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

Pranta Ray, Muhammad Torequl Islam*, Abul Bashar Ripon Khalipha, SM Hafiz Hassan, Md. Roich Khan, Razina Rouf

Department of Pharmacy, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj (Dhaka)-8100, Bangladesh

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 NH₂ derivative of PYT (D-NH₂) 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-NH₂ showed better preference in inhibiting to the protein 5KIR of COX-2 compared to other modified drugs and it can be claimed that D-NH₂ will be the best conformer for COX-2 induced inflammation.

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). PYT is evident in various important biological activities including anxiolytic (3), antinociceptive (4), antidiabetic (5), anti-atherogenic, antitumorogenic (7), anticonvulsant (8), anti-inflammatory (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 bi-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 hemodimer (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, -OCH₃, -CN and -NH₂ 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, -OCH₃, -CN and -NH₂ 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: η = (εLUMO – εHOMO)/2 and S = 1/η (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 PyMOI (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).

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-NH₂ 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-NH₂ 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-NH₂ 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, -OCH₃ 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-NH₂ 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-NH₂ and COX-2 (5KIR)

The binding affinity and binding specificity were increased due to the improved hydrogen bonding (23) in D-NH₂. In the D-NH₂-5KIR complex, multiple non-bonded interactions and
docked structure were observed. A strong hydrogen bond with CYS 41 (2.67 Å) and GLN 42 (2.59 Å) were observed in D-NH₂-5KIR complex (Figure 4). For enhancing the binding affinity of D-NH₂-5KIR, the strong hydrogen bonding is considered the most significant contributing factor. Several hydrophobic bonds were observed in the D-NH₂-5KIR 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-NH₂, and COX-2 (5KIR) were given in Table 3.

**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-OCH₃ and D-NH₂. On the other hand, D-Cl showed higher electronic energy and also the D-NH₂ exhibited higher electronic energy than the parent drug. The D-NH₂ 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-NH₂ 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).
and the docking results revealed that D-NH₂ 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-NH₂ for COX-2 (5KIR) will be the best conformer for 5KIR-induced inflammation in animals.

### COMPETING INTEREST
None declared.

### REFERENCES

1. McGinty D, Letizia CS, Api AM 2010, Review fragrance material review on phytol. Food Chem Toxicol. 48(3):59-63.
2. Ishibashi Y, Nagamatsu Y, Miyamoto T, Matsunaga N, Okino N, Yamaguchi K, Itô M. 2014, A novel ether-linked phytol containing digalactosyl glycerol lipid in the marine green alga, *Ulva pertusa*. Biochem Biophys Res Commun. 52:273-80.
3. Costa JP, de Oliveira GAL, de Almeida AAC, Islam MT, de Sousa DP, Freitas RM. 2014, Anxiolytic-like effects of phytol in a pilocarpine model in mice. Neurosci Lett. 523:115-8.
4. Bero J, Beauvy C, Hanaert V, Hérent MF, Michels PA, Quetin-Leclercq J. 2013, Antitryptosomal compounds from the essential oil and extracts of *Keetia leucantha* leaves with inhibitor activity on *Trypanosoma brucei* glyceraldehyde-3-phosphate dehydrogenase. Phytomedicine. 20:270-4.
5. Arnhold T, Elmazar MMA, Nau H. 2005, Biphasic effects of geranylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*. Agents Chemother. 49:1770-4.
6. Inoue Y, Hada T, Shiraishi A, Hirose K, Hamashima H, et al. 2005, Biphasic effects of geranylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*. Agents Chemother. 49:1770-4.
7. Amholde T, Elmaraz MM, El-Ahmar HS, Schaal MF, Faraj NA. 2013, Phytol/phytic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. PLoS One 8:45638.
8. Silva RO, Sousa FB, Damasceno SR, Carvalho NS, Silva VG, Oliveira FR, et al. 2014, Phytol, a diterpenic alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundam Clin Pharmacol. 28:455-64.
9. Minnetti L, Elmaraz MM, Nau H. 2004, Prevention of vitamin A teratogenesis by phytol or phytanic acid results from reduced metabolism of retinol to the teratogenic metabolite, all-transretinoic acid. Toxicol Sci. 66:274-82.
10. Vane JR. 1971, Inhibition of prostaglandin synthesis as a mechanism for action of aspirin-like drugs. Nat New Biol. 231:232-5.
11. Smith WL, Garavito RM, DeWitt DL. 1996, Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem. 271:33157-60.
12. Chick NA, DeWitt DL. 1992, Antiproliferative activity of beta-damascenone and E-PHY isolated from *Ipomoea pes-caprae*. Planta Med. 58:19-21.
13. Jordan J, Freitas RM. 2012, Anticonvulsant effect of phytol in a model of epilepsy and oxidative stress. Fundam Clin Pharmacol. 26:455-64.
14. Koga K, Matsumi K. 2002, Prevention of vitamin A teratogenesis by phytol or phytanic acid results from reduced metabolism of retinol to the teratogenic metabolite, all-transretinoic acid. Toxicol Sci. 66:274-82.
15. Bero J, Beauvy C, Hanaert V, Hérent MF, Michels PA, Quetin-Leclercq J. 2013, Antitryptosomal compounds from the essential oil and extracts of *Keetia leucantha* leaves with inhibitor activity on *Trypanosoma brucei* glyceraldehyde-3-phosphate dehydrogenase. Phytomedicine. 20:270-4.
tract. Gastroenterology. 109:285-301.
19. Gleeson MP, Gleeson D. 2009, QM/MM calculations in drug
discovery: a useful method for studying binding phenomena? J Chem
Information Modeling. 49:670-7.
20. Rahman A, Ali MT, Shawan MMAK, Sarwar MG, Khan MA,
Halim MA. 2016, Halogen-directed drug design for Alzheimer’s
disease: a combined density functional and molecular docking
study. SpringerPlus. 5:1346. 21. Parr RG, Yang W. 1989, Density-
Functional Theory of Atoms and Molecules (New York: Oxford
University Press).
22. Morris GM, Lim-Wilby M. 2008, Molecular docking. Methods Mol
Biol. 443:365-82.
23. Bissantz C, Kuhn B, Stahl M. 2010, A medicinal chemist's guide to
molecular interactions. J Med Chem. 53:5061-84.
24. Rehman S, Nabi B, Fazil M, Khan S, Bari NK, Singh R, Ali J.
Role of P-2017, Glycoprotein Inhibitors in the Bioavailability
Enhancement of Solid Dispersion of Darunavir. BioMed Res Int.
doi: 10.1155/2017/8274927.