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Research Article

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Molecular docking study of phytol and its derivatives against COX-2 induced inflammation: A combined density functional study

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ABSTRACT

This study aimed to determine the activity of PYT and its derivatives against COX-2, including 5KIR protein induced inflammation by using the computational tools. PYT and its derivatives have been designed by utilizing density functional theory (DFT) and the performance of the drugs was also evaluated by molecular docking study. Results suggest that the NH_2 derivative of PYT (D-NH_2) showed binding energy -6.4 (Kcal/mol) with protein 5KIR of COX-2 compared to the main drug (D) that showed binding energy -5.1 (Kcal/mol) with the same protein. HOMO and LUMO energy values were also calculated to determine the chemical reactivity of all the modified drugs. Non-covalent interactions of PYT and its derivatives were essential in improving the performance. In conclusion, D-NH_2 showed better preference in inhibiting to the protein 5KIR of COX-2 compared to other modified drugs and it can be claimed that D-NH_2 will be the best conformer for COX-2 induced inflammation.

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KEYWORDS: Phytol, 5KIR , COX-2, inflammation, molecular docking

INTRODUCTION

Phytol (PYT), a chlorophyll-derived diterpene essential oils found abundantly in nature (1). PYT is widely distributed in bacteria, especially cyanobacteria, plants and algae (2). PYTis evident in various important biological activities including anxiolytic (3), antinociceptive (4), antidiabetic (5), anti-inflammatory (6), anti-atherogenic, antiteratogenic (7), anticonvulsant (8), antispasmodic (9), antimutagenic (10), anti-protozoal (11), antimicrobial (12), hypolipidemic (13), and immunoadjuvant properties (14). Cyclooxygenase (COX) catalyzes the first committed step in the synthesis of prostanoids, known as prostaglandin (PG) H synthase (15). It has been demonstrated that, through the inhibition of COX enzymatic activity, non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory properties as well as inhibiting PG synthesis (16). COX remains central, as a unique enzyme, to the development of anti-inflammatory treatments of various pathologies, including neurodegenerative and neuroinflammatory diseases and COX produces two types of catalytic activities, firstly it catalyses PGG2 formation from arachidonic acid that is a bis-oxygenase activity (cyclooxygenase) and secondly COX reduces PGG2 to PGH2 which is a peroxidase activity (15). Two types of enzymatic activities include- external factors and interacting sites on the COX molecule can affect them (17) and COX undergoes a conformational rearrangement which gives rise to an inactive enzymatic species leading to an unstable intermediate during the cyclooxygenase activity (15). COX is involved in both enzymatic activities as an integral membrane glycoprotein which in the association of the heme group consisting of a homodimer (17). In many tissues, COX-1 is expressed as a constitutive enzyme, including the intestine and colon, whereas in macrophages, fibroblasts, and other cell types in inflammation COX-2 is expressed an inducible enzyme (18). In inflammatory reactions, COX-2 has emerged as a major player in peripheral tissues and COX-2 also functions in inflammatory and degenerative brain diseases (15). Some NSAIDs, including ibuprofen and mefenamic acid are the competitive inhibitors of COX-1 and -2 isoforms. The objective of this study is to determine the anti-inflammatory activity of PYT and its derivatives against COX-2, including 5KIR protein induced inflammation. The computational tools have been used, for this purpose, to investigate the best ligand and optimized receptor proteins as the best choice for the future scientists. The structure of PYT is modified with -OH, -F, -Cl, -OCH_3, -CN and -NH_2 in C-20 position.

COMPUTATIONAL METHODS

Optimization of Ligands by Quantum Mechanical Calculations

By using quantum mechanical (QM) methods, various types of complicated interactions between ligands and target proteins are interpreted and internal energy calculations were done (19), and all predictions were made by using Gaussian view 09 and Chem3D Pro12.0 program packages (20). The optimized structure of PYT (D) was modified with -OH, -F, -Cl, -OCH_3, -CN and -NH_2 groups (Figure 1). Considering Parr and Pearson interpretation with HOMO and LUMO energy (ε) (21), hardness (η) and softness (S) of all drugs were also calculated from the energies of frontier HOMOs and LUMOs. Hardness (η) and softness (S) of all the drugs were calculated according to the equation: $\eta = [\varepsilon LUMO - \varepsilon HOMO]/2$and $S=1/\eta$ (Table 1).

Protein Preparation

From the Protein Data Bank (PDB) database, the crystal structure of the protein (5KIR) of COX-2 of *Homo sapiens* was collected. Swiss-Pdb Viewer software package (version 4.1.0) has been utilized for the energy minimization of crystal structure and by using PyMOl (version 1.7.4.5) all the hetero atoms and water molecules of proteins (Figure 2) were removed before docking. For the analysis of docking results both the proteins and drug structures are taken into PDBQT format finally.

Docking Analysis and Binding Site

In computational drug design, molecular docking is an important tool that can predict the predominant binding mode(s) of a ligand with the target protein (22). By using CASTp the prediction of the active binding pocket of COX-2 was done and the docked pose of lowest binding free energy conformer with the respective protein was analyzed by PyMOL Molecular Graphics System (version 1.7.4.5).

Table 1: HOMO-LUMO, gap, hardness and softness

Molecules (Chair)	εHOMO	εLUMO	Gap	Hardness (η)	Softness (S)
Phytol (D)	-8.584	2.381	10.965	5.482	0.182
D-OH	-3.878	10.023	13.901	6.950	0.143
D-F	-4.201	10.075	14.276	7.138	0.140
D-Cl	-4.640	9.996	14.636	7.318	0.136
D-OCH_3	-6.620	9.890	16.51	8.225	0.121
D-CN	-6.460	9.504	15.964	7.982	0.125
D-NH_2	-3.236	9.991	13.227	6.613	0.151

RESULTS AND DISCUSSION

HOMO-LUMO, Gap, Hardness (η) and Softness (S) Analysis

Highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) refers frontier orbitals (FO), and the calculation of the quantity and chemical reaction in which drug molecules bind with the specific receptor have been performed by using these energy values. The structure of FO of PYT (D) and D-NH_2 were given in Figure 3. The energy gap, reveals the chemical stability and kinetic of the drug molecules, between HOMO and LUMO (20). The D-NH_2 exhibited lowest energy gap value and lowest η value and increased S value among the modified derivatives,which indicated that this drug has enhanced chemical reactivity. HOMO-LUMO, gap, η and S values were given in Table 1.

Binding Energy of the Protein-Ligands by Molecular Docking

To predict the stronger binder and virtual screen a database of compounds the docking is essential and D-NH_2 showed binding energy -6.4 (Kcal/mol) with protein 5KIR of COX-2 compared to the D (PYT) that showed binding energy -5.1 (Kcal/mol). The -OH, -F, -Cl, -OCH_3 and -CN group of PYT showed binding energy -6.3, -5.4, -5.4, -5.4 and -5.7 Kcal/mol, respectively and the D-NH_2 exhibited the lowest binding energy. The binding energy of ligand-proteins were given in Table 2.

Selected Non-Covalent Interactions Among Chair Ligands D, D-NH_2 and COX-2 (5KIR)

The binding affinity and binding specificity were increased due to the improved hydrogen bonding (23) in D-NH_2. In the D-NH_2-5KIR complex, multiple non-bonded interactions and

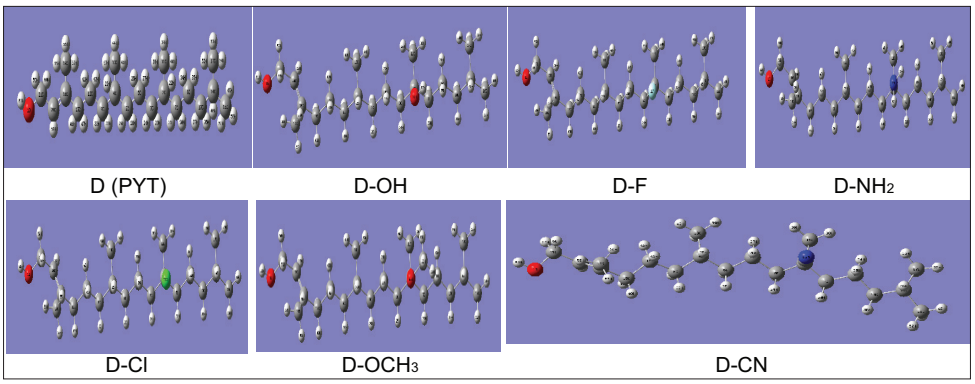

Figure 1: Modified structures of PYT (D)

docked structure were observed. A strong hydrogen bond with CYS 41 (2.67 Å) and GLN 42 (2.59 Å) were observed in D-NH_2-5KIR complex (Figure 4). For enhancing the binding affinity of D-NH_2-5KIR, the strong hydrogen bonding is considered the most significant contributing factor. Several hydrophobic bonds were observed in the D-NH_2-5KIR complex, including LYS 468 (4.52 Å), LEU 152 (5.03 Å), PRO 153 (4.69 Å), CYS 36 (4.01 Å), and TYR 130 (5.44 Å). Non-covalent interactions in D-5KIR complex were stabilized by several hydrophobic bonds, including LEU 93 (4.14 Å), VAL 116 (4.66 Å), ILE 112 (3.84 Å), VAL 89 (3.88 Å), ILE 92 (4.17 Å), and TYR 115 (4.61 Å). Selected non-covalent interactions among chair ligands D, D-NH_2 and COX-2 (5KIR) were given in Table 3.

Table 2: Free energy of binding values (Kcal/mol) for ligands –COX-2 (5KIR)

Ligands	Binding energy of ligands-proteins (5KIR) (Kcal/mol)
Phytol (D) (chair)	-5.1
D-OH (chair)	-6.3
D-F (chair)	-5.4
D-Cl (chair)	-5.4
D-OCH_3 (chair)	-5.4
D-CN (chair)	-5.7
D-NH_2 (chair)	-6.4

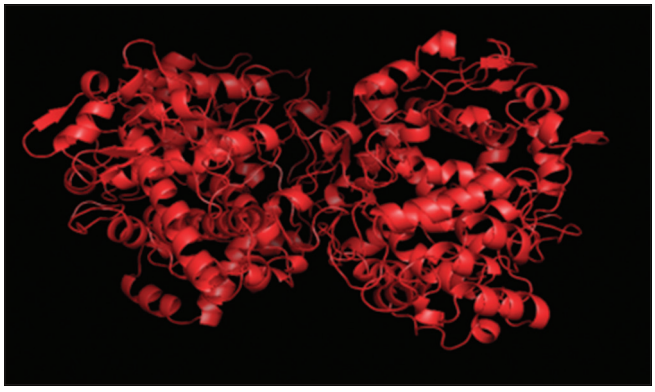

Figure 2: Crystal structure of 5KIR protein prior to docking

Pharmacokinetic Properties of PYT and Its Modified Derivatives

The modified drugs showed low acute oral toxicity, therefore, they are expected to be safe for use. The drugs will act positively, as the human intestinal absorption values of all the drugs were found positive in the bioavailability, drug metabolism and intestinal absorption (24). PYT and its modified derivatives showed weak inhibitory property for the human ether-a-go-go-related gene (hERG). AdmetSAR values of ligands were given in Table 4. Toxicity of all the compounds was predicted by PreADMET suggesting that all the compounds having a lower toxicity (Table 5).

Stoichiometry, Electronic Energy, Enthalpy, Gibb's Free Energy and Dipole Moment of PYT and Its Derivatives

The electronic energy, after modification, which indicates that the structures become more stable (Table 5). The highest Gibb's free energy is observed for D-OCH_3 and D-NH_2. On the other hand, D-Cl showed higher electronic energy and also the D-NH_2 exhibited higher electronic energy than the parent drug. The D-NH_2 showed Gibb's free energy, enthalpy, and dipole moment as 0.520518, 0.615199 1.6838, respectively; and these values would make the drug chemically more stable (Table 5).

CONCLUSION

This study showed that modified PYT drugs interact with 5KIR of COX-2 and some interesting characteristics related to free energy, dipole moment, charge distribution, and molecular orbital of the drug molecules were described by the DFT calculation. The electronic energy, enthalpy, Gibb's free energy and dipole moment of PYT and its derivatives indicate that these compounds are chemically more reactive than the main drug (PYT). The -D-NH_2 showed binding energy -6.4 (Kcal/mol) with the protein 5KIR of COX-2 compared to the main drug (D) that showed binding energy -5.1 (Kcal/mol)

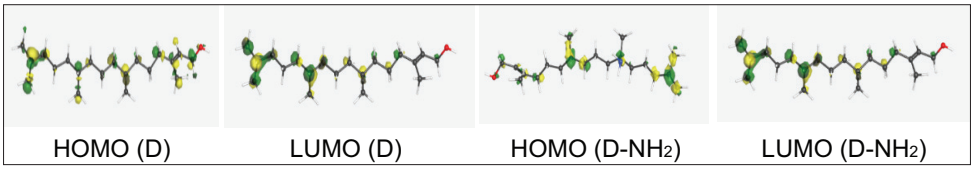

Figure 3: Frontier molecular orbitals of PYT (D) and D-NH_2

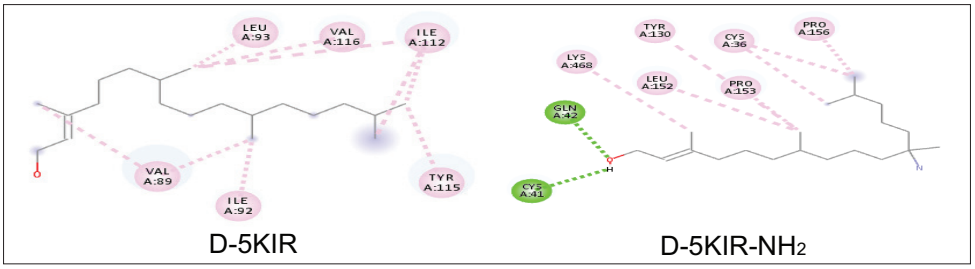

Figure 4: Binding site ligand-protein complexes

Table 3: Non-covalent interactions among chair ligands D, D-NH_2 and COX-2 (5KIR)

Systems	Contacts	Bond distances (Å)	Systems	Contacts	Bond distances (Å)
D-5KIR	Alkyl (LEU 93)	4.14	D-NH_2-5KIR	O...H (CYS 41)	2.67
	Alkyl (VAL 116)	4.66		O...H (GLN 42)	2.59
	Alkyl (ILE 112)	3.84		Alkyl (LYS 468)	4.52
	Alkyl (VAL 89)	3.88		Alkyl (LEU 152)	5.03
	Alkyl (ILE 92)	4.17		Alkyl (PRO 153)	4.69
	Pi-alkyl (TYR 115)	4.61		Alkyl (PRO 156)	3.65
	Alkyl (LEU 344)	3.41		Alkyl (CYS 36)	4.01
				Pi-alkyl (TYR 130)	5.44

Table 4: AdmetSAR values of ligands

Parameters	D	D-OH	D-F	D-Cl	D-OCH_3	D-CN	D-NH_2
Blood Brain Barrier	0.937	0.905	0.984	0.978	0.948	0.954	0.916
Human intestinal absorption	0.984	0.990	0.992	0.991	0.969	0.968	0.984
P-glycoprotein inhibitor	0.886 (NI)	0.863 (NI)	0.839 (NI)	0.888 (NI)	0.661 (NI)	0.829 (NI)	0.893(NI)
Human Ether a-go-go-Related (hERG) Gene Inhibition	0.783 (WI)	0.892 (WI)	0.945 (WI)	0.915 (WI)	0.835 (WI)	0.848 (WI)	0.956 (WI)
Acute Oral Toxicity	0.855	0.761	0.789	0.749	0.762	0.768	0.702

Table 5: Stoichiometry, electronic energy, enthalpy, Gibb's free energy in hartree and dipole moment (Debye) of PYT and its derivatives

Name	Stoichiometry	Electronic Energy	Enthalpy (Hartee)	Gibb's free energy (Hartee)	Dipole moment (Debye)
Phytol (D)	$C_{20}H_{40}O$	-910.112	0.495786	0.454336	1.2354
D-OH	$C_{20}H_{40}O_2$	-936.117	0.602311	0.507255	2.2188
D-F	$C_{20}H_{39}FO$	-960.152	0.589743	0.494326	1.4337
D-Cl	$C_{20}H_{39}ClO$	-1320.513	0.588849	0.493338	1.6486
D-OCH_3	$C_{21}H_{42}O_2$	-975.386	0.632000	0.534108	2.3188
D-CN	$C_{21}H_{39}NO$	-953.144	0.597589	0.501483	2.6719
D-NH_2	$C_{20}H_{41}NO$	-916.237	0.615199	0.520518	1.6838

and the docking results revealed that D-NH_2 shows the best performance on inhibiting human COX-2 (5KIR). The non-bonding interactions, help to develop new drug which can effectively target the COX-2. Pharmacokinetic calculation predicts that all the modified drugs are non-carcinogenic. D-NH_2 for COX-2 (5KIR) will be the best conformer for 5KIR-induced inflammation in animals.

COMPETING INTEREST

None declared.

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