

## Molecular Docking and Thermodynamic Studies of the Interactions between Aspirinate Complexes of Transition metals and Cyclooxygenase-2 Enzyme: Quantum Chemical Calculations based on the ONIOM method

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### Abstract

In the present research, molecular docking and thermodynamic properties of the transition metal complexes of aspirin were calculated against Cyclooxygenase-2 (COX-2) enzyme. Density functional theory with dispersion function (DFT-D) using LANL2DZ basis set calculation was carried out to study the structural and thermodynamic properties of the interaction between aspirinate complexes of transition metals and COX-2. The ONIOM2 (wB97X-D/LANL2DZ:UFF) method was applied to the interaction of transition metal complexes with COX-2 binding site. The Interaction enthalpies and the Gibbs free energies between aspirinate complexes of Cu(II), Zn(II), Fe(III), and In(III) as anti-inflammatory complexes and COX-2 enzyme in the gas phase were calculated. The structure as well as the thermodynamics of optimized metal complexes was debated from the biological point of view. In the gas phase, the interaction was relatively strong and transition metal complexes could be used as potential anti-inflammatory drugs.

**Keywords:** Aspirin, COX-2, Docking Simulations, Gibbs free energy, Metal complex, ONIOM2.

### 1. Introduction

Aspirin [Acetylsalicylic acid], ASA, belongs to the widely used class of non-steroidal anti-inflammatory drugs, NSAIDs, which are clinically tested as analgesic, antipyretic and anti-inflammatory agents (1). The therapeutic effect of NSAIDs is believed to be due to their ability to inhibit cyclooxygenase enzyme (COX). Although this enzyme exists in three isoforms, namely COX-1, COX-2 and COX-3 (2), but accumulating evidence indicates that only COX-1 and COX-2 isoforms have been mostly studied by

several groups worldwide (3, 4). There are only small differences in the amino acid sequences forming the cyclooxygenase active site that makes difference in biological activities (5, 6) in spite of the fact that COX-1 and COX-2 proteins are ~60% identical (7). COX-1 is a constitutively expressed protein formed in many different organs and is responsible for the production of protective prostaglandins. On the other hand, COX-2, which is rapidly up-regulated at inflammatory sites, leads to formation of pro-inflammatory prostanoids (8, 9). COX-2, as an inducible isozyme of cyclooxygenase, represents a potential target to prevent and treat a variety of pathological conditions (10).

A rapidly evolving field of bioinorganic chemistry is development in the coordination of

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molecules with biological and pharmaceutical activities like NSAIDs toward several metals. Not only does it increase the coordination potency of NSAIDs while minimizing their side effects, but its application also make it possible for new NSAIDs to be designed (11-14). Aspirinate metal complexes and its potential applications have been studied by several experimental and theoretical techniques. For illustration purposes, copper (II) aspirinate was observed to be a potent antioxidative compound possessing antioxidative activity in biological systems (15). Moreover, it has been proved to be very promising in becoming an anti-thrombotic drug in preventing and treating thrombotic diseases (16). Furthermore, during synthesis, spectroscopic and biological study showed that Ca(II), Mg(II), Sr(II) and Ba(II) aspirinate complexes possess more antibacterial activity than the free aspirin molecule (17). In another paper, the electrostatic potential of zinc(aspirinate)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> complex was calculated and analyzed through the 3D isopotential and molecular surface representations to highlight the active sites of the zinc-aspirinate complex (18).

Docking study of various NSAIDs were also carried out where these analogues were docked into the active sites of both COX-1 and COX-2. For instance, the origins of binding affinity and COX-2/COX-1 selectivity for analogues of celecoxib have been explored using an approach that combines docking with Monte Carlo (MC) simulations (19).

Virtual screening (VS) by means of docking drug-like molecules is a suitable technique which is able to segregate between active molecules and inactive structures. This method is considered as a frequent practice in drug discovery today. The main perspective of VS is to narrow down the number of compounds to be tested in vitro (20, 21).

Newly, exact molecular modeling for larger molecules, such as those in molecular biology, became more feasible due to new progresses in computational chemistry. One of the most popular hybrid theoretical mechanical quantum approach is the ONIOM (our N-layered integrated molecular orbital and molecular mechanics) method developed by Morokuma and co-workers (22-24).

ONIOM is a powerful and systematic method that divides the system into onion-like layers and makes it an accurate tool for studying large biological systems (25). In the ONIOM approach, a small part of a system, such as the inhibitor and a responding amino acid in the binding site of an enzyme is pitched at a high quantum chemical level, whereas the large surrounding region is made by employing a lower level of computations.

The objectives of this study are to i) optimize the structure of aspirinate complexes of some transition metals such as: Cu(II), Zn(II), Fe(III), and In(III) using Gaussian 09 software package, ii) evaluate the activity of these optimized complexes toward COX-2 by docking studies to gain more insight into the nature of the existing interaction between these compounds and active site of this enzyme and iii) select the most favorable pose within the binding site, in energy terms from docking investigation and study the electronic structure of the binding site and calculate thermodynamic quantities of interaction of these complexes using the ONIOM method at the wB97X-D/LANL2DZ:UFF level and establish the applicability of the ONIOM method for these systems. To the best of our knowledge, this is the first example of docking studies that has been explored involving metal aspirinate complexes being incorporated into the active site of the COX-2 enzyme.

## 2. Materials and methods

### 2.1. Geometry Optimization

Electronic structure using wB97X-D (26)/LANL2DZ level of theory was used to explain the structure of aspirinate transition metal complexes. Density functional theory with dispersion function (DFT-D) studies were performed by the Gaussian 09 Quantum chemistry package (27). The basis set LANL2DZ (Los Alamos National Laboratory 2 double zeta) includes an ECP plus DZ on metals and D95 on O and H (28-30). Because of the large system size, the geometry optimization at this level of theory was done without any symmetry restriction over geometry optimizations. The minimum energy is recognized by subsequent frequency calculations. Geometry optimization is one of the most important steps in theoretical studies. Positive values for all the calculated vibrational wave

numbers validated the geometry to be located at the true local minima on the potential energy surface.

### 2.2. Docking Simulation

Docking studies were carried out to provide insight into the interaction of the studied optimized aspirin and aspirinate complexes at different ionization states with the active site of COX-2 enzyme. All docking simulations were performed by means of an *in house* batch script (DOCKFACE) for automatic running of Autodock4.2 (31) in a parallel mode using all system resources. For this purpose, the PDB structure of Cyclooxygenase-2 (3NT1) was retrieved from the Brook haven protein database (RCSB) (<http://www.rcsb.org>) such as a complex bound with naproxen as inhibitor. Afterwards, all water molecules and the co-crystallized ligand were eliminated from the PDB structure. DOCKFACE was designed to facilitate docking studies in a stepwise mode including ligand preparation, receptor preparation, conf.txt preparation and finalization of docking runs. In all experiments, genetic algorithm search method was used to find the best pose of each ligand in the active site of the target enzyme (32). The grid box dimension was considered as 75×75×75 and coordinates of the grid center were -40.486, -51.053, and -20.415 respectively. Random orientations of the conformations were generated after translating the center of the ligand to a defined position within the receptor active site. No attempt was made to minimize the ligand-receptor complex (rigid docking). All visualization of protein ligand complexes was done using VMD software (33).

### 2.3. The QM/MM Simulation

It is celebrated that the molecular mechanics (MM) gives us an extremely powerful tool for analyzing the mechanistic, structural, and energetic properties of biomolecules. However, the MM is not able to calculate and/or simulate the break or formation of chemical bonds. Whenever the interaction of biomolecules with small molecules is considered, the quantum mechanical (QM) prescription must be employed. QM simulations are inherently unbiased and can accurately describe

the ionic, hydrogen and covalence binding reactions. In other words, QM methods are based on solving the Schrödinger equation, taking directly into respect the electronic structure of a molecule and therefore allow access to chemical interactions (34). Unfortunately, accurate QM calculations are very expensive and cannot directly be used for studying enzyme reactions. A solution to these constraints is the application of hybrid quantum mechanical/molecular mechanical (QM/MM) methods, as have been openly used in readings of drug-enzyme interactions. It is well known that, the ONIOM allows us to split the total system into two and/or three layers and applies an extrapolation to compute the total energy. In the present study, the two-layer ONIOM method was utilized. In the two-layer ONIOM (high: low), the real system energy ( $E_{\text{QM/MM}}$ ) can be calculated by the following equation (35)(Eq. 1):

$$E_{\text{QM/MM}} = E_{\text{MM}}(\text{MM} + \text{QM}) + E_{\text{QM}}(\text{QM}) - E_{\text{MM}}(\text{QM}) \quad \text{Eq. 1}$$

Where “real” refers to the full system, and “model” refers to the chemically most important (core) region. Superscripts low, and high mean low (molecular mechanic, MM) and high-level (quantum mechanics, QM) methodologies were used in the ONIOM calculations. The most significant footstep whenever using the ONIOM is in what way to select the methods that will combine, and the divide of the system into high and low level layers. These two factors are carefully related. An arbitrary select of model and method combination usually does not work. In addition, the quality of the *ab initio* results counts commonly on the Gaussian basis set and whether electron correlation is taken into consideration sufficiently. For the QM layer analyzed here, the DFT function with dispersion corrections was adopted (36), for the purpose of an accurate explanation of non-covalent hydrogen bonds at less computational cost. The MM layer of the systems was used in the UFF force field. The optimizations of the model systems are in standard capable of computing hydrogen and non-covalent bonding energies. Thus, the QM layers of ONIOM optimized geometries were extracted for single point energy computations at LANL2DZ basis set. This level of theory recovers important electron correlation energy and has

been shown to be successful in modeling the base pairs (29). The interaction energies between the base pairs and studied ligands were measured by a developed version of the Gaussian 09 program (27). The Gauss View version 5.0.8 was used as a graphical medium.

### 2.3.1. ONIOM Systems

In accordance with position within the binding site and docked binding energy value, the best inhibitor structure was chosen. After that, for understanding the change of energy in the different ligand receptor complex regions, ONIOM based calculations were done.

[Cu(Asp)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]: The model in this study was obtained from the 1.73 Å resolved crystal structure of copper aspirinate bound to COX-2 (3NT1.pdb) (37). Based on this structure, we adopted the system consisting of 14 amino acid residues within a 10 Å diameter centered at copper aspirinate. Amino acid residues included are Lys68, Pro71, Val74, Leu78, Val102, Ser105, Arg106, Leu109, Tyr341, Leu345, Phe456, Met457, Glu510, and Pro514. According to Morokuma method (38), all amino acids, assumed to be in their neutral form were terminated, if not connected to another amino acid in the selected model, by link H atoms at the N- as well as at the C-terminal with their bond and torsion angles assumed to be the same as in the X-ray structure. Hydrogen atoms were added to the X-ray structure to generate the complete structure of the model and their positions were optimized with the UFF force field. This structure was utilized as the starting geometry for all calculations.

[Zn(Asp)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]: Similarly, for this complex the system consists of 13 amino acid residues within a 10 Å diameter centered at Zinc aspirinate. The amino acid residues included are Pro69, Thr70, Pro71, Val74, Leu78, Tyr101, Val102, Ser105, Arg106, Tyr341, Phe343, Leu345, and Glu510.

[Fe(Asp)<sub>2</sub>Cl<sub>3</sub>]: Herein the system consisting of 13 amino acid residues within a 10 Å diameter centered at Iron aspirinate. The amino acid residues included are Val74, Ile77, Leu78, Trp85, Ile98, Tyr101, Val102, Ser105, Arg106, Val335, Tyr341, Phe343, and Leu345.

[In(Asp)<sub>2</sub>OH]: For this system there are 11 amino acid residues within a 10 Å diameter centered at Indium aspirinate. These amino acids are Pro69, Val74, Leu78, Tyr101, Val102, Ser105, Arg106, Tyr341, Leu345, Val509 and Ala513.

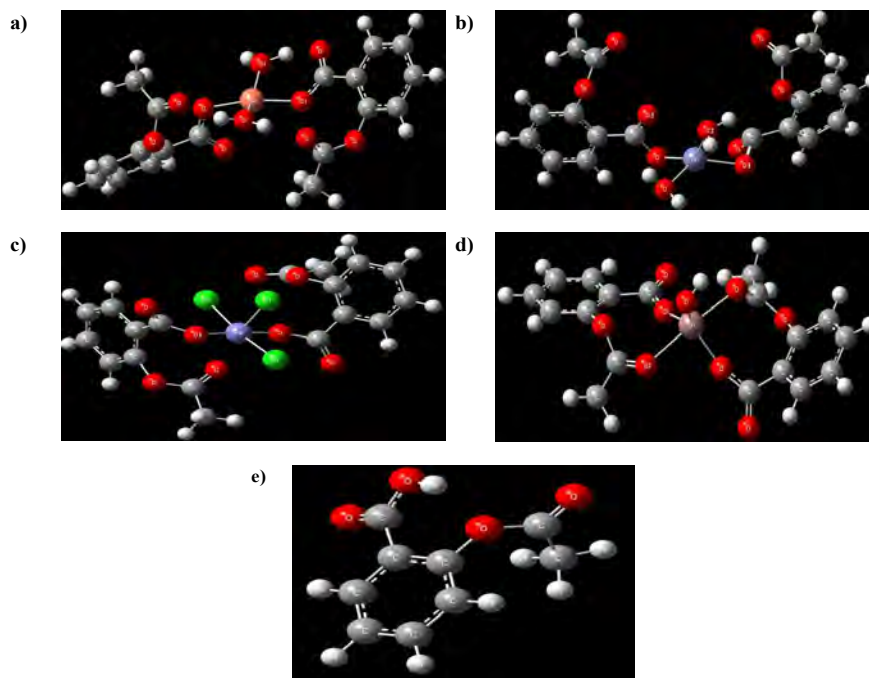
Therefore, the first task is to test how the single transition metal aspirinate structure is calculated by the normal (non-ONIOM) method. Different low level calculations were used to represent the best ONIOM model for these complexes. The geometry of the complexes was optimized with wB97X-D/LANL2DZ, PM6 and UFF. Some optimized geometrical bond lengths of the transition metal complexes are listed in Table 1. In general, any combination of methods gives great value agreement with the corresponding high level non-ONIOM computations.

## 3. Results and Discussion

### 3.1. Optimization

**Table 1.** Selected bond lengths in Å by theoretical calculations for the title compounds at wB97X-D/LANL2DZ and UFF and PM6 level of theory.

	wB97X-D/LANL2DZ	UFF	PM6	Experimental
Cu-O1	1.95	1.93	2.16	1.84-1.96 (40, 42-44)
Cu-O2	1.99	1.93	2.08	2.02 (45)
Zn-O1	1.99	1.95	1.93	1.95-2.03 (39-41)
Zn-O2	2.02	2.00	2.05	2.02 (39)
Zn-O3	2.54	2.56	3.20	2.53 (39)
Fe-O	1.87	1.88	1.85	1.87-1.94 (45-47)
Fe-Cl	2.38	2.28	1.80-2.46	2.23-2.31 (45-47)
In-O1	2.21	2.16	2.25	2.10-2.23 (48-50)
In-O2	1.96	2.04	2.10	



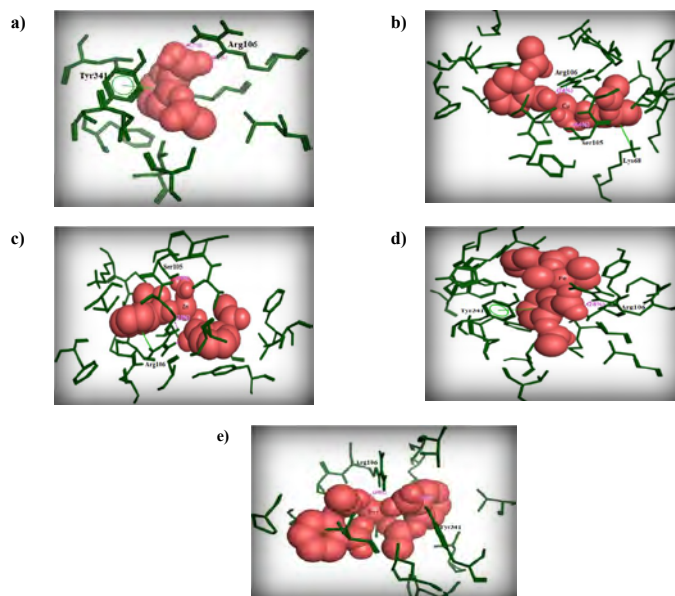
**Figure 1.** The optimized structure of the aspirinate complexes obtained at wB97X-D/LANL2DZ level of theory. (a)  $[\text{Cu}(\text{Asp})_2(\text{H}_2\text{O})_2]$ , (b)  $[\text{Zn}(\text{Asp})_2(\text{H}_2\text{O})_2]$ , (c)  $[\text{Fe}(\text{Asp})_2\text{Cl}_3]$ , (d)  $[\text{In}(\text{Asp})_2\text{OH}]$ , (e) Aspirin optimized with wB97X-D/6-31+G(d,p).

The structure of four complexes containing Cu, Zn, Fe and In metals as center of the molecule have been optimized with wB97X-D/LANL2DZ level of theory. The optimized stable molecular structure of the desired complexes with wB97X-D/LANL2DZ is shown in Figure 1(a)-(d). Selected geometrical parameters, bond lengths of the compounds are listed in Table 1. All the bond lengths and bond angles of the phenyl rings are in the normal range. In order to check the validity of the method applied, X-ray diffraction data was used. As a result, X-ray diffraction data of zinc (39-41), copper (40, 42-45), iron (46-48) and indium (49-51) complexes were used to compare the optimized structures of these complexes. The agreement between the computed structures by the DFT-D method and X-ray diffraction data was excellent. The little differences may have resulted from gas phase calculation which implies not many body interaction typical of the solid state was included. As a result, despite slight differences, the wB97X-D/LANL2DZ level of theory can produce acceptable results for the systems studied here. Therefore, the calculations at wB97X-D/LANL2DZ level of theory as represented above can reproduce the crystal struc-

ture of these complexes, and make the bases for the following discussion.

### 3.2 Docking Simulation Study

In order to explore the binding mode for interaction of the target compounds with COX-2, docking studies were performed. First the optimized ligand was docked back into the binding site of the enzyme and superposed with the native ligand. In table 2 a summary of docking results is listed. Also the binding mode for all systems as well as aspirin in the active site of COX-2 enzyme are depicted in Figure 2. The most important interactions observed for aspirin with COX-2 include a  $\pi$ - $\pi$  interaction between Tyr341 and the phenyl ring of aspirin as well as hydrogen bond between Arg106 and carboxylic acid moiety of the ligand (Figure 2(a)). Docking of copper complex with COX-2 revealed interactions between Lys68, Ser105 and Arg106 (Figure 2(b)). In case of Zinc complex the interactions were observed with Ser105 and Arg106 (Figure 2(c)). As displayed in Figure 2(d), the residues involved in the interaction of Fe complex with COX-2 were Tyr341 and Arg106. Finally, the two residues Arg106 and Tyr341 were involved in the interaction of In com-



**Figure 2.** Superposition of docked structure of ligands in the active site of COX-2. (a) Aspirin, (b) [Cu(Asp)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>], (c) [Zn(Asp)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>], (d) [Fe(Asp)<sub>2</sub>Cl<sub>3</sub>], (e) [In(Asp)<sub>2</sub>OH]. All visualizations were prepared by VMD.

plex with the enzyme (Figure 2(e)).

### 3.3 Application of ONIOM

Since the ligand-enzyme complexes formed by all amino acids and aspirinate transition metal are too large for high level (wb97X-D) calculations, the complexes have been divided into two parts. Accordingly, the ONIOM method was applied to the aspirinate transition metal complexes, with or without the effects of other amino acid residues. In other words, the main focus of the copper aspirinate part is the specific interaction of copper aspirinate with Lys68, Ser105, and Arg106, in the copper aspirinate-COX-2 complex. In order to reduce the computational demand, Copper aspirinate with Lys68, Ser105, and Arg106 were chosen such as model in high level and other amino acids as model in low level. In case of [Zn(Asp)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] system; Arg106, Ser105 and

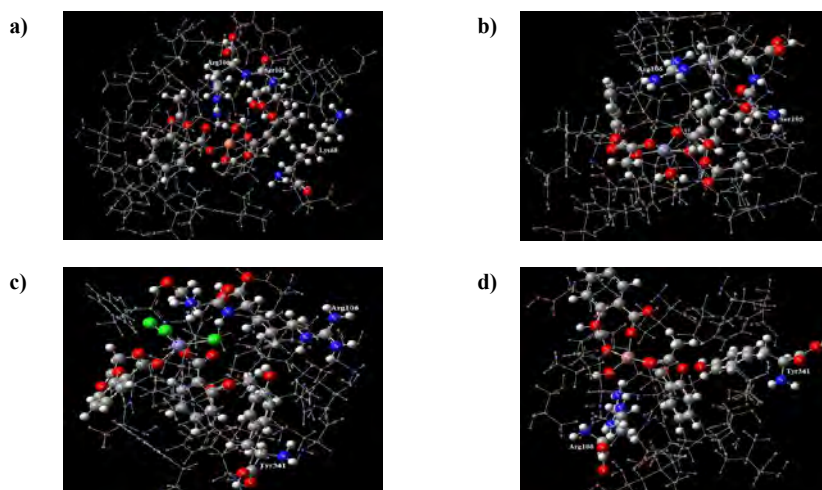
zinc aspirinate complex were selected in high level (wb97X-D) and other residues were selected in low level (UFF). In the [Fe(Asp)<sub>2</sub>Cl<sub>3</sub>] system; Tyr341, Tyr101, Arg106 and iron aspirinate were considered as high level while other amino acids were in low level. For the [In(Asp)<sub>2</sub>OH] system; Tyr341, Arg106 and Indium aspirinate complex were selected as high level and other amino acids as low level (See Figure 3(a)-(d)).

### 3.4 Stability and Binding Energy

Since polypeptide chains have the large size, information of the covalent structure is not sufficient to fully characterize proteins. The three-dimensional structure is the result of many simultaneous interactions that take place among different parts of the molecule (52). These non-covalent interactions not only determine the structure of a protein but also mediate its function with the envi-

**Table 2.** Ligand binding modes obtained by the docking methodology.

Ligand	Mean Binding Energy (Kcal mol <sup>-1</sup> )	Hydrogen bonding and $\pi - \pi$ interaction residues	Min (max) rmsd( $\text{\AA}$ )
Aspirin	-5.20	Arg(106), Tyr(341)	0.03 (1.41)
[Cu(Asp) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	-8.81	Lys(68), Ser(105), Arg(106)	0.85 (1.99)
[Zn(Asp) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	-8.70	Ser(105), Arg(106)	0.79 (1.99)
[Fe(Asp) <sub>2</sub> Cl <sub>3</sub> ]	-9.13	Arg(106), Tyr(341)	0.32 (2.00)
[In(Asp) <sub>2</sub> OH]	-9.33	Arg(106), Tyr(341)	0.01(0.12)



**Figure 3.** Optimized geometries of the (a)  $[\text{Cu}(\text{Asp})_2(\text{H}_2\text{O})_2]$ , (b)  $[\text{Zn}(\text{Asp})_2(\text{H}_2\text{O})_2]$ , (c)  $[\text{Fe}(\text{Asp})_2\text{Cl}_3]$ , (d)  $[\text{In}(\text{Asp})_2\text{OH}]$  in active site obtained at the two layer QM/MM level.

ronment, which is with water, ions, other proteins, membranes, *etc.* These interactions are based on the same set of non-covalent forces that, for convenience are usually classified as: van der Waals interactions, electrostatics interactions and hydrogen bonds (53).

Accordingly, it should be noted here that this study executed the quantum mechanical calculation of the Binding Energy between ligand and receptor in the gas phase, without using the contribution of the desolvation energy to the binding free energies. To this end, we are expanding intuitions into the ligand-receptor binding mode by applying the ONIOM method which is the more general collective method and much easier to execute compared to the other QM/MM approaches. The hybrid ONIOM (QM/MM) technique is one of the best methods to study complex molecular interactions (54). Conventionally, proteins and other large biological molecules have been out of the reach of electronic structure methods. The ONIOM method not only overcomes these constraints, but also pro-

vides the possibility of using higher accurate quantum calculations to obtain more accurate results for the ligand-receptor interactions. (i.e., excited state and transition state calculations for the reaction mechanism in enzyme complex)(55, 56).

The binding energy (BE) components distributed from each layer can be expressed with the following equations for ONIOM2 (Eq. 2):

$$E_{\text{Model}}^{\text{MM}} \quad (\text{Eq. 2})$$

In which the structure of the ligand-enzyme complex was optimized for the whole complex, and the structures of the QM model and MM model separately were taken to be the same as in the optimized complex and kept fixed throughout the calculations.

The results of BE calculated from different aspirinate complexes in ONIOM models are listed in Table 3. Results show that the interactions are strong, emphasizing the fact that these transition metal complexes can be utilized to improve biological and anti-inflammatory activity.

**Table 3.** Energies of ligand-enzyme complex, binding, QM and MM model ( $\text{kcal mol}^{-1}$ ).

Ligand	$E_{\text{Complex}}^{\text{ONIOM}}$	Binding Energy	$E_{\text{Model}}^{\text{QM}}$	$E_{\text{Model}}^{\text{MM}}$
$[\text{Cu}(\text{Asp})_2(\text{H}_2\text{O})_2]$	-1812995.62	-56.48	-1813102.29	163.15
$[\text{Zn}(\text{Asp})_2(\text{H}_2\text{O})_2]$	-1533628.17	-50.20	-1533690.92	112.95
$[\text{Fe}(\text{Asp})_2\text{Cl}_3]$	-1897765.94	-100.40	-1897822.42	156.88
$[\text{In}(\text{Asp})_2\text{OH}]$	-1638303.11	-81.58	-1638296.83	75.30

**Table 4.** Gibbs Free Energy Values of ligand-enzyme complex, QM model, MM model and ligand-enzyme Interaction (kcal mol<sup>-1</sup>)

Ligand	G <sub>Complex</sub> <sup>ONIOM</sup>	G <sub>Model</sub> <sup>QM</sup>	G <sub>Model</sub> <sup>MM</sup>	ΔG <sub>int</sub>
[Cu(Asp) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	-1877547.57	-1879781.51	2277.86	-43.92
[Zn(Asp) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	-1531249.90	-1533295.58	2108.43	-62.75
[Fe(Asp) <sub>2</sub> Cl <sub>3</sub> ]	-1895632.41	-1897420.81	1876.25	-87.85
[In(Asp) <sub>2</sub> OH]	-1636339.00	-1638234.08	1920.18	-25.10

As shown in Figure 4, it can be seen that the predicted data by ONIOM (wB97X-D: UFF) calculation is in accordance with the docking results. To confirm the results, thermodynamic quantities of ligand-enzyme interaction were calculated using the wB97X-D method.

### 3.5 Thermodynamic Properties

One of the methods for growing new drug compounds is to improve their pharmacological effects. Study of the thermodynamic properties of the interaction between aspirinate complexes of transition metals as anti-inflammatory and COX-2 enzyme is biologically important for the expectation of pharmacological properties of these complexes. Spontaneous changes in protein structures, such as the binding of a ligand to an enzyme or receptor, are characterized by a decrease of free energy. Despite the recent improvements in calculating power and methodology, it remains a challenge to accurately estimate free energy changes. Major issues concerned with the accuracy of the underlying model to designate the protein system and how well the calculation in fact emulates the behavior of the system are still being considered.

This paper is on the whole concerned with the characteristic of current free energy calculation methods as applied to enzyme-ligand systems. Several methodologies were utilized to calculate Gibbs standard free energies of binding for a collection of enzyme-ligand complexes. According to the multi-layered approach (ONIOM) developed by Morokuma (22), the following relationships can be proposed for the enthalpy and Gibbs free energy of ONIOM2 (QM/MM) (Eq. 3, Eq. 4):

$$G_{\text{ONIOM}} = G_{\text{QM(Model)}} + G_{\text{MM(Real)}} - G_{\text{MM(Model)}} \quad (\text{Eq. 3})$$

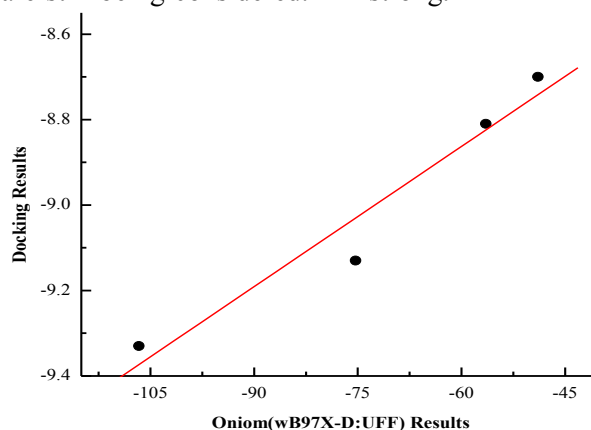
$$E_{\text{QM/MM}} = E_{\text{MM}}(\text{MM} + \text{QM}) + E_{\text{QM}}(\text{QM}) - E_{\text{MM}}(\text{QM}) \quad (\text{Eq. 4})$$

Therefore, the enthalpy and the Gibbs free energy of interaction QM/MM between QM and MM model can be obtained as follows (Eq. 5, Eq. 6):

$$\Delta G_{\text{int}} = G_{\text{ONIOM}} - G_{\text{QM,Model}} - G_{\text{MM,Model}} \quad (\text{Eq. 5})$$

$$\Delta H_{\text{int}} = H_{\text{ONIOM}} - H_{\text{QM,Model}} - H_{\text{MM,Model}} \quad (\text{Eq. 6})$$

As shown in table 4 and 5, the calculated Gibbs free energies and enthalpies of the interaction values were negative and relatively high indicating that this interaction is spontaneous and strong.



**Figure 4.** Docking Results of Binding Energy (kcal mol<sup>-1</sup>) values versus ONIOM (wB97X-D: UFF) level values for the Aspirinate Complexes (R<sup>2</sup>=0.94)



**Table 5.** Enthalpy Values of ligand-enzyme complex, QM model, MM model and ligand-enzyme Interaction (kcal mol<sup>-1</sup>).

Ligand	H <sub>Complex</sub> <sup>ONIOM</sup>	H <sub>Model</sub> <sup>QM</sup>	H <sub>Model</sub> <sup>MM</sup>	ΔH <sub>int</sub>
[Cu(Asp) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	-1530898.50	-1533214.00	2390.81	-75.30
[Zn(Asp) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]	-1877152.24	-1879681.10	2585.34	-56.48
[Fe(Asp) <sub>2</sub> Cl <sub>3</sub> ]	-1895287.28	-1897301.59	2127.26	-112.95
[In(Asp) <sub>2</sub> OH]	-1636006.42	-1637826.20	1869.98	-50.20

#### 4. Conclusions

The current study was carried out to be under investigation the interaction binding energy between aspirinate complexes of transition metals and COX-2 enzyme.

The estimated binding energy with ONIOM (wB97X-D: UFF) method confirmed docking results.

The calculated results showed that the interaction is spontaneous ( $\Delta G < 0$ ) and exothermic ( $\Delta H < 0$ ) in gas phase based on ONIOM method. Therefore, these systems have relatively high level of stability and the aspirinate complexes of transition metal can be used as anti-inflammatory drugs. The radioisotope of all metals used in this study

have been discovered (<sup>64</sup>Cu, <sup>65</sup>Zn, <sup>52</sup>Fe, <sup>111</sup>In) (57-60). Radioisotope complexes of the aforementioned metals can be also considered as radiotracers of COX-2 enzyme. Overexpression of COX-2 in some inflammatory diseases and cancers makes them a good choice for diagnosis methods based on nuclear medicine like SPECT and PET.

#### Acknowledgements

The authors are grateful to the research council at Shiraz University of Medical Sciences for providing computational facilities for this work.

#### Conflict of Interest

None declared.

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