Anti-oxidant Mechanism of Aminoantipyrines:

A Computational Investigation

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July-2019

CERTIFICATE

This is to certify that the dissertation entitled, **"Anti-oxidant Mechanism of Aminoantipyrines: A computational investigation"**, being submitted by **Ms. Sanya Singh** in partial fulfilment of requirement for the award of the degree of **Masters of Science** in **Chemistry** and being submitted to the School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is a bonafide work carried out by her under my supervision. The work has reached the standard necessary for submission, and the contents of this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

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CANDIDATE'S DECALARATION

I hereby declare that the thesis entitled **"Anti-oxidant Mechanism of Aminoantipyrines: A computational investigation"** is an authentic record of my work carried out as requirements for the award of the degree of Master of Science in Chemistry at Thapar Institute of Engineering and Technology, Patiala under the supervision of Dr. Debasish Mandal, Assistant Professor, School of Chemistry & Biochemistry, Thapar Institute of Engineering and Technology, Patiala during July' 2017 to July' 2019. No part of the matter embodied in this report has been submitted to any other university or institute for the award of any degree.

Sanya Singh

Date: 13/08/19

It is certified that the above statement made by the student is correct to the best of my knowledge and belief.

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Sanya Singh

ABSTRACT

Mechanism for the reaction of aminoantipyrines with OH radical have been studied using M062X/6-31G(d,p) and M08HX/6-311+G(d,p) level of theory. Two types of possible reaction pathways are discussed: Hydrogen atom abstraction and OH addition on the aromatic ring. Further, the Hydrogen abstraction is studied from three different channels. The PESs are computed using the same level of theories. The H-atom abstraction channels possess lower energetics than that of addition and the product is obtained from demethylation reaction. The enthalpies of formation were computed and compared with available experimental results to verify the reliability of the used methods. Thus, it was found that hydrogen atom abstraction mechanism was thermodynamically favorable in comparison with hydroxyl addition mechanism. Our results are also in good agreement with the experimental observation as both the investigations shows that the N-demethylation product is the major product.

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LIST OF ABBREVIATIONS AND SYMBOLS

1.	**ROS**	Reactive Oxygen Species
2.	**RNS**	Reactive Nitrogen Species
3.	**HAT**	Hydrogen Atom Transfer
4.	**PCET**	Proton-Coupled Electron Transfer
5.	**SET-PT**	Sequential Proton Loss Electron Transfer
6.	**IP**	Ionization Potential
7.	**PDE**	Proton Dissociation Enthalpy
8.	**PA**	Proton Affinity
9.	**HF**	Hartree-Fock Theory
10.	**SCF**	Self-Consistent Field
11.	**DFT**	Density functional theory
12.	**HF**	Hohenberg-Kohn theorems
13.	KS	Kohn-Sham Theorem
14.	**LDA**	Local Density Approximation
15.	**GGA**	Generalized Gradient Approximation
16.	**AO**	Atomic Orbitals
17.	**STO**	Slater-Type Orbitals
18.	**GTO**	Gaussian-Type Orbitals
19.	**LCAO-MO**	Linear Combination of Atomic Orbitals
20.	**ZPVE**	Zero Point Vibrational Energies
21.	**TS**	Transition State
22.	**IRC**	Intrinsic Reaction Coordinate
23.	**BS1**	6-31G(d,p)
24.	**BS2**	6-311+G(d,p)

CHAPTER 1: INTRODUCTION

In our body, free radicals are generated during aerobic metabolism. The molecular entities that possess unpaired electron(s) in their atomic or molecular orbitals are called free radical [1]. The present unpaired electron(s) in free radicals are responsible for their high reactivity. They can be classified as ROS or RNS. The most reactive free radicals in living system are produced by radicals derived from oxygen [2]. The examples of "oxygen-derived species" are •OH (hydroxyl), $RO_2^{\bullet}$ (peroxyl), $RO^{\bullet}$ (alkoxyl), $O_2^{\bullet-}$ (superoxide) and $HO_2^{\bullet}$ (hydroperoxyl) radicals. The non-radicals like HOCL (hypochlorous acid), H_2O_2 (hydrogen peroxide), $ONOO^-$ (peroxynitrite), $^1O_2$ (singlet oxygen), and O_3 (ozone) can simply initiate free-radical reactions in living organisms. Thus, the collective term ROS (reactive oxygen species) contains both radical and non-radical species [3]. Overproduction of ROS can make potential damage to biomolecules (e.g., lipids, amino acids, protein and DNA) and is termed as oxidative stress [4]. The harmful effect is due to presence of extra amount of free radicals than the amount of antioxidants present in our body [5]. Oxidative DNA damage may account in the development of ageing process as well as other human diseases such as cancer, diabetes mellitus, cardiovascular disease, neurodegenerative disorders (Alzheimer's disease), rheumatoid arthritis and other conditions [6-10].

Free radical scavengers are the antioxidant which defenses by the removal of the free radical. Antioxidants are the 'superheroes' of the new generation as they scavenges the formation of excess radical species and reduces the oxidative stress. Anti-oxidant can be defined as the substance that prevents biomolecules from undergoing the process of oxidation, even when they are present in low concentration [11]. The characteristics of good antioxidants should be that they stop the harmful chain reactions caused by the radical in numerous pathways, which consist of specific quenching of ROS [12].

Antioxidant inhibits the activity of the free radicals through the following three significant pathways: HAT (hydrogen atom transfer) and PCET (proton-coupled electron transfer), SET-PT (single electron transfer followed by proton transfer) and SPLET (sequential proton loss electron transfer) [13-15]. The HAT mechanism is governed by the homolytic cleavage of O-H bond (i.e. transfer of hydrogen atom). The important feature of this mechanism is that the

transfer of proton and electron of H-atom occurs in same set of orbital. It is favoured in non-polar media as it does not incorporate charge separation.

$$R_1^{\bullet} + R_2OH \rightarrow R_1H + R_2O^{\bullet} \quad (1)$$

The SET-PT and SPLET mechanism are more favourable for radicals with higher electron affinity and is preferred in polar media due to charge separation. The SET-PT is a two-step mechanism which leads to indirect H-abstraction. Firstly, transfer of electrons take place followed by a proton release. Also in this case, the most significant factor is the IP (ionization potential) and PDE (proton dissociation enthalpy). The abstraction of electron is easier when the value of ionization potential is less.

$$R_1^{\bullet} + R_2OH \rightarrow R_1^{-} + R_2OH^{\bullet+} \quad (2)$$

$$R_1^{-} + R_2OH^{\bullet+} \rightarrow R_1H + R_2O^{\bullet} \quad (3)$$

The SPLET mechanism is characterized by the PA (proton affinity) as well as ETE (electron transfer enthalpy). This mechanism is originated by proton loss and it is the reverse of SET-PT mechanism. The three steps mechanism is initiated by proton loss followed by electron transfer and then re-protonation.

$$R_2OH \rightarrow R_2O^{-} + H^{+} \quad (4)$$

$$R_2O^{-} + R_1^{\bullet} \rightarrow R_2O^{\bullet} + R_1^{-} \quad (5)$$

$$R_1^{-} + H^{+} \rightarrow R_1H \quad (6)$$

The net results and thermodynamic properties of these three mechanisms are same and further it gives information about which of these radical scavenging mechanism is thermodynamically favourable.

4-(N,N-dimethyl)-aminoantipyrines is the pyrazolone derivative which possesses analgesic, antipyretic and anti-inflammatory properties [16]. The scavenging activity against ROS and RNS as well as neutrophil-produced oxidative burst has been studied with the help of the biological activity of these compounds [17]. It was established that aminopyrines are highly effective scavengers of hydroxyl radical (•OH) while antipyrines was inefficient against it. However, besides the well-known therapeutic benefits, pyrazolones derivatives have been related to the harmful effects named as leukopenia.

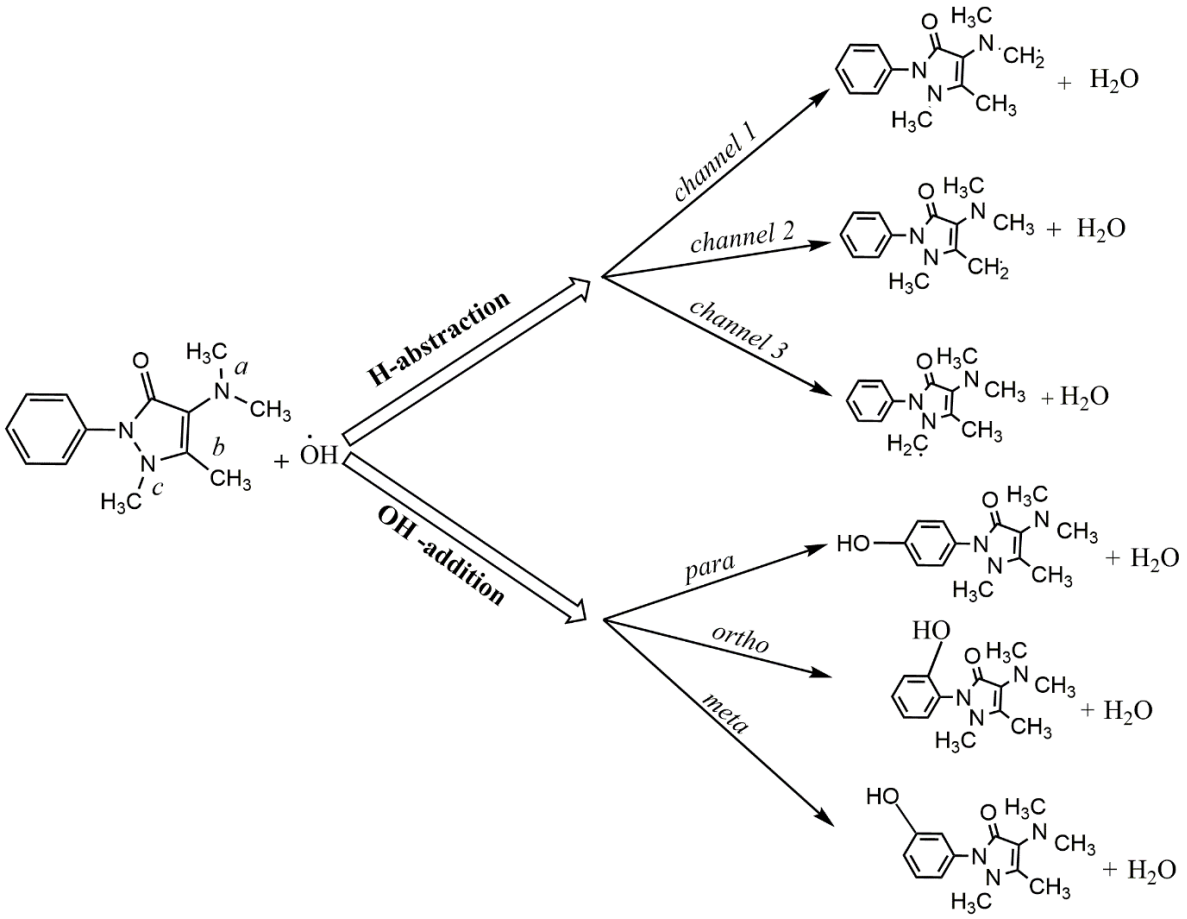
Few computational studies on the scavenging activity of anti-oxidant and mechanisms are mentioned here. *Mandal et. al* [18] computationally investigated the kinetics and mechanism of the OH radical with vinyl acetate in the presence of oxygen and nitrous oxide at M06-2X

density functional method. It was found that the hydrogen atom transfer channel possess the very high energy value than the OH addition. *Atkinson and his co-workers* [19-20] published and reviewed a kinetics study of the reaction between OH radical with toluene. The scavenging activity of OH radical with edaravone considering the possible mechanisms and reaction site was evidently studied by *Pérez-González et al* [21]. *Annia Galano and co-workers* [22] have performed the computational study for OH hydrogen abstraction in asparagine. She also studied the kinetics and mechanism of the OH radical with methionine [23]. In the recent study, *Agnihotri et al.* [24] shows the scavenging mechanism of OH radical by curcumin which further leads to the formation of two other antioxidants, namely ferulic acid and vanillin. The kinetically and thermodynamically favourable mechanism for the abstraction and addition of thiourea with OH radical was evaluated by *Kanbanda et al.* [25] by using M06-2X and other methods. Another study for the anti-oxidant mechanism was examined by *Dejan Milenković et al.* [26] and it was suggested that the most favourable pathway for the antioxidant action of dihydroxybenzoic acid in benzene was HAT mechanism.

The main focus of the present thesis is to computationally explore the antioxidant mechanism of the aminoantipyrines against OH radical. The reaction between OH radical and aminoantipyrines can proceed in two ways as: hydroxylation of OH radical to the molecule or abstraction of H atom from the molecule. Both the addition and abstraction mechanism are shown in scheme 1.

Hydrogen-Atom Transfer: There are three possible channels (1, 2, 3) for hydrogen atom abstraction from three different sites (*a, b, c*) of the molecule 4-(N,N-dimethyl)-aminoantipyrines.

Addition Reaction: The addition of OH radical on 4-(N,N-dimethyl)-aminoantipyrines can happen on the benzene ring on meta, ortho or para position.



Scheme 1: Possible Competitive Pathways for the hydrogen atom transfer and hydroxyl group addition mechanism of 4-(N, N-dimethyl)-aminoantipyrines.

CHAPTER 2: COMPUTATIONAL CHEMISTRY

Computational chemistry can be broadly described as the part of chemistry where computer programming and algorithm are used to solve chemical problems. These computer programs decipher the theoretical chemistry into efficient computer codes to reproduce complex chemical structures along with their molecular geometries, interactions and other properties [27]. Quantitative algorithms are formulated using an array of data obtained in vitro to qualitatively analyze the course and extent of any chemical phenomena which is difficult to study directly. The quantum mechanical behavior of a particle as a wave function of any system was proposed by Erwin Schrödinger in the year 1926. He outlined that the square of the wave-function defines the probability of getting a particle at any point. The analytical solution of the Schrödinger equation is only possible for a number of simple single electron systems, shown below in Equation 2.1

$$\hat{H}\Psi = E\Psi \qquad 2.1$$

In Equation 2.1, E is the energy and $\hat{H}$ is the corresponding Hamiltonian operator of the system and Ψ represents the wave function [28].

2.1 Born- Oppenheimer Approximation

This approximation proposes the separation of variables caused by the motion of electrons and nuclear particles is due to their large difference in mass. Hence, the motion of nucleus is considered to be relatively stationary with respect to electrons. So, the Schodinger equation for a molecule becomes separable and thus the total wave-function can mathematically be described as the multiplication of the electronic and nuclear wave functions. The most important significance of this approximation is that it leads us to potential energy concept that gives both conceptual and computational base for molecular chemistry [27].

2.2 Hartree–Fock Theory

HF (Hartree-Fock) theory, specifically known as SCF (self-consistent field) theory is based on an approximation that endeavors to unfold the Schrodinger wave equation to iteratively calculate the set of one electron orbitals that minimize the energy [28]. It optimizes the orbitals in single determinant. The self-consistent energy minimization is performed using variational method which gives the total electronic energy of the system. HF method is used

to solve Schodinger equation for systems that have more than one electron and helps in reducing N-electron system to N number of one-electron system. The major limitation arises in case of multi-electron system and they cannot be solved easily due to the coulombic electron-electron repulsion terms. These repulsive terms are responsible for the inseparability of Schrodinger wave equation. It is assumed that every electron is moving independently with respect to the other electrons and they are facing no specific interactions rather an average field by all other electrons. Thus, it can be said that HF method utilizes the mean field approximation. In other words, all the electrons present in a system experience mean field of other electrons.

Post - HF methods

Post-HF methods quest to improve HF method by incorporating the factor electron correlation.

The Hatree-fock method could not provide the accuracy as it does not include the correlation of electrons with different spins. Thus, it results in error which is known as correlation energy and it can be expressed as,

$$\mathbf{E}_{corr} = \mathbf{E}_{total} - \mathbf{E}_{HF}$$

where, E_{total} is the total energy of the system and E_{HF} is the HF energy.

2.3 Density functional theory (DFT)

DFT [29] provides different approach for solving the multi-electron systems. Recently, DFT has been extensively used by the chemists for the evolution of quantum chemistry. It is primarily based on the electron density of the system which depends on only 3 coordinate. So, it is faster than wave-function method which needs to define 3n coordinate, where n is the number of electrons. The DFT protocols are not strictly *ab initio* methods and they may also need some empirical expressions. So, this method computationally less inexpensive than the ab-initio method as it provides lots of reliable data related to geometry, energy barrier of reaction, and several properties of molecules at the cost of lower time.

Hohenberg-Kohn Theorems

In 1964, DFT has been developed on the basis of Hohenberg-Kohn theorems (HK) [30] that marked the foundation for modern DFT. The first theorem state that there must be a unique relation between the properties of ground state of a many-electron system with corresponding electron density. Therefore, this theorem proves that energy can be a *functional* form of one electron density and uniquely determines the Hamiltonian of a system, and hence its energy and wave function.

$$E_0 = E_0[\rho_0]$$

where the zero (0) subscript represents the system in its ground state.

The second theorem is basically the use of variational ansatz for obtaining electron density. It is stated that the true ground state density for a system is the one that minimizes the energy functional.

Kohn-Sham theorem

Kohn and Sham (KS) [31] formulated a method analogous to Hartee-Fock method which takes a fictitious non-interacting reference system as a route to find ground state electron density. Unlike the mathematical form of HF orbital, the electron density can be described as a linear combination of basis functions. These functions then form a determinant known as Kohn-Sham orbitals. The energy is calculated by the electron density from this Kohn-Sham orbitals.

2.4 Functionals

The exchange-correlation functional is an approximation which is key to the success of DFT. The one of the most simplest and popular approximation to better describe the exchange-correlation functional is Local Density Approximation (LDA) [32]. It is assumed that the electronic density of an uniform electron gas can be used locally. The constant value of the electronic density forms exactly or very highly accurate exchange and correlation energy. The LDA functional can be expressed by

$$E_{XC}^{LDA}[\rho(r)] = \int \rho(r)\varepsilon_{xc}[\rho(r)]dr$$

where ε_{XC} is known as exchange-correlation energy per particle in a uniform electron gas with ρ electronic density.

The LDA gives better results than the HF method and is used in the determination of many molecular properties such as geometry, vibrational frequencies but it is not adequate to predict the energetics information.

The more advanced exchange-correlation functional was introduced in the form the generalized gradient approximation (GGA) [33]. In this model, the gradient of the electronic density is used to determine ε_{XC}. Thus, the non-uniformity of the true electron density is rather explained better by the use of the electron density gradient.

The GGA splits into exchange and correlation term which can be solved individually.

$$E_{XC}^{GGA} = E_X^{GGA} + E_C^{GGA}$$

Some of the most commonly used GGA exchange functionals are the Lee-Yang-Parr (LYP) correlation functional, Becke (B) exchange functional, Becke 88 correlation functional [34-35]. One of the most popular functional is hybrid density functional (HF-DFT) method which uses the combination of exchange and correlation function. For example, B3LYP [36] functional shows very good agreement with respect to experimental outcomes in numerous cases.

Meta-GGA: The meta-GGA functionals are an extension of the GGA which includes the dependence on non-interacting kinetic energy density in addition to the local density as well as its gradient. Common examples are M06-2X, M08HX. The M06-2X & M08HX are Minnesota functional developed by D.G. Truhlar [37]. M062X is a hybrid meta-GGA functional with 54% of HF exchange while M08HX with 52.23% of HF exchange.

2.5 Basis set

Basis set is a collection of functions which are linearly combined to form the atomic orbitals (AO) or molecular orbitals. Generally, basis functions are related to atoms and especially to their atomic nucleus but there are chances to use these basis functions specifically on bonds and expand them for linear combination of atomic orbitals approximations (LCAO-MO).

The two key components of wave-functions are both radial and spherical but the radial part is the important component of the basis functions. The two different forms of basis functions are used in chemical computation i.e., Slater-type orbitals (STOs) and Gaussian-type orbitals (GTOs) which are used to describe the radial part.

The Slater-type orbitals [38] are an exact depiction of the hydrogen-like radial functions and have the functional form of, $e^{-\zeta r}$, where r is the distance from nucleus and ζ is the slater

exponent. The state of many electron system can be well describes by STOs, however, these functions are difficult for the evaluation of the integrals. Thus, the Gaussian-type orbitals which differ from STOs in the exponent term and have radial dependency$e^{-\alpha r^2}$ are used. So, calculations for the computational point of view are easy in case of GTOs but they lacks a cusp at nucleus. Thus, we have used GTOs as it is easy for calculation.

CHAPTER 3: COMPUTATIONAL METHODOLOGY

The electronic structure calculations of all the entities related to this investigation have been optimized using density functional theory method. M06-2X and M08HX functionals in conjugation with pople's 6-31G(d,p) (BS1) and 6-311+G(d,p) (BS2) basis set were used for the calculation. All the computations have been done using GAUSSIAN 16 package [39].

Harmonic vibrational frequencies are computed at the corresponding level of theories. Zero imaginary frequency identifies local minima whereas presence of one imaginary frequency indicates the transition states. For further verification of the transition state an intrinsic reaction coordinate (IRC) calculations were also been performed [40]. Zero point vibrational energies (ZPVE) are incorporated in the calculation of energy barriers. The enthalpies of formation ($\Delta_f H^{\circ}_{298.15}$) are calculated using M08HX methods for all the species at 298.15 K.

Chemcraft Program was used to visualize the molecular structure and analyze the computed results [41].

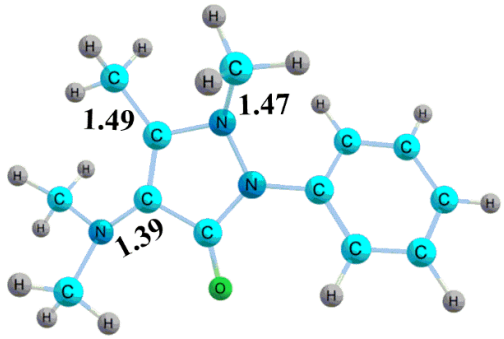
CHAPTER 4: RESULTS AND DISCUSSION

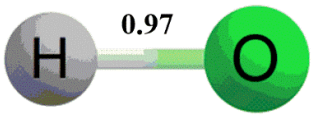
The reaction of hydroxyl radical (•OH) and 4-(N,N-dimethyl)-aminoantipyrine (R) can progress through two possible pathways: the hydrogen atom abstraction (HAT) and hydroxylation on the benzene ring. The step by step study of these two pathways is discussed here.

The geometries of the stationary states are presented in Figure 1. The optimized geometries of all the transition states along with the potential energy surface computed at the M062X/BS1 and M08HX/BS2 level of theory are depicted in Figure 2. All the important structural parameters are indicated in Figure 1 & Figure 2.

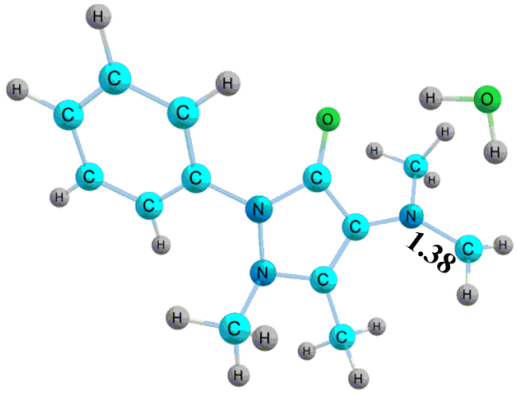
H-Atom Abstraction Pathways. From R, hydrogen atom abstraction can be possible via three different channels as presented a, b, and c as shown in scheme 1. The energy values is given in both the method as M062X (M08HX). The enthalpy of formation of all the species are also calculated at the M08HX/BS2 level of theory.

O-H addition reaction Pathways. The addition of the OH radical on the aromatic ring can take place on para, ortho and meta positions. The study of OH addition on meta and ortho position was excluded due to the high energetics. Thus, the addition on para position was computed at the M062X/BS1 and M08HX/BS2 level of theory.

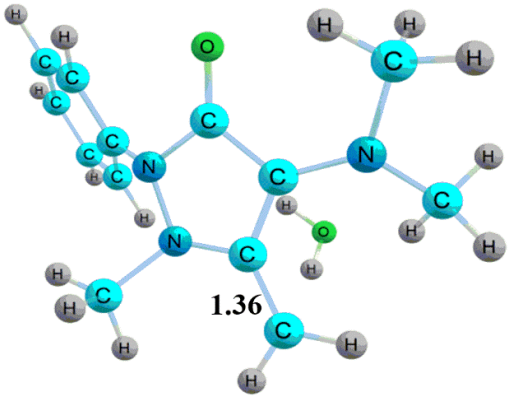




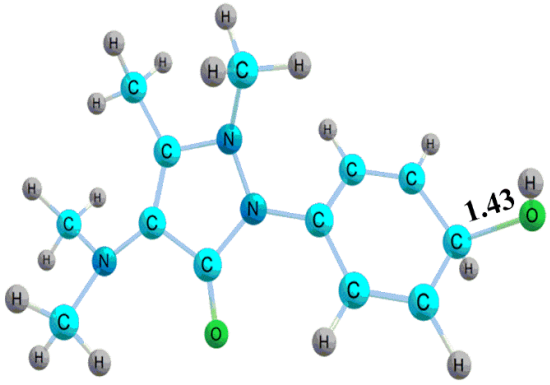
OH



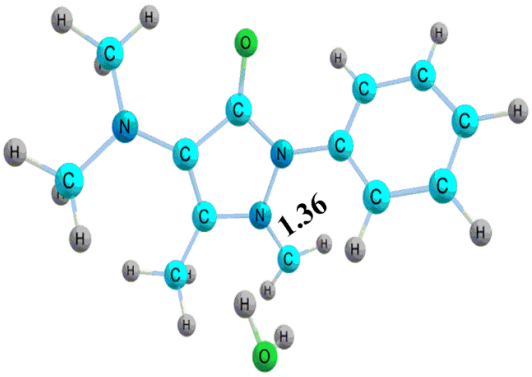
P1-H



P2-H



P3-A



P4-H

Figure 1. Optimized geometries with geometrical parameter of the reactants and products computed using M062X/BS1 and M08HX/BS2 level of theory.

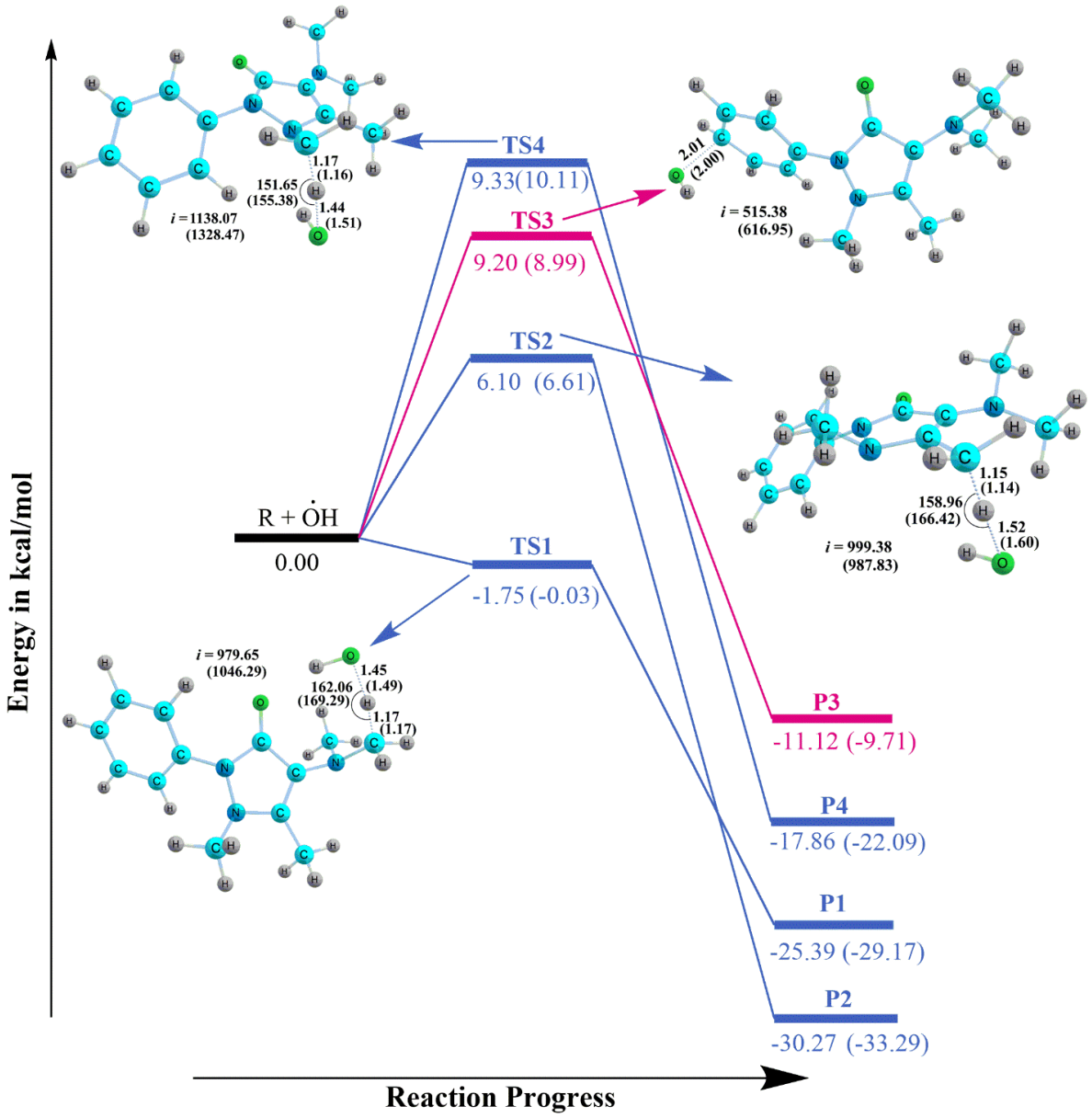


Figure 2. Potential Free-Energy profile for the reactions of 4-(N, N-dimethyl)-aminoantipyrines with OH radical along with optimized geometries with key geometrical parameters of the transition state at the M062X/BS1 and M08HX/BS2 level of theory. The energies are given as M062X/BS1(M08HX/BS2).

H-Atom Abstraction from channel 1: The abstraction of H atom by the OH radical exists via the transition state TS1 located at a 1.75 kcal/mol (-0.03 kcal/mol) lower point than the reactant in the PES. TS1 was confirmed by the value of imaginary frequency *i=979.65*. In TS1, the breaking of C-H bond occurs with distance 1.17 Å and forming of O-H bonds with distance 1.45 Å. There are 2 methyl groups present on this '*a*' site resulting in 6 H, all of them having the same chemical environment resulting in same abstraction value. The angle during the formation of transition state TS1 is C-H-O with bond angle 162.06. The exothermicity of the product formation results in stable products P1-H and water having the delta G value -25.39 kcal/mol (-29.17 kcal/mol).

H-Atom Abstraction from channel 2: In channel 2, the H-atom abstraction results in the production of P2-H and water through the transition state, TS2, which possesses 6.10 kcal/ mol (6.61 kcal/mol) energy of activation and imaginary frequency value *i=999.38*. In TS2, the C-H distance is 1.15 Å and the formation of O-H bond distance is 1.52 Å. The bond angle C-H-O of TS2 is 158.96. This exothermic reaction results with the product P2-H and water with energy value -30.27 kcal/mol (-33.29 kcal/mol).

H-Atom Abstraction from channel 3: This channel results in the highest free energy barrier, TS4, 9.33 kcal/mol (10.11 kcal/mol) which further results in the products P4-H and water -17.86 kcal/mol (-22.09 kcal/mol). The C-H and O-H bond distances are 1.17 and 1.44 Å respectively. On comparing all the H-atom abstraction channels, it can be suggested that the order of preference a > b > c is favorable according to the lower energy barrier value of transition state.

O-H addition on para position. The hydroxylation on the para position of the aromatic ring results in the transition state TS3 with energy barrier 9.20 kcal/mol (8.99 kcal/mol). The formation of the C-O bond distance results in 2.01 Å and the imaginary frequency *i=515.38*. Further, the product P3 with energy of activation -11.12 kcal/mol (-9.71 kcal/mol) is formed.

Thermochemistry: Standard gas phase enthalpies of formation also calculated at M08HX/BS2 computational method and the data are tabulated in Table 1.

Table 1: Standard Enthalpies of formation ($\Delta_f H^\circ_{298.15}$) computed at M08HX/BS2 level of theory.

species	$\Delta_f H^\circ_{298.15}$ **(kcal/mol)**	**Experimental (kcal/mol)**
R	52.57	
•OH	10.50	
TS1	54.14	
TS2	61.83	
TS3	74.57	
TS4	64.83	
P1-H	26.72	
P2-H	22.97	
P3-A	43.73	
P4-H	34.80	
NH_3	-9.27	-10.97[a]
•CH_3	37.91	34.82[b]

[a]Reference 42. [b]Reference 43

The calculated values were compared with the available experimental findings and that helps to verify that the chosen method is quite well or not to define the behavior of this type of systems. For example the experimental enthalpies of formation at 298K of CH3 radical is ~35 and we have found theoretically ~38 kcal/mol. Though the error is not so small but we can say that it is quite a good agreement between theory and experiment and maybe higher level composite method will require getting more accurate results.

CHAPTER 5: CONCLUSION

The anti-oxidation mechanism of the 4-(N,N-dimethyl)-aminoantipyrine has been established at M062X/BS1 and M08HX/BS2 computational level. The potential energy surface was constructed to comprehend the reaction mechanism systematically.

The reactions of OH radical with molecule R was initiated by the H-atom abstraction mechanism which gives three competitive channels. Another possible pathway for the reaction between R and OH is the hydroxylation on the aromatic ring. The addition of OH radical on the aromatic ring also results in the exothermic reaction. The H-atom abstraction channels possess lower energetics than that of addition.

From the observation, it was concluded that the Hydrogen atom abstraction from N-a site (channel 1) of the molecule 4-(N,N-dimethyl)-aminoantipyrine is much more favorable than the other abstraction or addition pathways. The reaction through this pathway basically produces the N-demethylation product. In experimental observation also the N-demethylated product was found as major product. So, we can say that our theoretical study provides a good agreement with the experimental results. It is also established from this study that OH radical can be potentially scavenged by the 4-(N,N-dimethyl)-aminoantipyrine. Consequently, the compound selected for this study might work as potential anti-oxidant.

CHAPTER 6: REFERNCES

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