In Silico anti-inflammatory activity of Natural phytoconstituents and its molecular mechanism

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Abstract

The aim of the present study is to reveal the possible mechanism of anti-inflammatory activity of natural analogues from several medicinal plants to claim folkoric use of the plant in inflammation disease conditions. In silico screening of ***Stigmasterol****,* ***Epicatechin****,* ***Hesperidin****,* ***Lupeol****,* ***Beta-sitosterol*** *and* ***Diosgenin*** *against crucial inflammatory molecular target such as COX-2 (PDB ID: 4COX). The docking score of these natural anti-inflammatory analogues compared with inflammatory drug* ***Diclofenac****. Whereas the 3D assembly of anti-inflammatory target protein were downloaded from Protein Data Bank, and molecular interaction studies amongst target protein and ligands were done with AutoDock Vina software. The study more exposed that all the inhibitor drugs acquired with negative dock energy against the target protein. Molecular docking investigation displayed that natural flavonoid phytosterol analogue* ***Stigmasterol*** *displayed remarkable inhibition ability with the binding energy values of -8.4 kcal/mol than other compounds and anti-inflammatory drug* ***Diclofenac*** *(Binding energy -8.2 Kcal/mol). Based upon the results of manifold sequence alliance, it is obvious that ligand binding sites were preserved. The present in silico examination thus, delivers structural awareness about the anti-inflammatory target protein and also its molecular relations with some of the recognised protease inhibitors.*

Keywords: *Anti-infammatory, Diclofenac, Diosgenin, Hesperidin, Molecular docking.*

1. Introduction

Inflammation is almost like a biological reaction that is usually triggering infective pathogens and physical or chemical injuries often. In addition, the inflammatory responses to the resistant structures are inherent non-specific in design. Inflammatory mediators activate the inflammatory immune responses such as mobilization and activation of inflammatory cells at the injury site and

then remove toxic agents and restore the compromised tissue [1,2]. The non-steroidal anti-inflammatory medications (NSAIDS) have been used widely to combat inflammation to date. Nonetheless, their use has reduced the harmful toxic effect of NSAIDS [3,4]. The serious effects of such NSAIDS include the underlying conditions of ulcer and gastrointestinal tract [5]. Therefore, a new natural medicinal agent without any side effects is ideal to replace the presently accessible chemical therapies to manage different inflammatory disorder disorders that are healthy, pretty affordable and feasible for several patients [6-8]. Insight of pharmaceutical sciences and phytoconstituent mode of action is very small, and is still under review, reducing attempts for standardization, assessment, and further usage to produce new pharmaceutical molecules [9]. The indigenous healing plant is a contractual and principal source of natural secondary metabolites used for the design of novel therapeutic agent [10,11] .Ecological features can significantly stimulus the exudation of secondary metabolites like phytochemicals from tropical plants. Consequently, secondary metabolites concealed by plants in tropical areas prodigious consideration and that may be advanced as remedies [12,13]. Numerous phytochemicals from therapeutic plants, have been testified for antiviral activity [14-16]. The phytosterol derivative Stigmasterol isolated from the hexane extract of medicinal plant *Eryngium foetidum* was displayed significant anti-inflammatory activity [17]. The phytosterol compound β-sitosterol isolated from the petroleum ether extract of medicinal plant *Nyctanthes arbortristis Linn*. (Oleaceae) was revealed remarkable anti-inflammatory activity [18]. The flavonoid analogue Epicatechin isolated from aqueous methanol extract of the bark of *Cinnamomun sieboldii Meissn*. (Lauraceae) showed significant anti-inflammatory activity [19]. The flavonoid derivative Hesperidin extracted from medicinal citrus plant displayed remarkable anti-inflammatory activity [20]. Natural analogue Lupeol isolated from the chloroform extract of medicinal plant, *Crateva adansonii* revealed significant anti-inflammatory activity [21]. A plant derived sapogenin derivative Diosgenin isolated from traditional medicinal plant, *Costus speciosus* (Koen ex.Retz.) displayed significant anti-inflammatory activity [22]. The natural anti-inflammatory analogues were represented in Figure. 1. Keep this in mind, we examined natural anti-inflammatory analogues **Stigmasterol**, **Epicatechin**, **Hesperidin**, **Lupeol**, **Beta-sitosterol** and **Diosgenin** as potential inhibitor candidates for crucial inflammatory molecular target such as COX-2 (PDB ID: 4COX) and compared with anti-inflammatory drug **Diclofenac**. The outcomes of this study will offer other investigators with prospects to find the precise medication against inflammation.

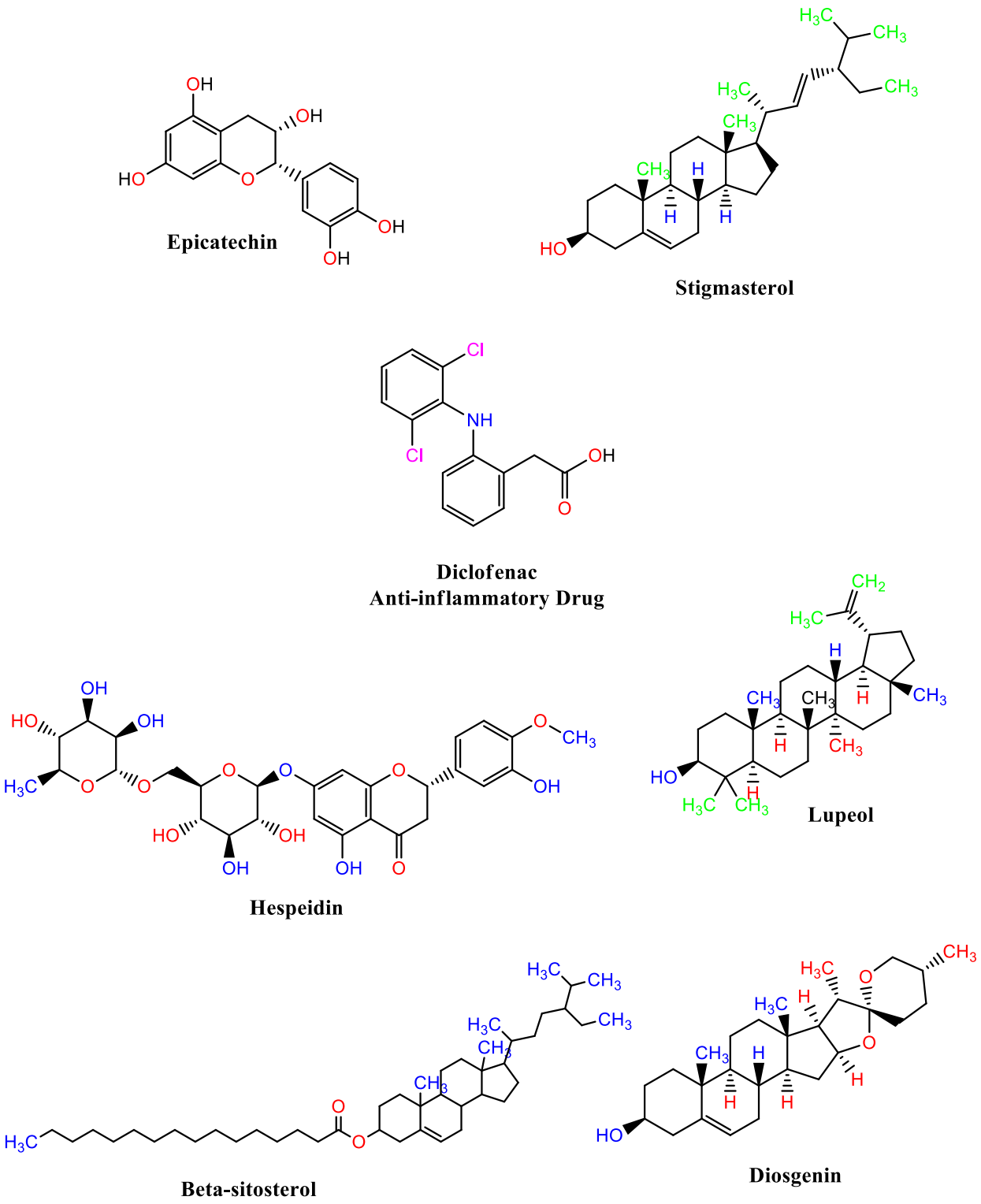

Figure 1. Natural anti-inflammatory analogues

2. Experimental

2.1. Molecular docking

Molecular docking studies were used to inspect the binding mode, interaction between compounds **Stigmasterol**, **Epicatechin**, **Hesperidin**, **Lupeol**, **Beta-sitosterol**, **Diosgenin** and anti-inflammatory drug **Diclofenac** with protein 5N5O using Autodock vina 1.1.2 [23]. The crystal structure of crucial inflammatory molecular target such as COX-2 (PDB ID: 4COX) were downloaded from Protein Data Bank (http://www.rcsb.org). The 3D structures of the inhibitors were drawn and energy minimized via ChemDraw Ultra 12.0 and Chem3D Pro 12.0 softwares. AutoDock Tools 1.5.6 program package was used to create the input files for Autodock Vina. The binding pocket of main protease (PDB ID: 4COX) was identified by using co-crystallized ligand via discovery studio program and the amino acid residues Arg120, Val349, Leu352, Ser353, Tyr355, Leu384, Tyr385, Trp387, Met522, Val523, Gly526, Ala527, Ser530 and Leu531 were situated in the binding pocket. The search grid of 4COX protein was recognized as center_x: -16.704, center_y: 51.851, and center_z: 69.392 with size dimensions x: 20, y: 20, and z: 20 with 1.0 Å spacing. The value of exhaustiveness was set to 8. The further constraints were fixed to default for Autodock Vina and not stated. The compound devouring smallest binding affinity value is the best-scoring compound and the fallouts were visually investigated with Discovery studio 2019 program.

3. Result and discussion

3.1. Docking studies

In order to advance perception into the plausible mechanism of biological activities docking, simulations were performed. The compounds **Stigmasterol**, **Epicatechin**, **Hesperidin**, **Lupeol**, **Beta-sitosterol**, **Diosgenin** and anti-inflammatory drug **Diclofenac** were studied for their docking behaviour with protein 4COX via Autodock Vina program. All of this tested inhibitors shows negative binding energy. The natural phytosrerol derivative **Stigmasterol** demonstrates remarkable inhibition ability with the binding energy value of -8.4 kcal/mol than other compounds **Diosgenin** (-3.4 kcal/mol)**, Epicatechin** (-8.0 kcal/mol)**, Hesperidin** (-6.2 kcal/mol)**, Lupeol** (-0.2

kcal/mol), **Beta-sitosterol** (-6.5 kcal/mol) and anti-inflammatory drug **Diclofenac** (-8.2 kcal/mol) in 4COX protein respectively. Hydrogen bonding plays a major role in bonding stability between protein and ligand, and the approving bond distance is less than 3.5 Å amongst H-donor and H-acceptor atoms [24]. The hydrogen bond distances of relevant compounds were less than 3.5 Å in target protein indicates the resilient hydrogen bonding between protein and ligands. Compound **Stigmasterol** does not show any Hydrogen bonding interactions with the receptor 4COX. The residues of amino acids Val89, Leu93, Val116, Arg120, Val349, Leu352, Tyr355, Leu359, Val523, Ala527 and Leu531 were tangled in hydrophobic contacts. The hydrogen bonding and hydrophobic contacts of amino acid residues in 4COX protein with compound **Stigmasterol** was shown in Figure 2. The anti-inflammatory drug **Diclofenac** shows one Hydrogen bonding interaction with the receptor 4COX. The residues of amino acid Val349 was tangled in hydrogen bonding interaction with the bond length of 2.45 Å. The residues of amino acids Tyr348, Leu352, Tyr385, Val523, Ala527 and Leu531 were tangled in hydrophobic contacts. The hydrogen bonding and hydrophobic contacts of amino acid residues in 4COX protein with anti-inflammatory drug **Diclofenac** was shown in Figure 3. The fallouts displayed that the compound **Stigmasterol** having the remarkable inhibition ability than other compounds in respective target protein. The outcomes were abridged in Table 1.

Table 1. Molecular docking interaction of compounds (**1a-1g**) against inflammatory molecular target protein COX-2 (PDB ID: 4COX).

Compounds	Inflammatory molecular target protein COX-2 (PDB ID: 4COX)		
	Binding affinity (kcal/mol)	No. of H-bonds	H-bonding residues
Hesperidin (1a)	-6.2	3	Arg120, Val349
Stigmasterol (1b)	-8.4	0	-
Epicatechin (1c)	-8.0	2	Val349, Tyr355
Lupeol (1d)	-0.2	0	-
Beta-sitosterol (1e)	-6.5	0	-
Diosgenin (1f)	-3.4	30	-
Diclofenac (1g)	-8.2	1	Val349

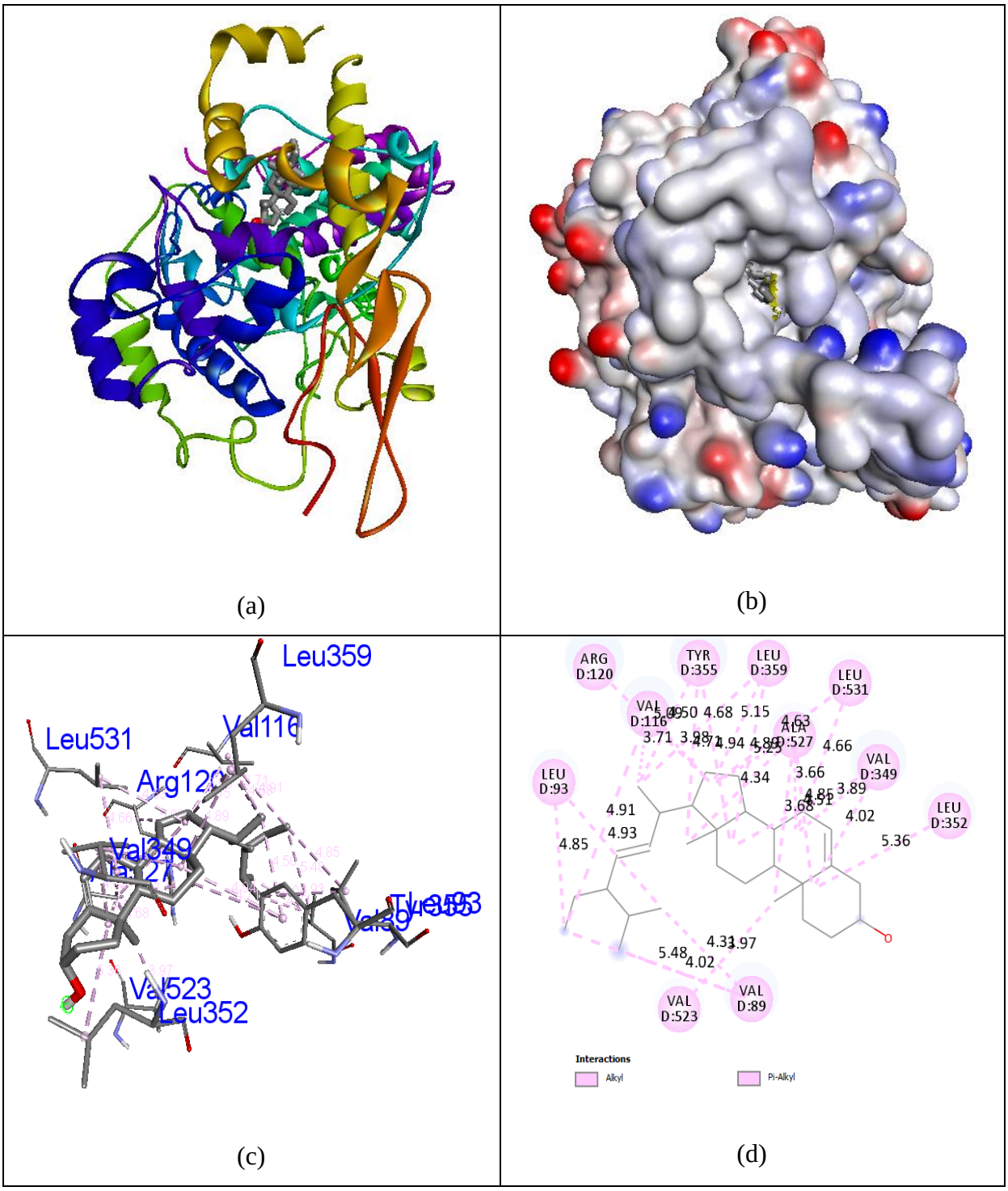

Figure 2 Docked complex (a), molecular surface (b), 3D (c), and 2D (d) interaction modes of ***O*-Stigmasterol** within the binding site of **4COX** protein.

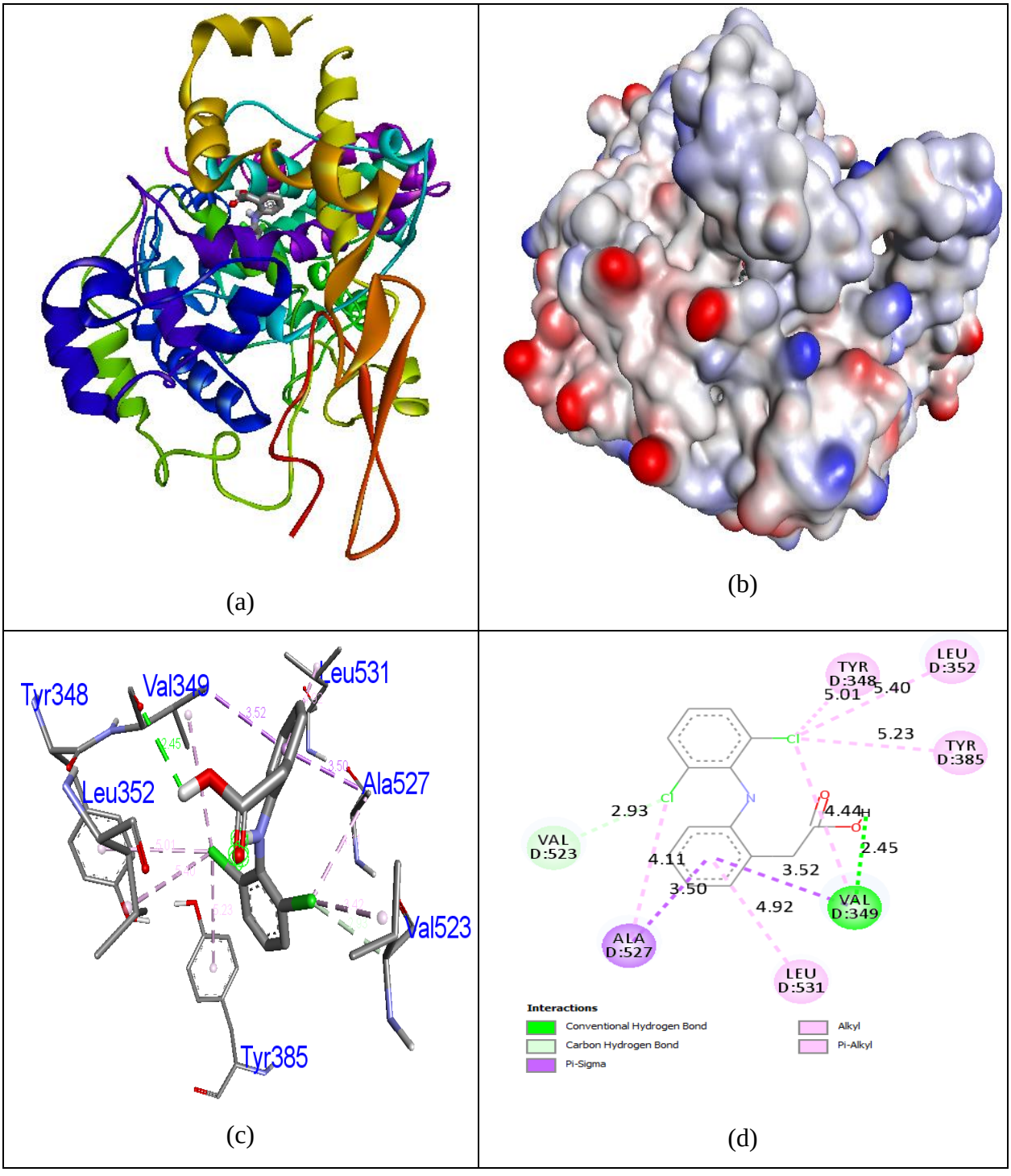

Figure 3 Docked complex (a), molecular surface (b), 3D (c), and 2D (d) interaction modes of anti-inflammatory drug **Diclofenac** within the binding site of **4COX** protein.

4. Conclusion

The purpose of the present study was to inspect some therapeutic plant-derived natural analogues that may be cast-off to combat inflammation. **Stigmasterol**, **Epicatechin**, **Hesperidin**, **Lupeol**, **Beta-sitosterol** and **Diosgenin** with negative binding energy values were the utmost suggested compounds originate in therapeutic plants that might act as significant inhibitors of crucial inflammatory molecular target such as COX-2 (PDB ID: 4COX). Molecular docking studies showed that phytosterol analogue **Stigmasterol** displayed remarkable inhibition ability with the binding energy values of -8.4 kcal/mol than other compounds and anti-inflammatory drug **Diclofenac** (Binding energy -8.2 Kcal/mol). Yet, advance investigation is essential to inspect the probable uses of the medicinal plants having these compounds.

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