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Density functional theory and molecular docking of salicylic acid and its derivatives reveal their potential as amyloid β fibril inhibitors in the treatment of Alzheimer's disease

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Abstract: Studies have shown that aggregation of amyloid β (Aβ) protein contributes towards the growth of Alzheimer's disease. Besides treating diseases like arthritis, pain, fever etc. salicylic acid based compounds like aspirin and diflunisal have been found to be potent Aβ aggregation inhibitors. Herein, we report the potential of salicylic acid (**1**) and three of its derivatives: salsalate (**2**), fosfosal (**3**) and fendosal (**4**) towards inhibition of Aβ fibrils by using density functional theory (DFT) and molecular docking studies. DFT, studying structural and electronic properties of all the mentioned compounds was carried out using the Becke three-parameter Lee-Yang-Parr function (B3LYP) with 6-311G (d,p) level of theory. Molecular electrostatic potential (MEP) map and Frontier orbital analysis (FOA) was carried out. HOMO-LUMO energy gap was calculated that permitted the calculation of global reactivity descriptors like chemical hardness, chemical inertness, chemical potential, nucleophilicity and electrophilicity which is required to get insights in to the reactive centre4s of these compounds. To know the binding interactions and binding affinities of the target compounds with Aβ fibrils, molecular docking using Autodock 4.2 was carried out. It was observed that fosfosal (**3**) has the highest binding tendency with ΔG_b = -2.45 kcal/mol and inhibition constant, K_i of 17.22 μM. However salicylic acid (**1**) was predicted to be least potent, as it displayed no bonding interactions with Aβ fibrils. This study led us to conclude that fosfosal (**3**) has the potential to act as Aβ fibril inhibitor, an important step in the treatment of Alzhemeir's disease.

Key words: Alzheimer's disease; Fendosal; Fosfosal; Salicylic acid; Salsalate

Introduction

Alzheimer's disease (AD), a neurodegerative disorder, commonly leads to deterioration of memory, cognition and behavior (Möller & Graeber 1998). Globally 24 million people suffer from dementia, and major part of such population is said to have Alzheimer's disease (AD). This dementia is said to increase more because of less prevalent treatment approaches (Kumar *et al*. 2016). It is said that aggregation of amyloid beta (Aβ) fibrils inside brain is the most common pathological event in Alzheimer's disease which in turn leads to neuronal dysfunction and death (Awasthi *et al*. 2016) because aggregation of amyloid fibrils leads to formation of plaques, which are highly neurotoxic (Sandberg *et al*. 2010). Currently there is no cure for Alzheimer's disease. Instead, there are few treatment strategies, which include use of acetylcholinesterase inhibitors (galanthamine, donepezil etc) and memantine, which is a non-competitive inhibitor of NMDA receptors (Allian et al. 2003). Owing to the prevalence of less number of effective treatment approaches, scientists all over the globe are routinely scrutinizing newer treatment methods (Gurung *et al.* 2017). One such treatment option describes the use of non-steroidal anti-inflammatory drugs (NSAIDS) in the treatment of Alzheimer's disease, besides being used for the treatment of pain, fever and

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arthritis (Umar *et al*. 2016). A recent study shows that salicylates like aspirin and diflunisal or compounds belonging to aryl/heteroaryl acetic acids have the potential to inhibit Aβ fibrils (Azam *et al.* 2017). In view of the aforementioned facts, we envisaged to explore salicylic acid (**1**) and its three derivatives salsalate (**2**), fosfosal **(3)** and fendosal (**4**) for structural, electronic and molecular properties, besides studying them as predicted Aβ fibril inhibitors in the treatment of Alzheimer's disease.

Materials and Methods

All the computations were carried out using GAUSSIAN 09 software program suite (Frisch *et al.* 2010). The DFT modelling method, using the hybrid B3LYP (Becke, 1993) functional was used to calculate theoretical parameters for target compounds with the basis set combination 6-311G (d,p) (McLean and Chandler, 1980). Geometry optimization was carried out until global minima were achieved. The crystal structure of amyloid fibril was obtained from Protien Data Bank web site (http://www.rrscb.org/pdb) bearing PDB ID: 2BEG in its gz format (Luhrs *et al*. 2005). The same file was saved and opened using Discovery studio visualizer R2 client program. Water molecules were removed and the file saved as Target.pdb. The fibril is pentameric and has been described as proto-fibril by (Fan *et al.* 2016). The structures of salicylic acid (**1**) and its derivatives: salsalate (**2**), fosfosal (**3**) and fendosal (**4**) (**Fig 1**) were drawn in ChemDraw Ultra 12.02. and saved as mol files. Mol file of each molecule was subjected to optimization using Gaussian 09 package. Autodock 4.2 was used to carry out docking studies. During docking simulation a blind docking approach was used in which maximum space around the protein was covered so that maximum binding interactions can be viewed. (Kumar *et al*. 2016). The best docked conformers as revealed by free energy of binding and inhibition constant were studied to know the actual amino acid residues of Aβ fibrils involved in interactions between docked molecules and Aβ fibrils. The docking results were analyzed and processed using Pymol. 1.7.5.

Results and discussion

Molecular electrostatic potential (MEP) which reveals size, shape and variation of electron density based on dipole moment, partial charges, electronegativity and chemical reactivity sites present in the molecule (Abhishek *et al*. 2015) was carried out for all the target compounds. **Fig 2** shows MEP for compounds **1, 2, 3, 4** etc calculated using DFT. The pictorial representation with rainbow color scheme of electrostatic potential for compound **1** lies in the range of -6.512e-2 to 6.512e-2. for compound **2** between -7.257e-2 to +7.257e-2, for compound **3** between -6.796e-2 to +6.796e-2. and for compound **4** between -7.096e-2 to 7.096e-2. As can be seen in the MEP of all the compounds carbonyl oxygen atoms have highest electron density while as the H-atom of COOH and OH groups along with the phosphorus atom of fosfosal **(3)** are highly electron deficient centers. To get insights about the reactivity/stability of compounds, frontier molecular orbital (FMO) analysis was carried out (**Fig 3**). HOMO-LUMO energy gap was calculated for compounds **1**, **2**, **3** and **4** respectively **(Table 1**). Compound **4** has the lowest HOMO-LUMO energy gap while as compound **1** has the highest HOMO-LUMO energy gap. Using HOMO-LUMO energy gap the important chemical descriptors like softness, hardness, electronegativity, chemical potential, electron affinity and ionization

energy were evaluated. Chemical hardness and softness is basically the measurement of chemical reactivity to which the addition of charge stabilizes the system (Costa *et al.* 2017) and chemical potential μ gives an idea about the transfer of charge from higher potential to lower potential. Another important descriptor, electronegativity (χ) represents the tendency to attract electrons. These properties have been defined by Par *et al.* 1999 as: $\eta = \frac{(I-A)}{2}$, $\mu = \frac{-(I+A)}{2}$, $\chi = \frac{(I+A)}{2}$, where I and A represent ionization potential and electron affinity of the compound, which are actually obtained from HOMO and LUMO energies as I = $-E_{HOMO}$ and A = $-E_{LUMO}$ as per Janak theorem (Janak, 1978) and Perdew *et al.* (1982). A large HOMO-LUMO gap represents a hard molecule while as a small gap indicates a soft or more reactive/less stable molecule. As can be seen the HOMO-LUMO energy gaps in compounds **1, 2, 3, 4** are 0.25714, 0.25485, 0.147905 and 0.00046 ev respectively which indicate the order of reactivity among the target compounds is **4** > **3** > **2** > **1** and stability as **1**> **2** > **3** > **4**. The global electrophilicity index (ω), a global reactivity index that is related to chemical hardness and chemical potential is given by $\omega = \mu^2/2\eta$ was found to be 0.06642, 0.06424, 0.21329 and 24.7915 e.v for **1**, **2**, **3** and **4** respectively. All these parameters have been calculated for the target compounds and are depicted in **Table 1**. Having successfully optimized the structures along with their properties, we explored their potential towards binding with Aβ fibrils. Since Aβ fibril used in this study (PDB ID BEG) lacks the ligand/co-crystallized molecule, therefore we employed ovine COX-1 protien complexed with ibuprofen (PDB ID 1EQB) to validate our protocol as has been demonstrated earlier (Azam *et al.* 2017). Hence we redocked the co-crystallized ibuprofen with COX-1. The

Table 1: Calculated parameters for compounds **1**, **2**, **3** and **4**.

Parameter	Salcylic acid (1)	Salsalate (2)	Fosfosal (3)	Fendosal (4)
Energy (a.u)	-496.1885	-915.8801	-1063.71122	-1244.8733
Dipole moment (Debye)	0.7279	3.5231	2.9768	3.4309
E_{HOMO} (eV)	-0.25926	-0.25538	-0.24824	-0.10702
E_{LUMO} (eV)	-0.00212	-0.00053	-0.10335	-0.10656
$E_{HOMO-LUMO}$ (eV)	0.25714	0.25485	0.147905	0.00046
Hardness (η)	0.12857	0.127425	0.072425	0.00023
Chemical Potential (μ)	-0.13069	-0.127955	-0.175795	-0.10679
Electronegativity (χ)	0.13069	0.127955	0.175795	0.10679
Electrophilicity index (ω)	0.06642	0.06424	0.21329	24.7915

Table 2. Binding free energies (ΔG_b), inhibition constant (Ki) and H-bonding interactions of docked compounds with amino acid residues of Aβ fibrils.

Compound	(ΔG_b) kcal/mol	(Ki, μM)	H-Bond interactions with amino acids of chains A, B, C, D and E of Aβ fibril	No. of H Bonds
Salicylic acid (**1**)	+0.95	-	-	0
Salsalate (**2**)	-1.68	149	ASP23 (Chain D), GLU22 (Chain C)	2
Fosfosal (**3**)	-2.45	17	ASP23 (Chain A), ASP23 and GLU22 (Chain B) ALA21 (Chain C),	4
Fendosal (**4**)	-2.12	43	ALA21 (Chain B), ALA21 (Chain C), GLU22 (Chain A)	3

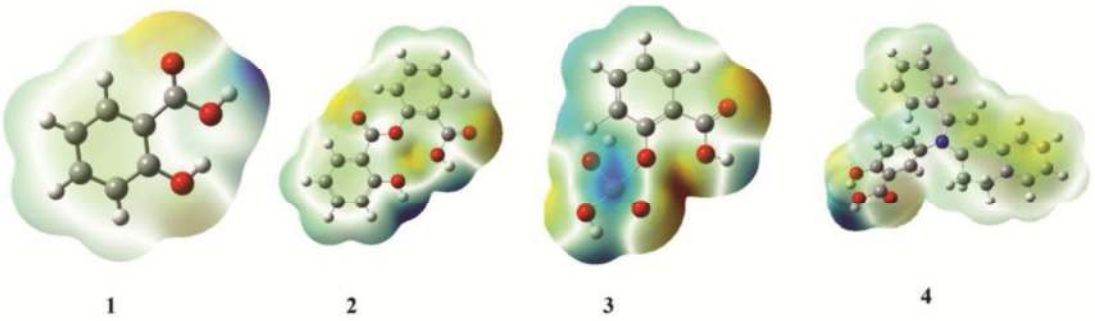

Fig 2. Molecular electrostatic potential maps of compounds **1**, **2**, **3** and **4**.

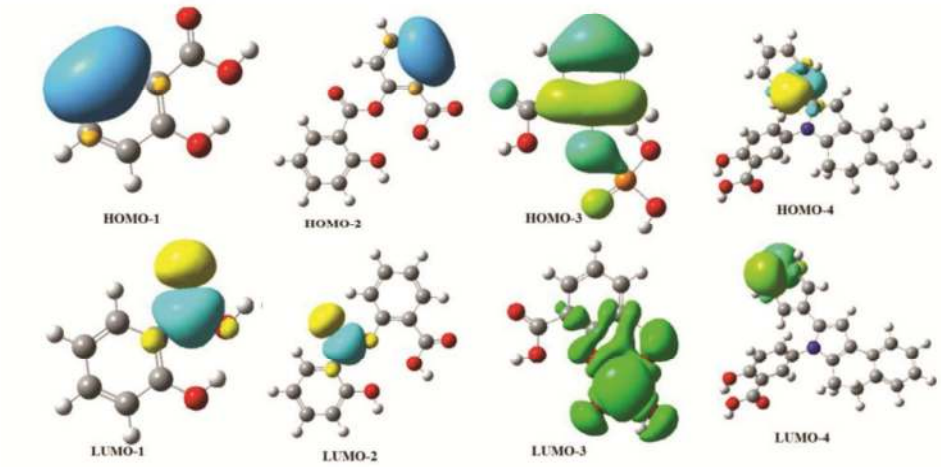

Fig 3. HOMO-LUMO orbitals of compounds **1, 2, 3** and **4**

superposition of the docked and co-crystallized

ibuprofen recorded an RMSD of 1.6 A°. All the four target compounds were therefore docked to Aβ fibrils as per this protocol (**Fig 4**). It was observed that among all the compounds, fosfosal (**3**) has the highest binding tendency with ΔG_b = -2.45 kcal/mol and inhibition constant, K_i of 17.22 μM and thus seemed to be potent (**Table 2**).

In short, a DFT study of salicylic acid (**1**) and its derivatives: salsalate (**2**), fosfosal (**3**) and fendosal (**4**) was carried out. This theoretical study helped in assessing reactivity descriptors like hardness, chemical inertness, chemical potential, nucleophilicity and electrophilicity of all the four compounds. Molecular docking led us to conclude that fosfosal (**3**) has the highest binding tendency, suggesting it's potential to act as Aβ fibril inhibitor.

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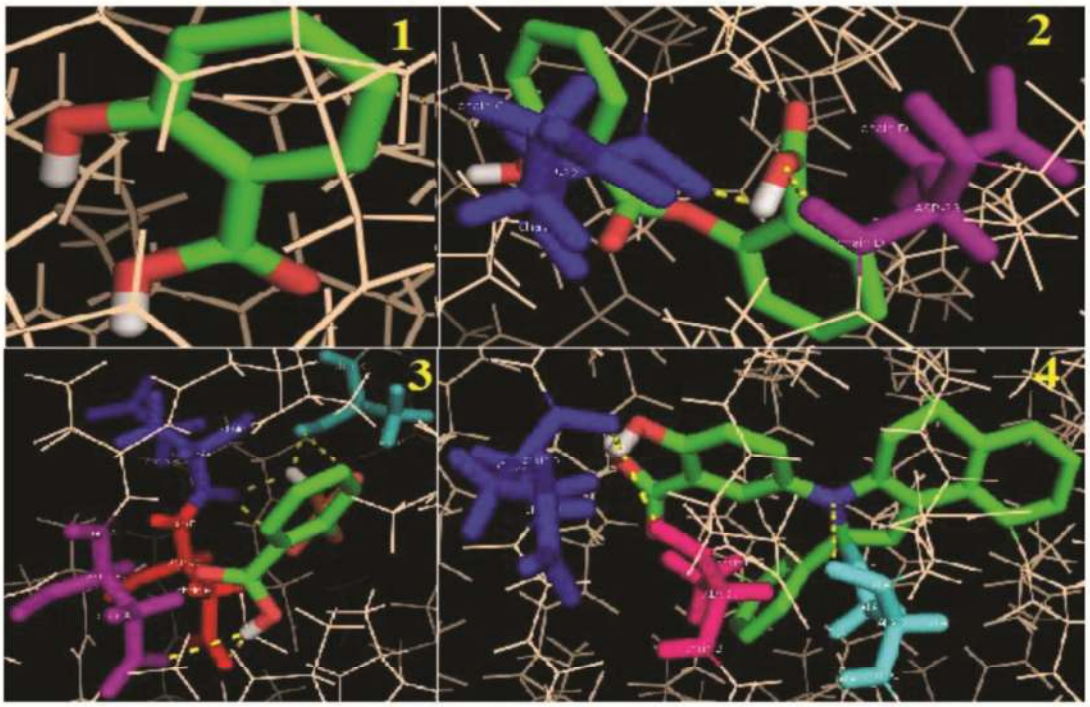

Fig. 4. Best docking conformers of all four compounds showing bonding interactions with amino acid residues **1**). Lowest energy docked conformation of salicylic acid where no interaction with any amino acid residue was seen. **2**) Salsalate and its interactions with ASP23 (chain D, purple) and GLU22 (chain C, blue) ones GLU22 of chain C. **3)** Fosfosal and its interactions with ASP23 (purple, chain A), ASP23 (red, chain A), ALA21 (cyan, chain C) and GLU22 (Blue, chain B) **4**). Fendosal and its interactions with GLU22 (chain A, Blue), ALA21 (chain B, Hot Pink), ALA21 (chain C, cyan).

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