intermediate state and C-O bond formation step to be the rate determining step of the reaction.

Table 4.1: Spin densities and charges of Cpd1 & substrate (4-nitrophenol) using DFT at the B3LYP/6-31G level of theory.

Reactant Complex										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	1.10	0.91	0.44	0.00	0.53	0.50	-0.37	0.44	0.00	0.53
M_2	1.36	0.75	-0.58	0.00	-0.53	0.53	-0.45	-0.01	-0.05	-0.03
TS1										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	1.41	0.64	0.06	0.51	0.36	0.46	-0.42	-0.28	0.21	0.03
M_2	1.15	0.46	-0.41	0.12	-0.32	0.41	-0.40	-0.22	0.23	-0.02
Intermediate1										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	2.06	0.25	-0.13	0.85	-0.03	0.49	-0.53	-0.29	0.29	0.04

Intermediate 2										
	Spin Density					Charge				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	2.84	0.04	0.07	0.04	0.05	0.45	-0.74	0.12	0.51	-0.05
M_2	1.05	-0.01	-0.07	-0.02	0.04	0.30	-0.72	-0.19	-0.51	0.01
Transition State 3										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M4	2.66	0.03	0.04	0.03	0.26	0.48	-0.74	0.13	-0.51	-0.1
Product										
	Spin Density					**Charge**				
	Fe	**O**	**Por.**	**Sub.**	**SH**	**Fe**	**O**	**Por.**	**Sub.**	**SH**
M_4	2.50	0.00	0.04	-0.00	0.47	0.52	-0.68	-0.36	-0.00	-0.15

This step clearly shows that it requires less energy in initiating the reaction at both spin surfaces. Finally, the product at high spin state surface (HS), is formed, the reaction followed patterns of two state reactivity (TSR) mechanism and energy landscape for doublet and quartet were close and parallel with each other, but this pattern bifurcated at IM1. TSR behavior is transformed to single state reactivity (SSR) showing that reaction is possible only for high spin surface (HS). This process is not

regioselective, so the product is formed directly from intermediate. Reaction mechanism of 4-nitrophenol with cpd1 is one of the crucial process, but its reaction pathway was still hidden. Our work gives energy pathway of reaction mechanism with very good accuracy.

Chapter-5 Computational Study of Electrical Properties of Various Drugs that Metabolized via Cytochrome P450

Several drugs are metabolized through the Cytochrome P450. Here, we computationally investigated the physical properties of some drugs that are metabolized through the P450 enzyme. Diacetylmorphine is also known as heroin or diamorphine, which is used as a drug due to its euphoric effect. In several countries, it is used as a pain relief like, during childbirth, heart attack, or opioid replacement therapy. It is taken in the form of injection and can also be smoked or inhaled. It is also found in the tablet form.

Losartan is a drug which is used in the treatment of high blood pressure, a diabetic patient with kidney failure, and heart failure patients. It may take six months for the complete treatment of the disease. There are also some side effects of Losartan medication like cramps, cough, anemia, stuffy nose, low blood pressure, and angiotensin. The above drug is not recommended during breastfeeding and pregnancy, because the blockage of the angiotensin II.

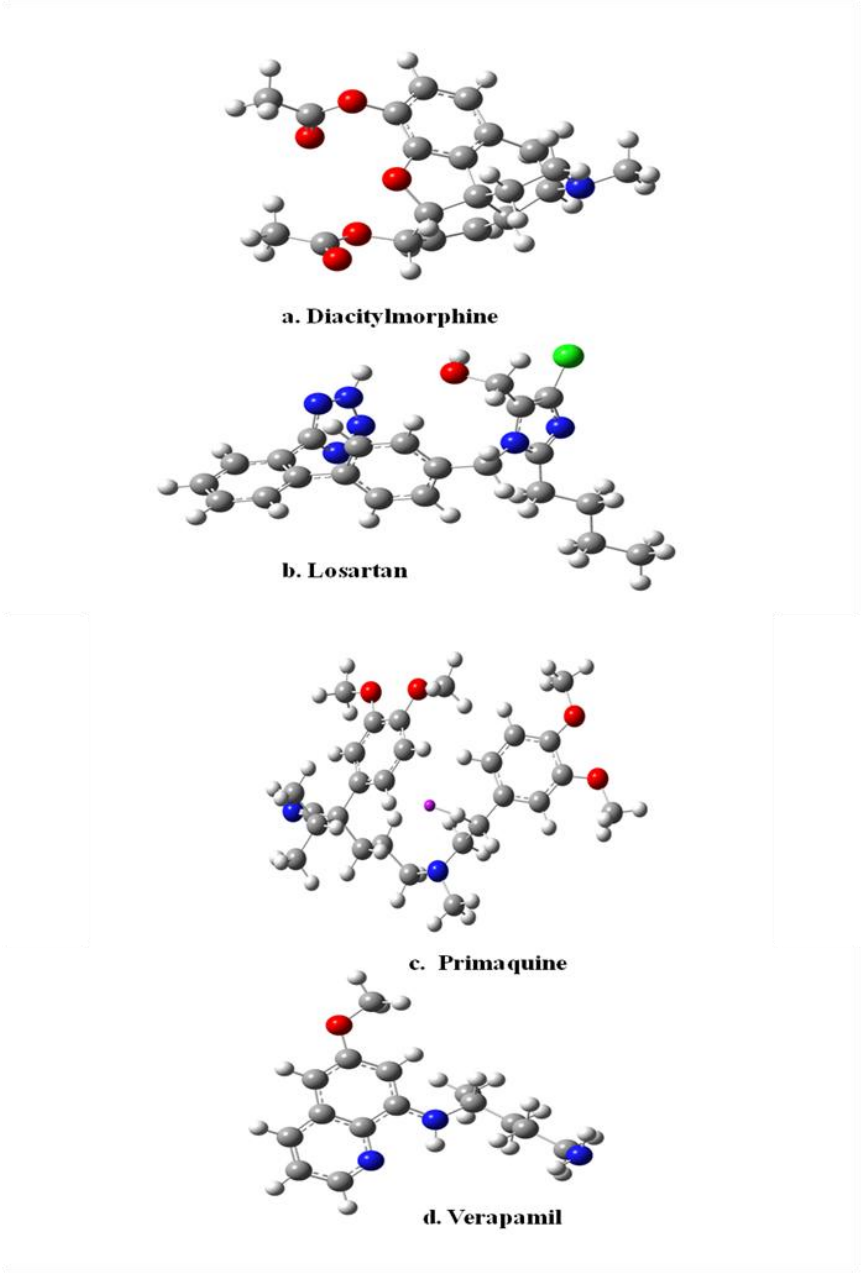

Figure 5.1: Optimized molecular structures of , **a.** Diaacetylmorphine, **b.** Losartan, **c.** Primaquine, **d.** Verapamil

It is an essential drug that is listed in World Health Organization (WHO) and approved by the United States in 1995. It is also highlighted as a generic medication. In the United States, it is top ninth most prescribed drugs in 2017. It is also combined with hydrochlorothiazide and form a new version of it.

This new version is 67th most prescribed drug in the United States, in 2017. For studying the most stable configuration of the molecules, the widely used method is Quantum Mechanical (QM) method. Basically, in this, various quantum mechanical theories are compiled to finding the ground state energy or can say the stable structure of the molecule. In this method, the Schrodinger wave equation for multi-electron system is solved and their energies are analyzed.

Table 5.1: Optimization energy and HOMO-LUMO bandgap of Diacetylmorphine, Losartan, Primaquine, Verapamil drugs

S. No.	**Name of drug**	**Optimization Energy (in eV)**	**HOMO-LUMO bandgap (in eV)**
1.	Diacitylmorphine	-33,865.29	5.36
2.	Losartan	-46,684.46	4.70
3.	Primaquine	-22,407.43	3.79
4.	Verapamil	-39,772.58	5.36

The optimization energy of Furafylline is the highest amongst the useless, due to this; it has a less stable configuration. But Miconazole has the lowest energy (Table 5.2), that's why may be said to have the most stable configuration amongst all azoles. Optimization results of Azoles-antifungal drugs shows that all azoles have good stability. Miconazole has the most stable structural configuration which means that it can bind tightly with the enzymes. This result acts as an aid in understanding the reaction mechanism of many biochemical reactions. So, it is concluded that

Miconazole is a good anti-fungal drug as well as a good substrate to react with enzymes.

Table 5.2: Optimization energy and HOMO-LUMO bandgap of Azoles

S. No.	Name of drug	Optimization Energy (in eV)	HOMO-LUMO bandgap (in eV)
1.	Azaconazole	-46,230.40	6.0168
2.	Bifonazole	-26,070.31	3.6079
3.	Clotimazole	-38,592.54	5.0266
4.	**Miconazole**	**-74,008.74**	**3.5855**
5.	Furafylline	-24,741.05	5.0392

Further research work in such a field may reveal a wide range of mysteries in the understanding of metabolism, detoxification, and many other biochemical reactions as well as help in search of good anti-fungal drugs also.

Chapter-6 Conclusion and Future Plan

Nowadays computational quantum mechanical methods are highly accurate and reliable in enzymatic catalysis. Heme and non-Heme enzymes react fast with substrates, so that computational method can assist experimental studies with good accuracy and give a piece of proper information about the properties of enzymes that leads to the reaction mechanism and also provide the nature of the enzyme.

The present work presents the reaction mechanism of oxygen atom transfer from cpd1 to substrate through aromatic hydroxylation. The present thesis is also defined the

barrier energies of various complexes and the rate-determining step in each mechanism. Moreover, barrier height can be used to predict the rate of reaction or rate constant of the reaction. It also identifies the origin of the rate constant by transfer of an electron or proton. The present work established the reactivity pattern of hydroxylation of the substrate via cpd1 for various drugs.

Moreover, study of metabolism process of various drugs via P450 is large to an extent scene for compatible collaboration of computational modeling with experimental results for biomimetic, enzymatic work and defining the optoelectronic properties.The present thesis well explained the metabolism process through the hydroxylation of various drugs (substrate) mediated by cpd1.