



In-Silico Molecular Docking Study of Coumarin Derivatives in order to Investigate the Inhibitory Effects of Human Monoamine Oxidase Enzyme and DFT Studies

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Abstract

In the present study, DFT calculations of four coumarin derivatives and also their molecular docking with “human monoamine oxidase enzyme” (HMAO) were performed in order to study the inhibitory effect of these compounds. The optimized molecular geometries and vibrational frequencies were calculated at the B3LYP/6-31+G(d) level of theory without any imaginary frequency. The total energy, dipole moment and energies of the frontier molecular orbitals were calculated for all the compounds. All quantum calculations were performed using the Gaussian 03 software. The molecular docking of coumarin derivatives and phenelzine with HMAO enzyme were calculated and inhibitory effect of coumarins were compared with phenelzine. Also the binding free energy, amino acid residue and hydrogen bond interactions between all the compounds and HMAO enzyme were calculated. The binding energies for coumarins and Phenelzine are in the range of 7.04 – -6.15 Kcal/mol. The binding energy potency follows the order of: 4>2>3>1> Phenelzine. The binding energy of all the compounds with HMAO enzyme was stronger than Phenelzine.

Keywords: Coumarin, antidepressant, Monoamine oxidase, DFT study, molecular docking

1 Introduction

Coumarins (also known as 1, 2-benzopyrone or hydroxycinnamic acid-8-lactone) are categorized as an important class of natural compounds, mainly found in Rutaceae and Umbelliferae families. These compounds are divided into several classes such as simple coumarins, furanocoumarins, pyranocoumarins, biscoumarins, triscoumarins, and coumarinolignans. Coumarins have too many biological applications including antidepressant (1), antimicrobial (2), antiviral (3), anticancer (4), anti-inflammatory, antioxidant, and anticoagulant (5, 6). Coumarins like esculetin, fraxetin, daphnetin and other related coumarin derivatives are known as inhibitors, not only LOX and COX enzymes, but also the neutrophil-dependent superoxide anion generation. Many investigators reported that

various coumarin derivatives are able to block inflammation by inhibiting different targets but none of them has found a way to the clinics so far.(7) Fylaktakidou et al (2004) reviewed the capability of different natural and synthetic coumarins as anti-inflammatory and antioxidant molecules (8). Curini et al (2006) reported an array for the biological applications of prenyloxycoumarins and prenyloxyfuranocoumarins (9). In addition to anti-inflammatory properties, coumarins have also been considered as potential anticancers (10, 11) and also monoamine oxidase inhibitors (12).

Monoamine oxidase (MAO) is a flavoenzyme with iron that exists within cells, being connected to the surface membrane of mitochondria and involved in the degradation of biogenic amines. Two MAO isoenzymes i.e. MAO-A and MAO-B, are closely linked in opposite orientations to the X chromosome and expressed in the external part of mitochondrial membrane. MAO-A and MAO-B are able to oxidize neuro-transmitters and

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xenobiotic amines using the oxidative deamination process (13). MAO is abundantly found in noradrenergic nerve terminals but it is also present in many other places, like liver or intestinal epithelium. MAO-A, the primary type in fibroblasts, preferentially degrades serotonin, norepinephrine and dopamine. Meanwhile, MAO-B, found in platelets, human brain and other primates, preferentially degrades the phenyl ethylamine and benzylamine (12).

The activity of MAO helps maintaining the neuron firing rates throughout the body within homeostatic limits. Part of the biochemical activity of MAO produces hydroxyl radicals, very toxic members of the oxygen free radical group that might be involved in neurodegenerative disorders such as Parkinson's disease. As mentioned above, MAO plays a determining role in the metabolism of many neurotransmitters and can be utile in the treatment with several psychiatric and neurological diseases. These characteristics determine the pharmacological interests for the MAO inhibitors. In fact, human MAO-B inhibitors *e.g.* selegiline (R-(-)-deprenyl) and rasagiline are advantageous compounds for the treatment with Parkinson (14) and Alzheimer's diseases (15, 16). Besides, the selective MAO-A inhibitors, like clorgyline (irreversible) and moclobemide (reversible), are useful for the treatment with neurological disorders like depression and anxiety (17, 18). In this project, we have investigated the DFT calculations of some coumarin derivatives and their molecular docking with human monoamine oxidase enzyme in order to investigate the inhibitory effect of these compounds.

2 Materials and Methods

2.1 DFT study

One of the most important tasks for the computational works is to determine the optimized geometries of the compounds. In this study, the molecular structures of the all the compounds were optimized and the electronic properties were calculated. Besides, the optimized geometries of the molecules were visualized with ChemCraft program. All the calculations were performed applying the Becke's three parameters hybrid exchange functional with the Lee-Yang-Parr gradient corrected correlation functional (B3LYP hybrid functional). All the atoms were described with a split valence Pople basis set plus polarization and diffuse functions, 6-31+G(d) (19). Frequency calculations were performed for all the optimized geometries to ensure that the obtained structures represent local minima. All the calculations were established employing the Gaussian 03 program suite.

2.2 Molecular docking study

I. Protein preparation

PDB database was applied to retrieve the raw X-ray crystal structures of HMAO (PDB: 1GOS)(20), but this structure cannot be used directly for the molecular docking investigations. Since this structure involving the heavy atoms, cofactors, waters, metal ions and ligand and it fails to provide information on topologies, bond orders and formal atomic charges. Therefore, this PDB structure has been changed employing the protein preparation wizard available in chimera software. At the initial step, all the components, excluding the chain A of protein, were removed. All hydrogen atoms were added

to the protein and the structure was optimized and applied for molecular docking.

II. Molecular docking

The optimized geometries for all the ligands calculated by DFT calculations were applied as input files for the conformational search by systematic search method. The conformer with lowest energy was employed for the docking calculations. AutoDock 4.2 software, using the Lamarckian genetic algorithm together with the AutoDock tools, was used to set up and perform docking calculations of all compounds binding to the receptors.(21) In order to perform docking, the protein structure having pdb format is prepared as shown in part I and applied in the present study. Polar hydrogens are added for saturation while the non-polar hydrogens are merged and Gasteiger charges are computed. A grid box with grid spacing of 0.375 Å and dimension of 60 × 95 × 80 grid points along x, y and z axes are made around the active sites. In this regard, the grid center was set at 2, 16, and 38 Å. The grid box contains the complete active site of the protein receptor and offers enough space for the ligand translational and rotational walk.

AutoGrid was employed to make the grid map for different atoms in the ligands and receptor. After the completion of the grid map, Autodock was used for 50 runs with the following: maximum of the numbers for the energy evaluations are set to 2500000 and a maximum number of 27000 GA operations are produced by an initial population for 150 individuals. For each of the docking cases, the lowest energy value for the docked conformations was chosen as the binding mode on the basis of the AutoDock scoring function. Visualization of the docked pose has been performed by Discovery Studio and Chimera molecular graphics software.

3 Results and discussion

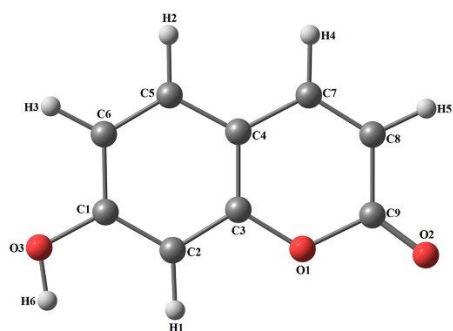
3.1 Computational study

The geometries of all the structures were completely optimized using the B3LYP/6-31+G(d) level of theory. The optimized structures are depicted in figure1. For all the structures, total energy, dipole moment, energy levels of HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) and HOMO-LUMO energy gap (ΔE) were calculated and are summarized in Table 1.

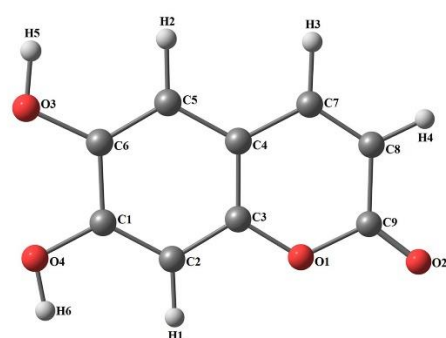
As a matter of fact, frontier molecular orbitals (FMOs) are the most important orbitals in a molecule. FMOs play determining roles in the interaction between the molecules and also in the electronic spectrum of a molecule. For all the compounds, the calculations indicated that the charge density distribution for the HOMO level is predominantly localized on the π -conjugated system of aromatic carbons together with the lone pairs on oxygen atoms of hydroxyl and methoxy groups. Meanwhile, the charge density of the LUMO is localized on the anti-bonding aromatic system. FMOs in all the optimized structures were visualized using the gauss view 5. The density plots of HOMO and LUMO orbitals are revealed in Figure 2. Vibrational frequency calculations were performed for all the compounds using the similar levels for which the imaginary frequencies were not observed. The detailed vibrational assignments for the main frequencies in all the compounds are listed in Table 2.

Table 1: The computed electronic properties for all compounds at B3LYP/6-31+G(d) level of theory.

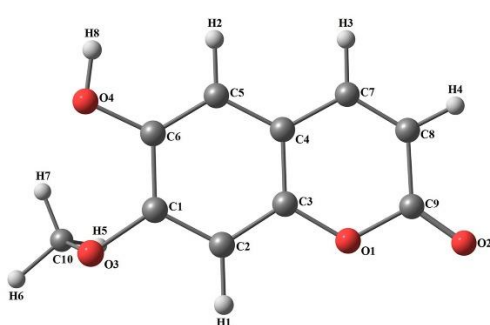
Compound	Properties				
	E_{B3LYP} (a.u.)	μ (Debye)	E_{HOMO} (a.u.)	E_{LUMO} (a.u.)	HOMO-LUMO gap (eV)
1	-572.2417	3.926	-0.2260	-0.0624	4.450
2	-647.4505	4.017	-0.2134	-0.0620	4.119
3	-686.7536	6.504	-0.2186	-0.0652	4.174
4	-726.0606	6.814	-0.2160	-0.0630	4.163



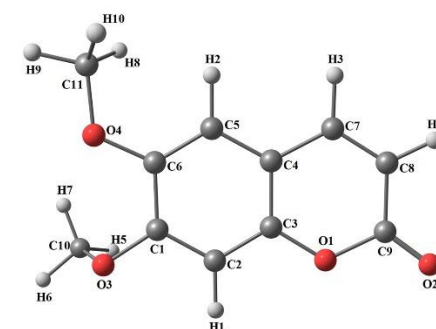
Compound 1



Compound 2



Compound 3



Compound 4

Figure 1: The optimized structure of the all compounds.

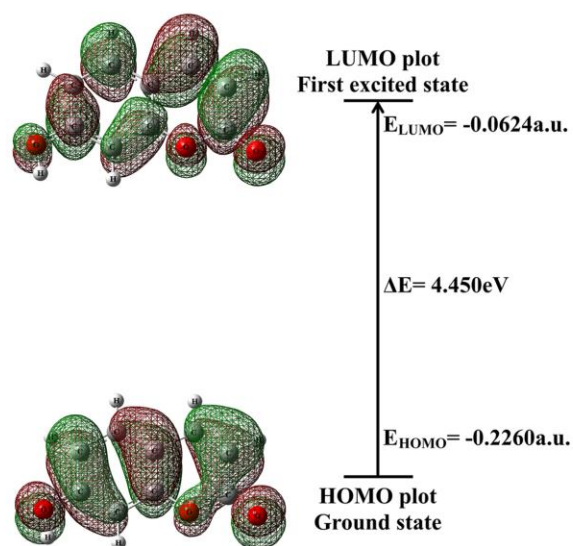
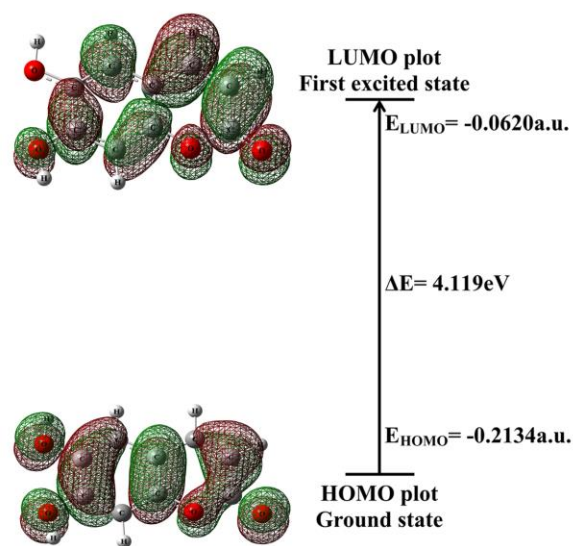
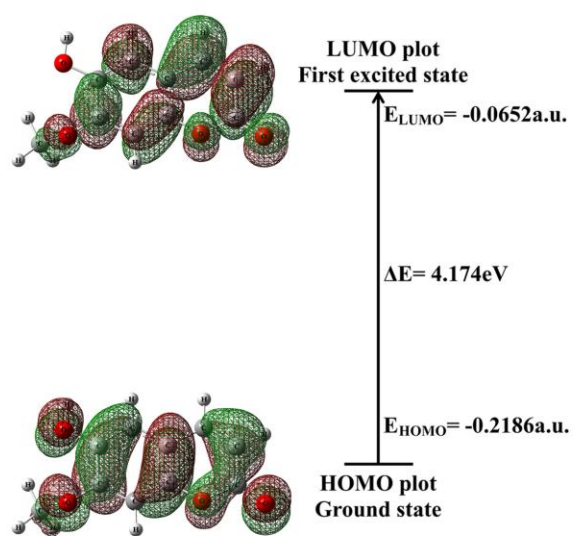
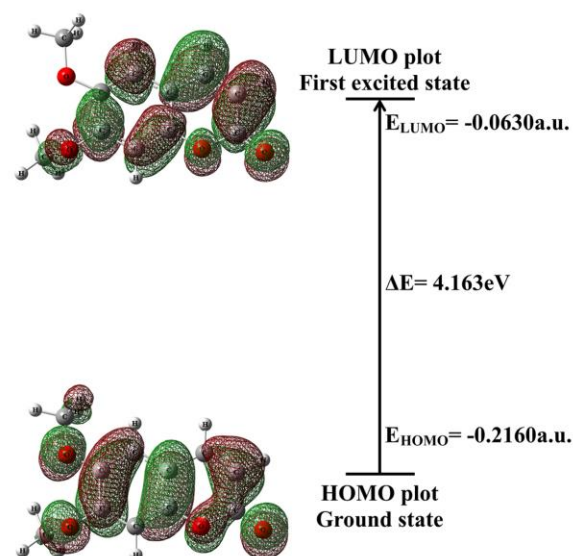
**Compound 1****Compound 2****Compound 3****Compound 4**

Figure 2: Density plot of HOMO and LUMO orbitals for all compounds

Table 2: The calculated vibrational frequencies at the B3LYP/6-31G(d) levels

Vibrational assignments	C-O	C=C(aromatic)	C=O	CH(aliphatic)	CH(aromatic)	OH
Compound 1	829.28, 1105.28 1364.69	1401.84, 1490.51, 1619.58, 1674.81, 1678.57	1844.77	-----	3187.18, 3195.15 3204.03, 3229.35 3249.33	3747.57
Compound 2	867.16, 931.73, 1104.11, 1177.87	1682.82, 1676.15 1630.37, 1573.10	1840.34	-----	3174.92, 3186.14 3204.28, 3241.44	3748.09 3755.64
Compound 3	867.06, 928.10 1030.40, 1108.48 1167.27	1563.20, 1605.09 1671.91, 1681.86	1839.62	3045.87, 3131.84 3167.82	3168.52, 3186.22 3239.79, 3240.85	3353.13
Compound 4	868.72, 921.77 1026.59, 1069.46	1596.85, 1665.90 1679.89	1838.89	3027.86, 3044.14 3088.35, 3128.28 3163.19, 3165.13	3184.86, 3221.97 3228.86, 3241.04	-----

3.2 Molecular docking study

The molecular docking operations for the coumarin derivatives and phenelzine with HMAO enzyme were performed. Phenelzine, having the molecular formula of β -phenylethylhydrazine, is a monoamine oxidase inhibiting antidepressant which is effective in the treatment with panic disorder and social anxiety disorder. Due to the mentioned reason, Phenelzine, as a commercial drug, was chosen to be compared with present the compounds in this study. Our studies started

with the examination of the known structure of HMAO in a non-covalent complex with four different ligands. Only complexes containing non-covalent ligands were selected which is due to that the bond length of a covalent bond is less than that of non-covalent bond. Thus, during the docking process for a covalent inhibitor, a ligand docked in a correct location will poorly be scored even its position may be correct, experimentally. At first, the crystal structure of HMAO (PDB: 1GOS) was optimized (See Figure 3 for the 3D structure). The

docking calculations were performed and the best conformation based on the binding energy value for each ligand was chosen for the analysis. The best conformation docked to the HMAO is shown in Figure 4 for all the compounds.

In all the cases, binding energy values, amino acid residue and amino acids involved in hydrogen bonding interactions for the best conformation are listed in table 3 and visualized in figure 5. The calculated binding free energies for the Phenelzine and compounds 1–4 are in the range between -7.04 and -6.15 Kcal/mol. The binding energy strength for the compounds obeys the order of: 4>2>3>1> Phenelzine. Compound 1 forms classical and non-classical hydrogen bondings with respectively ARG233 and VAL235 via oxygen head of its hydroxyl group. In this context, π - π interaction is established between THR 393 and aromatic system.

Compound 2 has four classical hydrogen bondings with HMAO (VAL10, LEU33, ALA35 and ARG233) and also one non-classical bond with VAL235 which are due to the presence of two hydroxyl groups located in different orientations. Moreover, π - π interactions are formed between THR 393 and aromatic system.

Compound 3 and 4 also exhibit two and three classical hydrogen bondings, respectively. Compound 4, despite of the lower number of hydrogen bondings rather than compound 2 and also the absence of π - π interactions, possesses the highest amount of energy, which is due to the orientation of the molecule. In this regard, Phenelzine also forms six hydrogen bondings with val10, leu33 and arg233 amino acids. Based on the docking results, the interactions in the compounds 1-4 with HMAO enzyme are stronger than Phenelzine.

4 Conclusion

According the molecular docking studies, all the compounds have higher binding affinities compared to that of phenelzine. Consequently, they can be applied as appropriate choices as an antidepressant drug. Also, this study showed that the orientation of the molecule has an important role in receptor-ligand interactions.

Acknowledgments

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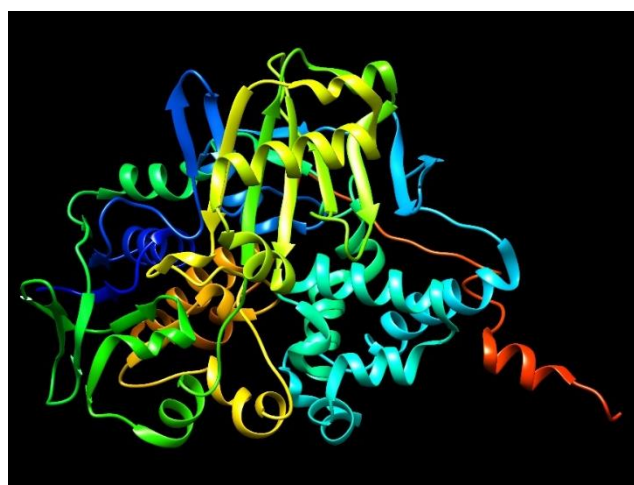


Figure 3: The optimize structure of HMAO, visualize with chimera

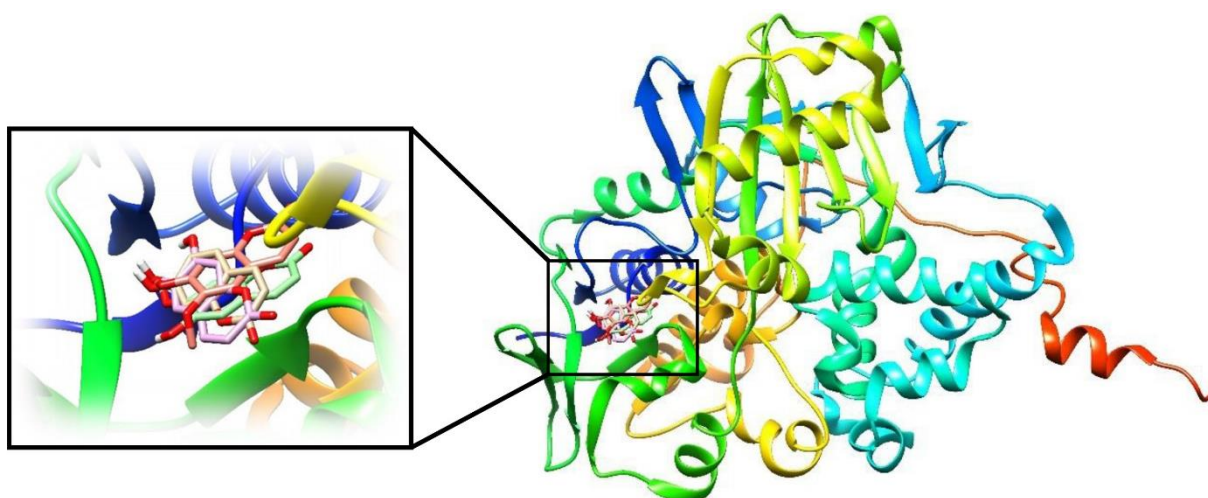


Figure 4: Docked of all the compounds in active site of HMAO

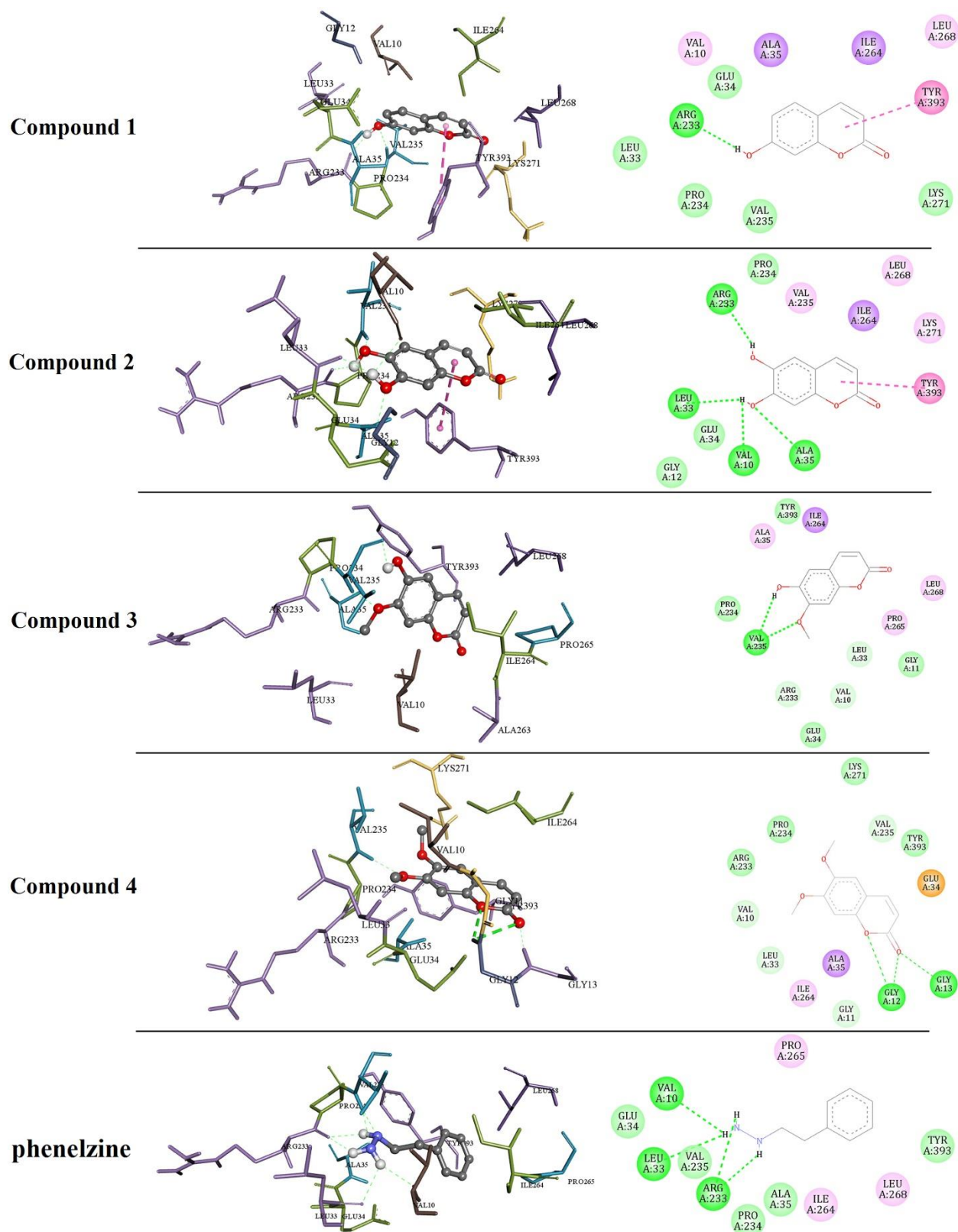


Figure 5: Hydrogen bond interaction and amino acid residue (3D and 2D)

Table 3: The results of molecular docking for all the compounds and HMAO enzyme

Compounds	Binding Energy (Kcal/mol)	Amino acid residue	Hydrogen bond
Compound 1	-6.66	VAL10, LEU33, GLU34, ALA35 ARG233, PRO234, VAL235, ILE264 LEU268, LYS271, TYR393	ARG233, VAL235
Compound 2	-6.91	VAL10, LEU33, ALA35, ARG233 VAL235, ILE264, LEU268, LYS271 TYR393	VAL10, LEU33 ALA35, ARG233 VAL235
Compound 3	-6.67	VAL10, LEU33, ALA35, ARG233 PRO234, VAL235, ALA263, ILE264 PRO265, LEU268, TYR393	VAL235
Compound 4	-7.04	val10, gly11, gly13, leu33, glu34, ala35 arg233, pro234, val235, ile264, lys271 tyr393	gly13
phenelzine	-6.15	val10, leu33, glu34, ala35, arg233 pro234, val235, ile264, tyr393, pro265 leu268	val10, leu33 arg233

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