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Review Article

Computational Models: A Sustainable Approach to Reveal the Inhibitory Potential of Benzo(a)anthracene and its Monohydroxy Derivatives Against Human Sex Hormone-Binding Globulin

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ABSTRACT

Benzo(a)anthracene (BaA), a major environmental pollutant, is metabolized *in vivo* and produces many hydroxy derivatives. The density functional theory calculations were performed to investigate the frontier molecular orbitals as well as the chemical reactivity descriptors of BaA and its monohydroxy derivatives using B3LYP/3-21G basis set. Using *in silico* tools of AutoDock 1.5.6, these compounds were docked into the active site cavity of human sex hormone-binding globulin (hSHBG) to evaluate their binding affinity. Docking results showed that the binding affinities of BaA and its monohydroxy derivatives lie in the comparable range (−8.5 kcal/mol to −9.04 kcal/mol) with dihydrotestosterone (DHT) (−10.94 kcal/mol), a known ligand of hSHBG. The combined results from both the computational models emphasized that BaA and its metabolites can structurally mimic the binding pattern of DHT, a known inhibitor to hSHBG.

Key words: Benzo(a)anthracene, Human sex hormone-binding globulin, Density functional theory calculations, Docking.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are often detected in the environment and are regarded as endocrine disruptors. Benzo(a) anthracene (BaA) is one of the important components of PAHs and is metabolized by cytochrome P450 enzymes to dihydrodiols, phenols, and quinone derivatives [1]. These hydroxylated compounds have a structural similarity to Dihyrotestosterone (DHT), the known inhibitor and can interact with receptor, i.e., human sex hormone-binding globulin (hSHBG). Sex hormone-binding globulin (SHBG) is a high molecular weight plasma protein that binds androgens and estrogens, it plays a key role in maintaining the balance between unbound and bound sex steroids [2]. Any changes in SHBG level affect the distribution of the sex steroids to target tissues. SHBG has also been reported to bind with several Endocrine-disrupting chemicals such as phthalate esters [3]. PAHs modulate various signaling pathways including receptor-mediated pathways, growth factor/cytokine-signaling pathways, and key-regulator/modulator-dependent pathways. B(a)A is reported to be involved in the reactive oxygen species/Mitogen-activated protein kinases pathway in cell migration [4], the peroxisome proliferator-activated receptors (PPARα)/PPARβ/δ pathway in carcinogenesis [5]. PAHs have an extensive body of literature describing their endocrine disruptive activity [6]. As the indirect evidence is suggestive of a possible endocrine disruption by monohydroxy derivatives of BaA, the present *in silico* study was undertaken to verify whether such a binding is energetically possible.

In this investigation, we reported the optimization and hSHBG inhibition pathway of BaA and its metabolites utilizing molecular docking and density-functional theory (DFT) calculations. BaA and few of its corresponding hydroxy-BaA taken for DFT and docking studies were: BaA, 1-HydroxyBenzo(a)anthracene (1-OHBaA), 2-HydroxyBenzo(a)anthracene (2-OHBaA), 3-HydroxyBenzo(a) anthracene (3-OHBaA), 4-HydroxyBenzo(a)anthracene (4-OHBaA), 5-HydroxyBenzo(a)anthracene (5-OHBaA), 9-HydroxyBenzo(a) anthracene (9-OHBaA), 10-HydroxyBenzo(a)anthracene (10-OHBaA) and 11-HydroxyBenzo(a)anthracene (11-OHBaA). Frontier molecular orbitals (FMOs) are the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The HOMO and LUMO, hardness, softness, and chemical potential were studied for every molecule. Molecular docking was performed to understand the binding affinity and binding modes of all structures with the receptor protein (hSHBG). As a positive control, a known ligand of hSHBG, DHT was docked and compared with binding energies of BaA and its monohydroxy derivatives.

2. MATERIALS AND METHODS

2.1. DFT Calculations

Quantum mechanical (QM) methods keep an important role for the calculation of molecular orbital properties [7]. In this investigation, QM calculation was implemented by using DFT employing Becke's (B) [8] exchange functional combining Lee, Yang, and Parr's (LYP) correlation functional [9] in Gaussian 09 program package for all pollutants. Pople's 3-21G basis set was used to optimize the pollutants and for other calculations [10]. Initial geometry of all the pollutants was drawn in GaussView 5.0 and was optimized (Figure 1) using Gaussian 09 software [11]. For every molecule's free energy, dipole moment and

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polarization were calculated. FMOs calculation was performed using the same level of theory (Figure 2). Hardness (η) and softness of all pollutants were also calculated from the energies of frontier HOMOs and LUMOs considering Parr and Pearson interpretation [12,13] of DFT and Koopmans theorem [14] on the correlation of ionization potential (I) and electron affinities (E) with HOMO and LUMO energy (ε). The following equations are used for the calculation of hardness (η) and softness (δ):

$\eta=[E_{LUMO}-E_{HOMO}]/2$; $\chi=[E_{LUMO}+E_{HOMO}]/2$; $\omega=\chi2/2\eta$; $\delta=1/\eta$

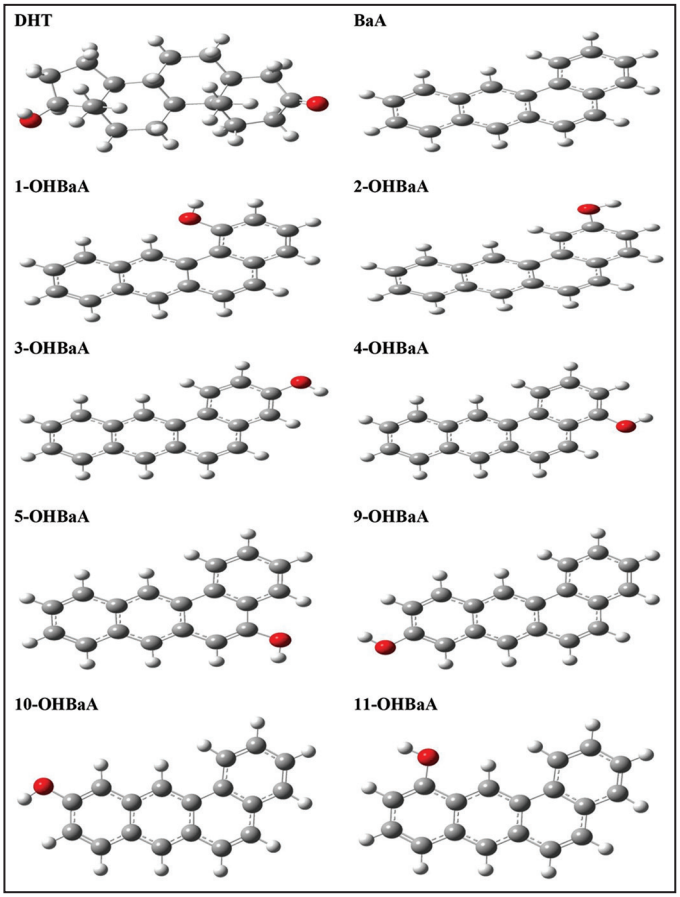

Figure 1: Three-dimensional representation of investigated pollutants and known inhibitor, dihydrotestosterone

2.2. Molecular Docking Procedure

Computational docking studies were performed in order to assess the interaction of BaA and hydroxyl derivatives of BaA with hSHBG. AutoDock 4.2.6 [15] was used to perform the docking of compounds with hSHBG. AutoDock utilizes a semi-empirical free energy force field to calculate the binding free energy of a small molecule to a macromolecule. The 3-D structure of hSHBG (protein data bank [PDB] ID: 1D2S) was retrieved in PDB format from online PDB. Receptor molecule was prepared by removing heteroatoms, also by adding explicit hydrogen molecules and associated Kollman charges (16.0) by utilizing the AutoDock Tools and saved in.pdbqt file format. As a positive control, the known inhibitor of hSHBG, DHT) was docked and compared with binding affinity scores of BaA and its metabolites. The ligands were prepared by adding hydrogen atoms and Gasteiger charges and then saved in.pdbqt format. Ligand flexibility was used to specify the torsional degrees of freedom in ligand molecule. For docking purpose, Lamarckian genetic algorithm and grid-supported energy evaluation method were adopted. The pose with the maximum binding affinity score and the corresponding interactions was selected and further visually inspected and analyzed in Ligplot.

3. RESULTS AND DISCUSSION

3.1. Molecular Orbital Properties

Chemical hardness (η) and softness (S) of a molecule can determine from the HOMO - LUMO gap [16]. Large HOMO-LUMO gap related to high kinetic stability and low chemical reactivity and small HOMO-LUMO gap is important for low chemical stability because the addition of electrons to a high-lying LUMO and/or removal of electrons from a low-lying HOMO is energetically favorable in any potential reaction [17]. In this study, DHT has the HOMO-LUMO gap 5.97 eV whereas BaA, along with all the monohydroxy derivatives showed the lower energy gap as compared to DHT (Table 1). In this study, 10-OHBaA among all the pollutants showed the lowest HOMO-LUMO gap 3.71 eV.

3.2. Molecular Docking

Molecular docking is an extensively used computational approach to validate the binding of the suitable orientation of small molecule with the receptor protein. Results of this study revealed that BaA and its metabolites have a binding energy in the range of −8.5–−9.04 kcal/mol which is comparable to DHT (−10.94 kcal/mol) as shown in Table 2. These toxicants occupied the active site cavity comprising of key

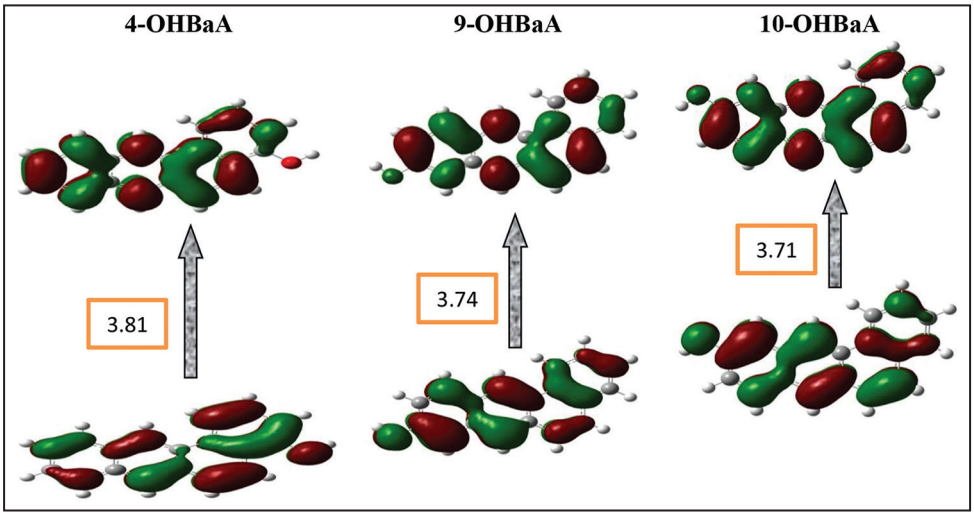

Figure 2: The calculated ground state isodensity surface plots for Frontier molecular orbitals for investigated pollutants

Table 1: Calculated electronegativity (χ), global hardness (η), softness (δ), global electrophilicity index (ω), the ionization potential (I) and the electron affinity (A) (in eV) of investigated pollutants.

Compound	Homo	Lumo	δe	η	χ	δ	ω	i	a
DHT	−6.14	−0.17	5.97	2.98	3.15	0.33	1.66	6.14	0.17
BaA	−5.45	−1.58	3.87	1.93	3.51	0.51	3.19	5.45	1.58
1-OHBaA	−5.30	−1.43	3.87	1.93	3.36	0.51	2.92	5.30	1.43
2-OHBaA	−5.27	−1.48	3.79	1.89	3.37	0.52	3.00	5.27	1.48
3-OHBaA	−5.35	−1.53	3.82	1.91	3.44	0.52	3.09	5.35	1.53
4-OHBaA	−5.32	−1.51	3.81	1.90	3.41	0.52	3.06	5.32	1.51
5-OHBaA	−5.26	−1.46	3.80	1.90	3.36	0.52	2.97	5.26	1.46
9-OHBaA	−5.28	−1.54	3.74	1.87	3.41	0.53	3.10	5.28	1.54
10-OHBaA	−5.23	−1.52	3.71	1.85	3.37	0.54	3.06	5.23	1.52
11-OHBaA	−5.23	−1.41	3.82	1.91	3.32	0.52	2.88	5.23	1.41

DHT: Dihydrotestosterone, 1-OHBaA: 1-HydroxyBenzo (a) anthracene, 2-OHBaA: 2-HydroxyBenzo (a) anthracene, 3-OHBaA: 3-HydroxyBenzo (a) anthracene, 4-OHBaA: 4-HydroxyBenzo (a) anthracene, 5-OHBaA: 5-HydroxyBenzo (a) anthracene, 9-OHBaA: 9-HydroxyBenzo (a) anthracene, 10-OHBaA: 10-HydroxyBenzo (a) anthracene, 11-OHBaA: 11-HydroxyBenzo (a) anthracene, BaA: Benzo (a) anthracene

Table 2: Details of molecular docking results: the summary of binding affinities (kcal/mol) and the H-bond or hydrogen bond as well as hydrophobic interactions of the BaA-hSHBG complexes

Compound	Binding Energy (kcal/mol)	Type of Interactions		Number of Bonds	
		H-Bond Residues	Hydrophobic Bond Residues	H-Bonds	Hydrophobic Bonds
DHT	−10.94	Asn82 (2.85 Å), Asp65 (2.81 Å)	Trp66, Phe67, Val105, Ser42, Thr40, Leu171, Phe56, Gly58, Ile141, Asp59	2	10
BaA	−8.8	-	Trp66, Phe67, Val105, Ser41, Thr40, Leu171, Gly58, Asp59, Asn82, Asp65, Met139, Met107, Lys106, Leu80	0	14
1-OHBaA	−8.56	-	Trp66, Phe67, Val105, Ser41, Thr40, Leu171, Gly58, Asp59, Asn82, Asp65, Met139, Met107, Lys106, Leu80, Val112	0	15
2-OHBaA	−8.54	-	Trp66, Phe67, Val105, Ser42, Gly58, Asn82, Asp65, Met139, Lys106, Ile141	0	10
3-OHBaA	−8.78	Asn82 (2.98 Å)	Trp66, Phe67, Val105, Ser42, Gly58, Met139, Lys106, Leu80, His81, Met107, Thr40, Ser41, Leu171, Asp59	1	14
4-OHBaA	−9.0	Asn82 (3.27 Å), Asp65 (2.85 Å)	Trp66, Phe67, Val105, Met139, Lys106, Met107, Thr40, Leu171, Asp59, Val112, Thr60	2	11
5-OHBaA	−8.58	-	Trp66, Phe67, Val105, Met139, Leu171, Asp59, Gly58, Asp65, Ser42, Asn82, Leu80	0	11
9-OHBaA	−8.81	Asn82 (3.32 Å)	Trp66, Phe67, Val105, Lys106, Thr40, Leu171, Phe56, Ile141, Ser41, Ser42, Asp65	1	11
10-OHBaA	−9.04	Asn82 (3.08 Å), Asp65 (2.92 Å)	Trp66, Phe67, Val105, Lys106, Thr40, Leu171, Ile141, Asp59, Gly58, Met139, Met107	2	11
11-OHBaA	−8.5	-	Trp66, Phe67, Val105, Leu171, Ile141, Gly58, Met139, Met107, Asn82, AWsp65, Ser41, Ser42, Thr40	0	13

DHT: Dihydrotestosterone, 1-OHBaA: 1-HydroxyBenzo (a) anthracene, 2-OHBaA: 2-HydroxyBenzo (a) anthracene, 3-OHBaA: 3-HydroxyBenzo (a) anthracene, 4-OHBaA: 4-HydroxyBenzo (a) anthracene, 5-OHBaA: 5-HydroxyBenzo (a) anthracene, 9-OHBaA: 9-HydroxyBenzo (a) anthracene, 10-OHBaA: 10-HydroxyBenzo (a) anthracene, 11-OHBaA: 11-HydroxyBenzo (a) anthracene, BaA: Benzo (a) anthracene

residues such as Asn82, Trp66, Phe67, and Val105, in the same manner as that of DHT as shown in Figure 3. Except ordinary hydrogen bonding, nonbonding interactions are frequently used term to determine the shape and behavior of molecules.

DHT, the known inhibitor of SHBG, showed the lowest binding affinity, i.e, −10.94 kcal/mol and interacted hydrophobically with residues Thr 40, Phe 56, Leu 171, Ser 42, Gly 58, Val 105, Ile 141, Phe 67, Trp 66 and Asp 59. The polar interactions were found to be with

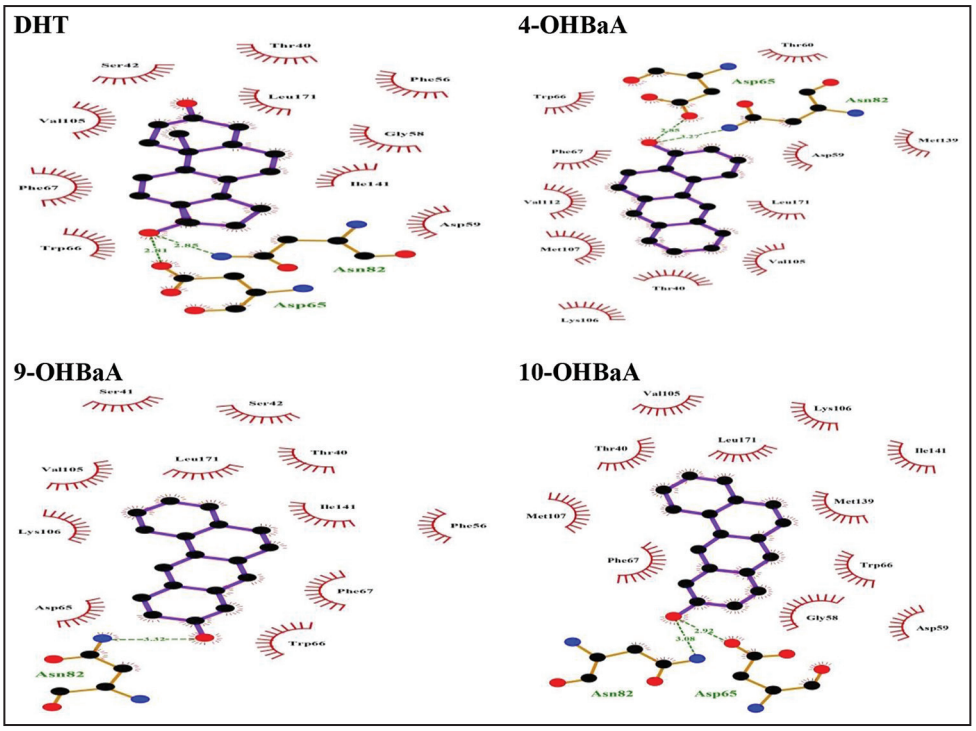

Figure 3: The binding interaction of selected HydroxyBenzo(a)anthracene that showed lower binding affinity compared to Benzo(a) anthracene with human sex hormone-binding globulin (hSHBG). The interacting residues of hSHBG are represented in red semi-circle form and the green dotted line shows the hydrogen bond interactions.

Asn 82 (2.85Å) and Asp 65 (2.81Å), shown in Figure 3. The presence of hydrogen bonds along with hydrophobic interactions between the DHT and the critical amino acids residues of the hSHBG contribute to the lowest binding affinity. These results suggest that all the studied pollutants can efficiently bind in the active site of hSHBG. Moreover, 10-OHBaA along with 4-OHBaA and 9-OHBaA seems to be the more potent inhibitor than BaA for hSHBG. Hence, these monohydroxy derivatives of BaA can potentially mimic the natural hSHBG ligand, DHT for the availability of binding sites resulting in altered androgen-estrogen homeostasis.

4. CONCLUSION

BaA is the ubiquitous environmental compound which have endocrine disruption and teratogenic properties. These compounds have been investigated as inhibitors for hSHBG by DFT and molecular docking. SHBG is a high molecular weight plasma protein that binds androgens and estrogens and plays a key role in maintaining the balance between unbound and bound sex steroids. This computational study shows that the BaA-hSHBG complexes have binding affinities similar to known inhibitor-protein complex, i.e., DHT-hSHBG. In this study, 10-OHBaA amongst all the pollutants showed the lowest HOMO-LUMO gap 3.71 eV, also showed the lowest binding affinity −9.04 kcal/mol. Hence, BaA and their metabolites can efficiently bind to hSHBG and inhibit its activity. It could be concluded that these parameters share together with different magnitudes and affect the degree of the binding affinity of these toxicants with the active protein sites to afford a certain degree of inhibition.

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***Bibliographical Sketch**

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