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Thermochemical, molecular docking and ADMET studies of aspirin metabolites

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Abstract

Aspirin (Asp) is a member of nonsteroidal anti-inflammation drug and widely used as an analgesic, antipyretic, and anti-inflammation agent. In this investigation, the inherent stability, chemical reactivity, and biological properties of Aspirin and its metabolites have been studied. Density functional theory (DFT) with B3LYP/3-21g has been employed to optimize the structures. Frontier molecular orbital features (HOMO-LUMO gap, hardness, and softness), dipole moment, electrostatic potential and thermodynamic properties (electronic energy, enthalpy, Gibb's free energy) of these metabolites have been investigated. Molecular docking has been performed against prostaglandin H2 (PGH2) synthase protein 5F19 to search the binding affinity and mode(s) of all compounds. It is found that, all compounds are thermodynamically stable; most of them are chemically more reactive and show better binding affinity than the parent drug. ADMET calculations predict the improved pharmacokinetic properties of all metabolites.

Introduction

Aspirin (Asp) is popularly used as analgesic, anti-pyretic, anti-inflammatory, and anti-platelet agent [1-4]. Recently, Asp and its modified derivatives are using in the treatment of cancer [5-7] stroke [8], and cardiovascular diseases [9,10]. It inhibit the prostaglandin synthesis by blocking cyclooxygenase [11,12]. It has some common side effects including asthma, kidney and stomach diseases [13]. Previously, some of the major metabolites of Asp are detected and reported by many researchers (Figure 1) [14-16]. Till now, the metabolites formation and their biological action is not completely understood. Attempts have taken to optimize the reported metabolites to understand their biochemical behavior on the basis of quantum mechanical methods. The free energy, enthalpy, dipole moment, HOMO-LUMO gap, and molecular electrostatic potential have been calculated to compare their thermal and chemical behavior. Molecular docking has been performed against human prostaglandin synthase protein 5F19 to predict their binding affinity and modes [17,18]. Pharmacokinetic prediction has been performed to compare their absorption, metabolism and toxicity. The main objective of our investigation was to understand the thermodynamic, molecular orbital, binding affinity, and ADMET properties.

Computational methods

Geometry optimization

In computer aided drug design, quantum mechanical methods are widely used to predict thermal, molecular orbital, and molecular electrostatic potential properties [19]. Initial geometry of Aspirin and its metabolites were taken from the online structure database named ChemSpider [20]. Geometry optimization and further modification of all structures carried out using Gaussian 09 program [21]. Density functional theory (DFT) with Becke's (B) [22] three-parameter hybrid model, Lee, Yang and Parr's (LYP) correlation functional [23] under 3-21g basis set has been employed to optimize and elucidate their

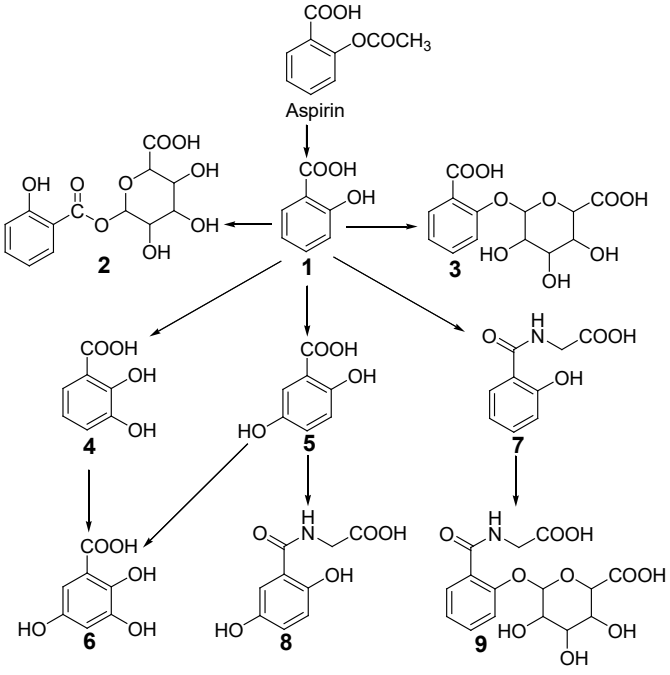

Figure 1. Chemical structures of Aspirin and its major metabolites

1=Salicylic acid, **2**= Salicylacyl glucuronide, **3**= Salicylphenol glucuronide, **4**= 2,3-Dihydroxybenzoic acid, **5**= Gentisic acid, **6**= 2,3,5-Trihydroxybenzoic acid, **7**= Salicyluric acid, **8**= Gentisuric acid, **9**= Salicyluric phenolic glucuronide

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thermal and molecular orbital properties [24]. Initial optimization of all compounds was performed in the gas phase. Dipole moment, enthalpy, free energy, and electrostatic potential were calculated for all the compounds.

Frontier molecular orbital features HOMO (highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital) were calculated at the same level of theory. For each of the metabolite, HOMO-LUMO energy gap, hardness (η), and softness (S) were calculated from the energies of frontier HOMO and LUMO as reported considering Parr and Pearson interpretation [25,26] of DFT and Koopmans theorem [27] on the correlation of ionization potential (I) and electron affinities (E) with HOMO and LUMO energy (ε). The following equations are used to calculate hardness (η), softness (S);

$$\eta = \frac{[\varepsilon LUMO - \varepsilon HOMO]}{2}; S = \frac{1}{\eta}$$

Molecular docking and ADMET prediction

Molecular docking simulation was performed to understand the mechanism of the prostaglandin H2 (PGH2) inhibition of Asp and its metabolites and their binding affinity and mode(s) with target protein [28]. The 3D structure of aspirin acetylated human cyclooxygenase-2 (PDB ID: 5F19) was obtained in pdb format from online protein data bank (PDB) database [29]. All hetero atoms and water molecules were eliminated using PyMol (version 1.3) software packages [30]. Energy minimization of the protein implemented by Swiss-Pdb viewer software (version 4.1.0) [31]. Then optimized drugs were subjected for molecular docking study against human prostaglandin synthase protein (5F19). Finally, molecular docking simulation was performed by PyRx software (version 0.8) [32] considering the protein as macromolecule and the drug as ligand. In this analysis, rigid docking was performed where, all rotatable bonds were converted into non-rotatable with the center grid box size 20.8612, 37.5501 and 59.3402 Å along x, y and z directions respectively.

AdmetSAR online database was utilized to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of all metabolites [33].

Result and discussion

Thermodynamic analysis

Simple modifications of molecular structure significantly influence the structural properties including thermal and molecular orbital parameters. Spontaneity of a reaction and stability of a product can be predicted from the free energy, and enthalpy values [34]. Highly negative values are more favorable for thermal stability. In drug design, hydrogen bond formation and non-bonded interactions also influenced by dipole moment. Increased dipole moment can improve the binding property [35]. The free energy of Asp is -644.986 Hartree, where the free energies of Salicylacyl glucuronide (2), Salicylphenol glucuronide (3) are almost same (-1174.083 and -1174.103 Hartree respectively), but slightly improved thermal and dipole moment are observed in 3 due to the presence of strong carboxylic (-COOH) group. The highest free energy (-1380.911 Hartree) and dipole moment (8.172 Debye) is observed in Salicyluric phenolic glucuronide (9) due to the presence of a bulky group which suggesting the possible improved binding affinity (Table 1).

Molecular orbital analysis

The HOMO-LUMO energies, hardness, softness of all metabolites are presented in Table 2. The electronic absorption relates to the transition from the ground to the first excited state and mainly described by one electron excitation from HOMO to LUMO [36]. The chemical hardness, softness, and chemical potential values depend on the energy of HOMO-LUMO [37,38]. Kinetic stability increases with the increase of HOMO-LUMO gap. As a result, removal of electrons from ground state HOMO to excited state LUMO requires more energy. In this study, the HOMO-LUMO gap of Asp (5.435eV) is greater than its metabolites. The lowest gap (4.263 eV) with highest softness (0.470 eV) is found in Gentisuric acid (Figure 2).

Molecular electrostatic potential

Molecular electrostatic potential (MEP) was calculated to forecast the reactive sites for possible electrophilic and nucleophilic attack of all metabolites [39]. Red colour represent maximum negative area which favourable site for electrophilic attack, blue colour indicate the maximum positive area which favourable site for nucleophilic attack and green colour represent zero potential area. MEP displays molecular size, shape as well as positive, negative and neutral electrostatic potential regions simultaneously in terms of colour grading. From MEP map (Figure 3), region having the negative potential are over electronegative atom (oxygen atoms) and having positive potential are over hydrogen atoms. Here, the potentiality of Asp is -0.211 a.u. and +0.214 a.u. where 9 has the highest potentiality (-0.260 a.u. and +0.215 a.u. respectively), which support the highest possible electrophilic and nucleophilic attack.

Molecular docking analysis

Binding affinities of metabolites and protein are summarized in Table 3. Greater negative values of binding value indicate stronger

Table 1. Molecular formula (MF), molecular weight (MW), enthalpy, free energy in Hartree and dipole moment (Debye) of Aspirin and its metabolites

	MF	MW	Enthalpy	Free energy	Dipole moment
Asp	$C_9H_8O_4$	180.157	-644.936	-644.986	4.344
1	$C_7H_6O_3$	138.121	-493.187	-493.228	0.666
2	$C_{13}H_{14}O_9$	314.245	-1174.015	-1174.083	2.856
3	$C_{13}H_{14}O_9$	314.245	-1174.035	-1174.103	5.178
4	$C_7H_6O_4$	154.120	-567.964	-568.008	4.325
5	$C_7H_6O_4$	154.120	-567.957	-568.002	3.900
6	$C_7H_6O_5$	170.120	-642.753	-642.801	5.743
7	$C_9H_9NO_4$	195.172	-699.981	-700.033	2.435
8	$C_9H_9NO_5$	211.171	-774.784	-774.840	5.629
9	$C_{15}H_{17}NO_{10}$	371.296	-1380.833	-1380.911	8.172

Table 2. Energy (eV) of HOMO, LUMO, energy gap, hardness and softness of Asp and its metabolites

	HOMO	LUMO	Gap	Hardness	Softness
Asp	-6.999	-1.564	5.435	2.717	0.368
1	-6.179	-1.352	4.827	2.414	0.414
2	-6.055	-1.370	4.685	2.344	0.427
3	-6.279	-0.965	5.314	2.657	0.376
4	-5.898	-0.979	4.919	2.460	0.407
5	-5.692	-1.122	4.570	2.285	0.437
6	-5.430	-0.981	4.449	2.225	0.450
7	-6.354	-0.937	5.417	2.708	0.370
8	-5.355	-1.092	4.263	2.132	0.470
9	-6.032	-1.095	4.937	2.468	0.405

binding between drugs and the receptor protein. Strong hydrogen bonding is the most significant contributing factor in increasing binding affinity of drugs with the receptor. Binding affinity and speciality change with the substitution/addition of different functional group. Here, the binding affinity of Asp is 7.0 kcal/mol, improved binding affinities are found for all metabolites except Salicylic acid (1). The binding affinity significantly improved due to the addition of glucuronide (2,3, and 9) and Salicyluric phenolic glucuronide shows the highest binding affinity (-9.5 kcal/mol) (Figure 4).

ADMET prediction

ADMET calculation has performed to compare the absorption, metabolism, and toxicity of all metabolites. According to AdmetSAR data (Table 3), Aspirin shows II category acute oral toxicity and rest of the metabolites show III category acute oral toxicity, which suggesting less toxicity of them than the parent drug. All the metabolites are non-carcinogenic, show positive response for blood brain barrier (BBB) and human intestinal absorption criteria. All drugs are P-glycoprotein

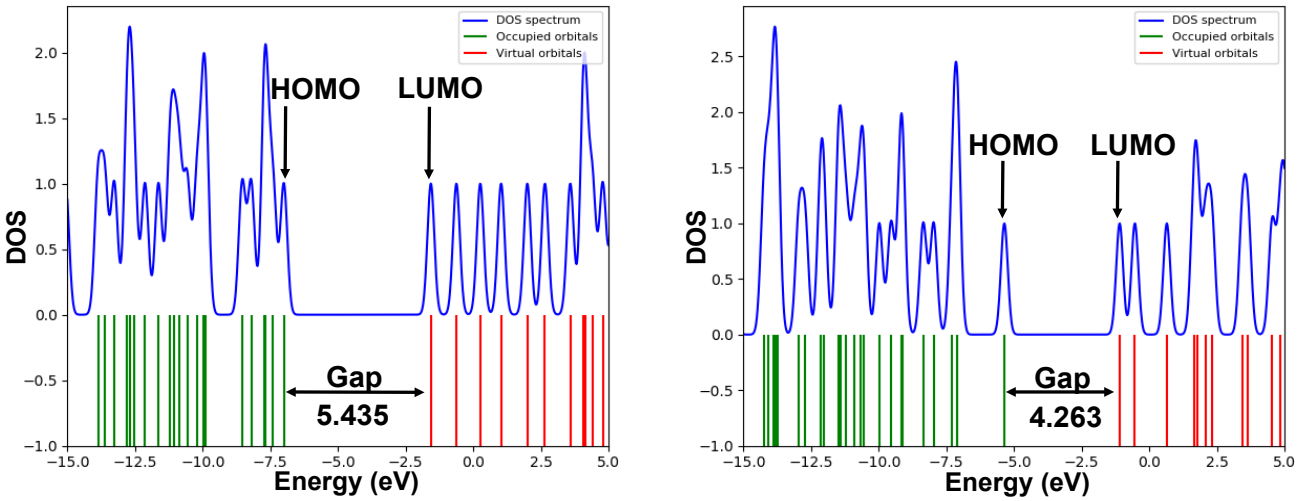

Figure 2. DOS plot and HOMO-LUMO energy gap of Aspirin and Gentisuric acid

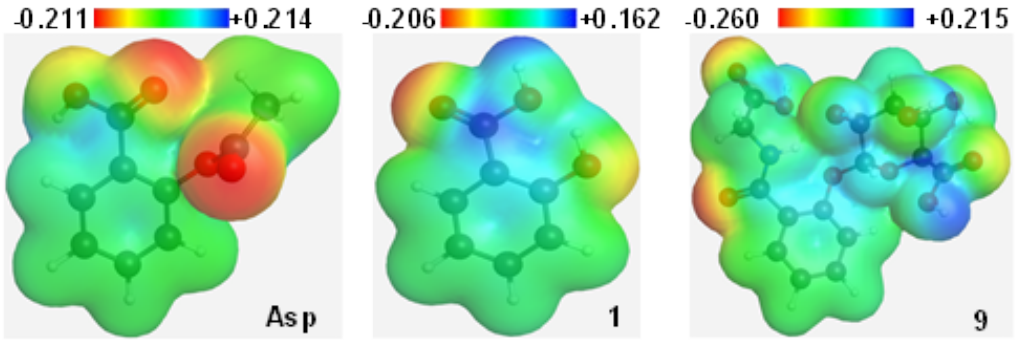

Figure 3. Molecular electrostatic potential map of Asp, **1** and **9**

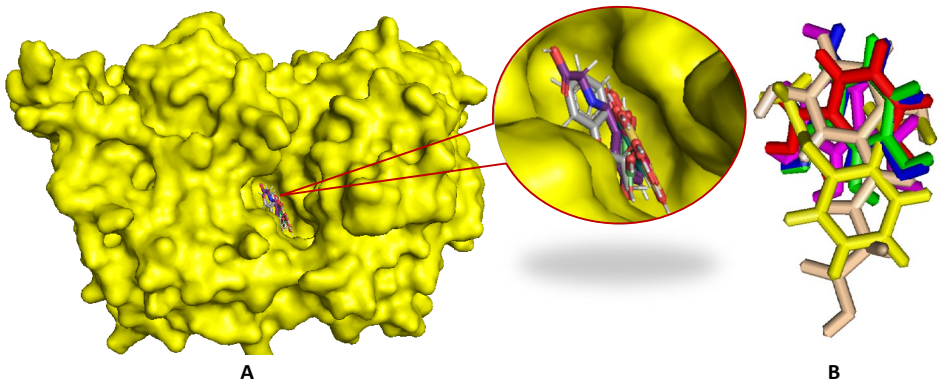

Figure 4. (A) Docked conformation of Asp, **1, 4, 5, 6**, and **8** at inhibition bounding site of 5F19; (B). Superimposed view of them after rigid docking

Table 3. Binding affinity and selected pharmacokinetic parameters of Asp and its metabolites

Drug	Binding affinity (kcal/mol)	BBB	Human intestinal absorption	P-glycoprotein inhibitor	hERG	Carcinogen	Acute oral toxicity
Asp	-7.0	+(0.945)	+(0.974)	NI(0.958)	WI(0.944)	NC(0.652)	II
1	-6.6	+(0.616)	+(0.976)	NI(0.994)	WI(0.963)	NC(0.863)	III
2	-8.8	+(0.691)	+(0.720)	NI(0.848)	WI(0.974)	NC(0.945)	III
3	-9.3	+(0.691)	+(0.720)	NI(0.848)	WI(0.974)	NC(0.945)	III
4	-7.2	+(0.623)	+(0.805)	NI(0.994)	WI(0.982)	NC(0.918)	III
5	-7.1	+(0.666)	+(0.922)	NI(0.994)	WI(0.965)	NC(0.882)	III
6	-7.6	+(0.622)	+(0.805)	NI(0.994)	WI(0.982)	NC(0.918)	III
7	-7.9	+(0.576)	+(0.723)	NI(0.964)	WI(0.971)	NC(0.928)	III
8	-8.7	+(0.576)	+(0.723)	NI(0.964)	WI(0.971)	NC(0.928)	III
9	-9.5	+(0.781)	+(0.716)	NI(0.614)	WI(0.983)	NC(0.945)	III

NI=Non-inhibitor, *WI*= Weak –inhibitor, *NC*= Non-carcinogenic

non-inhibitor where, P-glycoprotein inhibition can interrupt the absorption, permeability and retention of the drugs [40]. However, all the compounds show weak inhibitory feature for human ether-a-go-go-related gene (hERG) which can lead to long QT syndrome [41], so further more study of this aspect is necessary.

Conclusion

In this investigation, the inherent stability and biochemical interactions of Asp and its metabolites are studied. All the metabolites have lower HOMO-LUMO gap and have improved pharmacokinetic properties. All the metabolites (except 1) have better binding affinity with the receptor protein and most of them (2,3,7,8, and 9) are thermally more stable than the parent drug. Finally, this study may be helpful to understand the thermal, chemical, pharmacological and binding properties of Asp and its metabolites.

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Conflicts of interest

Authors declare no conflicts of interest.

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