Indian Journal of Natural Products and Resources
Vol. 15(3), September 2024, pp. 413-425
DOI: 10.56042/ijnpr.v15i3.7212

Computational insight into the antagonistic activity of some natural ligands against protease-activated receptors in psoriasis

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Received 11 December 2023; revised received 01 June 2024; accepted 05 August 2024

Psoriasis is a unique skin dermatoses characterised by autoimmune and inflammatory features in humans. Along with these features, pain and itching are the cardinal signs of psoriasis. Lately, many researchers investigated the implications of protease-activated receptors, especially PAR-1 and PAR-2, in skin diseases such as psoriasis. In the current study, molecular docking was carried out with AutoDock tools v 1.5.6 to find out binding interactions of some natural ligands, with PAR-1 and PAR-2, which have been preclinically evaluated as antipsoriatic agents, but their mechanism of action has not been established. For this purpose, three-dimensional structures of plant ligands were prepared using the ChemSketch 2015 free version and interactions were observed via Biovia Discovery studio version 4.5. This study demonstrated that Capsaicin and Dimethyl Fumarate, in terms of binding interactions, could behave as protease-activated receptor-1 and protease-activated receptor-2 selective antagonists, respectively. Moreover, molecular dynamic simulation was also performed employing the Desmond module (Schrodinger, LLC, Cambridge, USA) to examine the stability of the ligand-target complex at 100 ns.

Keywords: Autoimmune, Molecular docking, Molecular dynamics, Protease-activated receptor, Psoriasis

IPC code; Int. cl. (2021.01)−A61K 36/00, A61K 17/00

Introduction

Psoriasis is an autoimmune and chronic inflammatory skin disorder with complex pathophysiology, which includes prominent features such as pruritis, pain, inflammation, and scale formation on the skin. The proper treatment of psoriasis is not available as it tends to relapse. It significantly affects the social well-being of the persons suffering from it[1]. Many therapeutic targets have been identified through various research studies. From the Indian perspective, people are not aware that psoriasis disease may lead to many cardiovascular and other diseases related to morbidity and, subsequently, mortality. The rationale behind this might be the lack of research across the globe and its mysterious pathophysiology. PARs are expressed by keratinocytes and are involved in the generation of cytokines. After activation, PARs play critical roles in allergic reactions and inflammation. Thus, PARs may be implicated in skin patho-physiologies such as wound healing, cutaneous inflammation, pruritus, tumorigenesis (e.g. melanoma, squamous cell carcinoma), and bacterial infections[2]. Functional PAR-1 and PAR-2 were recently demonstrated on primary afferent neurons in the skin, thereby regulating neurogenic inflammation. PAR-2 is also believed to be involved in the generation of itch along with cutaneous inflammation[3]. Previously, PAR-1 was also known as a thrombin receptor. PAR-1 has been expressed increasingly in different pathophysiological conditions such as colon, breast, and prostate cancer, metastasis, and other kinds of cancer[4]. The discharge of protease in case of skin inflammation is due to the recruitment of inflammatory cells. These released proteases can activate the protease receptor, especially PAR-2. Besides, epidermal cells express protease, and some of them can activate protease-activated receptors. PAR-2 was more extensively studied in the case of psoriasis compared to PAR-1, although the involvement of both receptors in the development of this pathological condition has been precisely established; therefore, both receptors are promising targets for developing antipsoriatic molecules[5]. Epidemiological studies demonstrated the beneficial impact of phytochemicals in various diseases and thus paved the way for the development of many therapeutic candidates for different ailments. Therefore, in the current study,

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Proteease-activated receptors have been taken into consideration for molecular docking studies with few natural ligands. Many antioxidants such as Catechin, Epicatechin, Quercetin, Luteolin, Curcumin, Capsaicin, and Embelin have earlier been evaluated against psoriasis via different preclinical studies[6-11]. Apart from these antioxidant molecules, other plant molecules such as Hypericin, Caffeine, Gossypol, and Fumaric esters have also been investigated against psoriasis[12-15]. These molecules could alleviate psoriasis, but their mechanism of action is still being meticulously investigated. Hence, these mentioned plant ligands have been included in this study, and a molecular docking study was conducted to predict the binding interactions of natural ligands with PAR-1 and PAR-2 and to illuminate their possible inhibitors of them.

Materials and Methods

The crystallographic three-dimensional structures of PAR-1 and PAR-2 (PDB IDs: 3vw7 and 5nj6) were downloaded from the PDB data bank[16]. The chemical structures of ligands were prepared using the ChemSketch 2015 free version (Fig. 1). Further, these structures were cleaned for energy minimisation purposes and optimised to three-dimensional structures by exporting them into .mol format. For compatibility with AutoDock, the .mol format of ligands was converted into .pdb format via Open Bable version 3.1.1. The blind docking method was adopted for the evaluation of actual binding modes of the natural ligands on the targets, and for this purpose, X, Y, and Z coordinates were taken 126x126x126. For molecular docking purposes, the receptor was refined by removing water molecules, hetero atoms, etc. and adding hydrogen

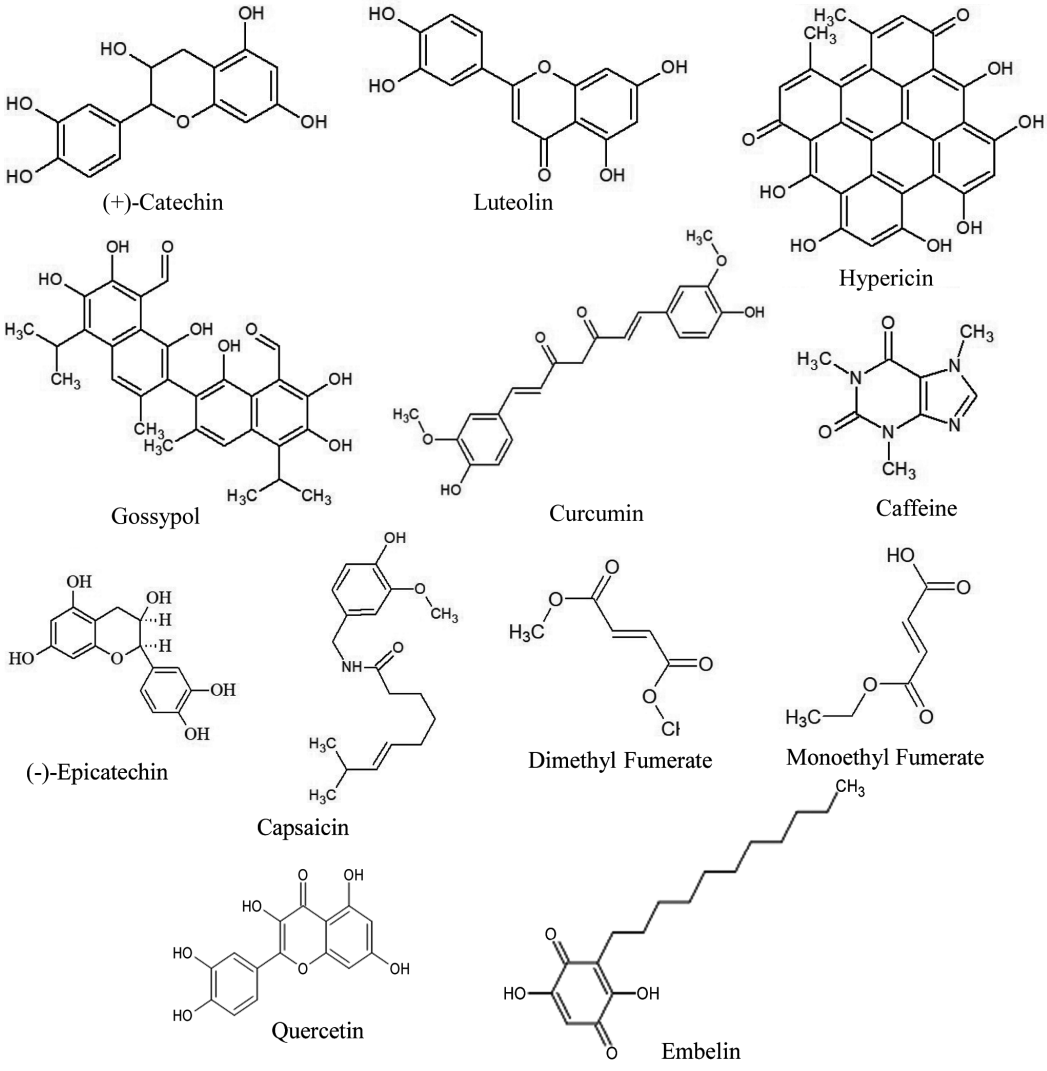


Fig. 1 — Structure of natural ligands.

Table 1 — Results of molecular docking study of Natural products with PAR-1 receptor

S. No	Ligands	Binding Energy at the active site (-kcal/mol) PAR-1	Amino acids involved in H-bonding in the active site
1	Dimethyl Fumarate	-3.76	Tyr337, His336, Thr261
2	Monoethyl Fumarate	-4.14	Thr215, Arg214
3	Caffeine	-4.62	Tyr337, Leu262
4	Embelin	-4.75	Asp256
5	Curcumin	-5.23	Asp256
6	Gossypol	-5.45	Ser153
7	Capsaicin	-6.02	Tyr337, Tyr353, Tyr183
8	Epicatechin	-6.43	Leu332, Leu258, Asp256
9	Catechin	-6.44	Tyr337, Gly233
10	Luteolin	-6.57	Leu258, Val257, Asp256
11	Quercetin	-6.64	Tyr353, Tyr183, His336, Thr261
12	Hypericin	-7.69	Lys240, Tyr267

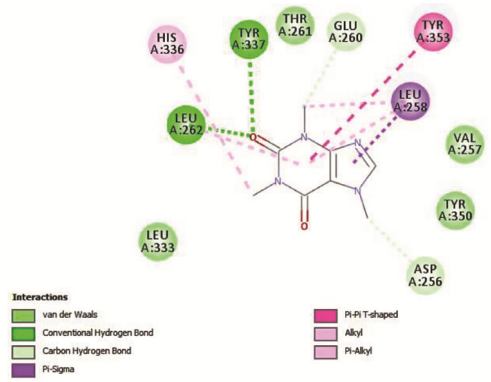
Table 2 — Results of molecular docking study of natural products with PAR-2 receptor

S.N	Ligands	Binding energy at the active site (-kcal/mol) PAR-2	Amino acids involved in H-bonding in the active site
1	Embelin	-3.60	Nil
2	Dimethyl Fumarate	-4.01	Lys131
3	Capsaicin	-4.68	Arg267
4	Catechin	-4.83	Ala355
5	Quercetin	-5.05	Ala255, Ala259
6	Luteolin	-5.26	Ala289, Thr301
7	Curcumin	-5.31	Ala259
8	Epicatechin	-5.72	Ala255, Ala259, Ala289, Thr301
9	Caffeine	-5.87	His135
10	Gossypol	-6.12	Tyr345
11	Hypericin	-6.27	Glu145, Ala146, Val211
12	Monoethyl Fumarate	-6.36	Tyr345, Lys280, Arg284, Lys287

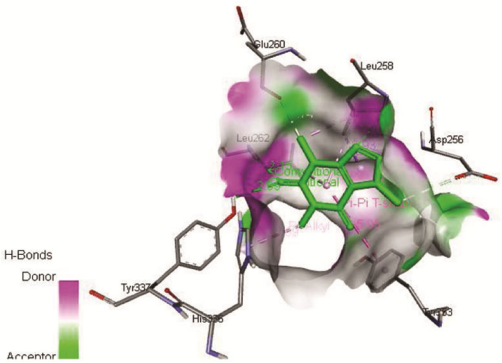
molecules using AutoDock. Then, ligands were prepared by detecting the root, choosing and setting the number of torsions, and finally, they were saved as .pdbqt. Furthermore, after gridding and docking processes with the Lamarckian algorithm with a short number of evaluations (250000), binding energy was obtained for each ligand individually. Two and three-dimensional structures were obtained by Discovery Studio 4.5[17]. A molecular dynamics simulation was performed to check the stability of the protein individually and with ligands and the changes that occurred within the protein-ligand complex concerning the time. The Desmond module (Schrodinger, LLC, Cambridge, USA) was used for molecular dynamics (MD) simulation at 100 ns. The output file of the MD comprises a simulation interaction diagram (SID) encompassing metrics such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and Protein-ligand contact analysis. The results of the above metrics define the stability of the protein-ligand complex or protein individually.

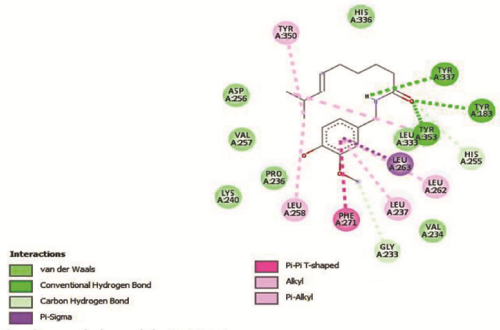
Results

In the current molecular docking study, the interactions between ligands and PAR-1 and PAR-2 were identified to propose a possible molecular mechanism of ligands to act as antagonists either for PAR-1 or PAR-2 or for both receptors. The major interactions related to inhibition of PAR-1 are found with some specific amino acids. Zhang *et al.* 2012 illustrated the interaction between Vorapaxar and PAR-1 and concluded that some specific amino acids interact with ligands, providing them the capacity to be selective antagonists for PAR-1. These amino acids are Tyr337 at the carboxy-terminal end of transmembrane 6, which forms a strong hydrogen bond with Vorapaxar, and Tyr353, Tyr183, and Phe271, which form the base of ligand binding pocket with Tyr183, which is located at the N-terminal end of transmembrane-7[18]. The major interactions related to PAR-2 inhibition were precisely described by Kakarala *et al.* 2014. They described some amino acids viz. Tyr82, Ser124, Lys131, Tyr156, Asn158, Met159, Tyr160, Ser162, Ile163, Asp228, Met295, Cys299, Ser303, and Leu330 are found in the active pocket of PAR-2 and interactions of these amino acids with ligands might be responsible for the any pharmacological actvity[18]. Tables 1, 2 and Fig. 2, 3 demonstrate the results of the molecular docking study of natural plant products with PAR-1 and PAR-2 receptors, respectively.

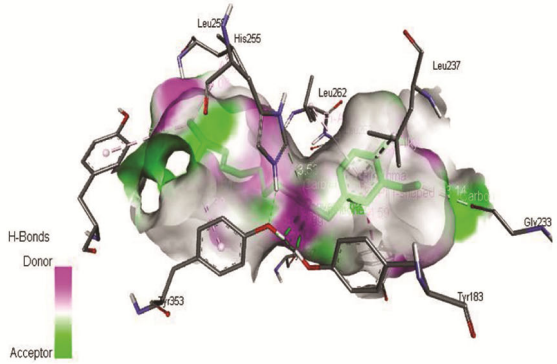


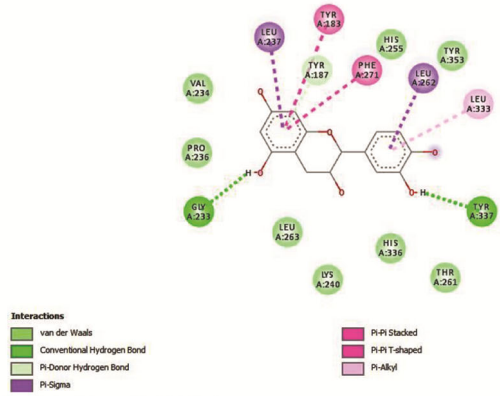
1. Caffeine with PAR-1



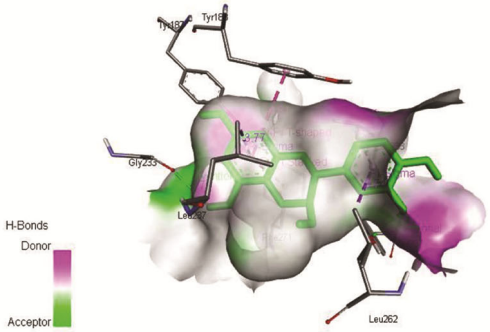


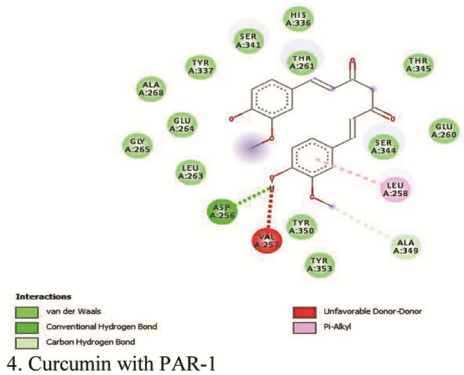
2. Capsaicin with PAR-1



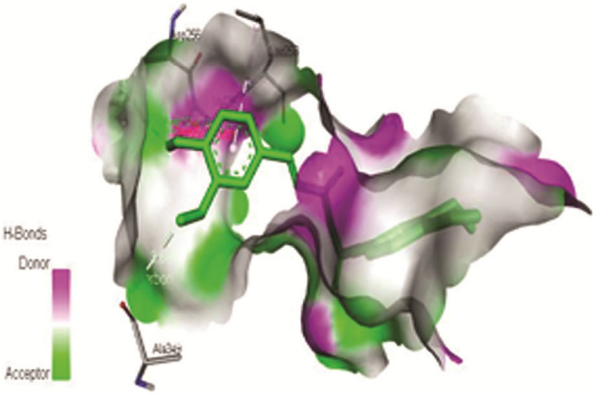


3. Catechin with PAR-1

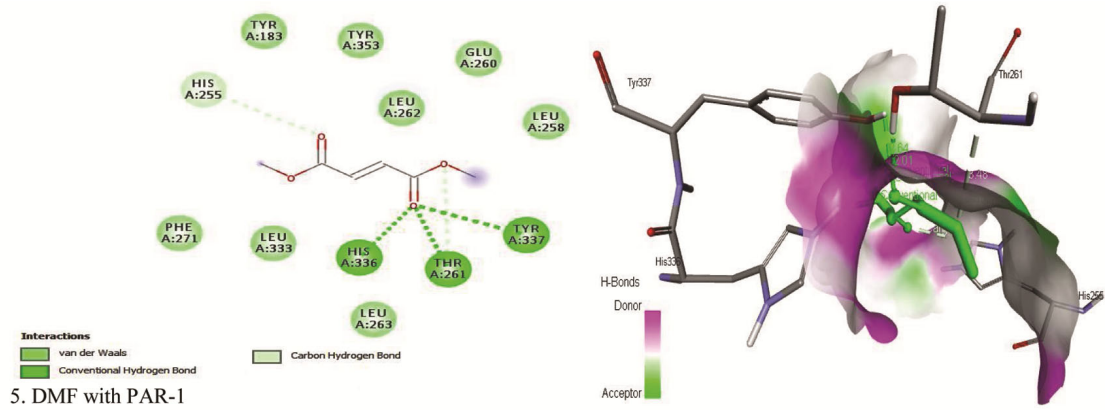




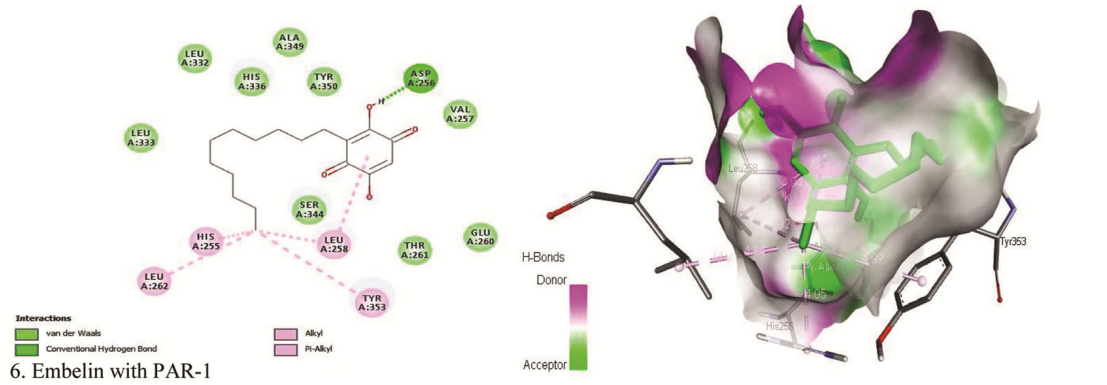
4. Curcumin with PAR-1



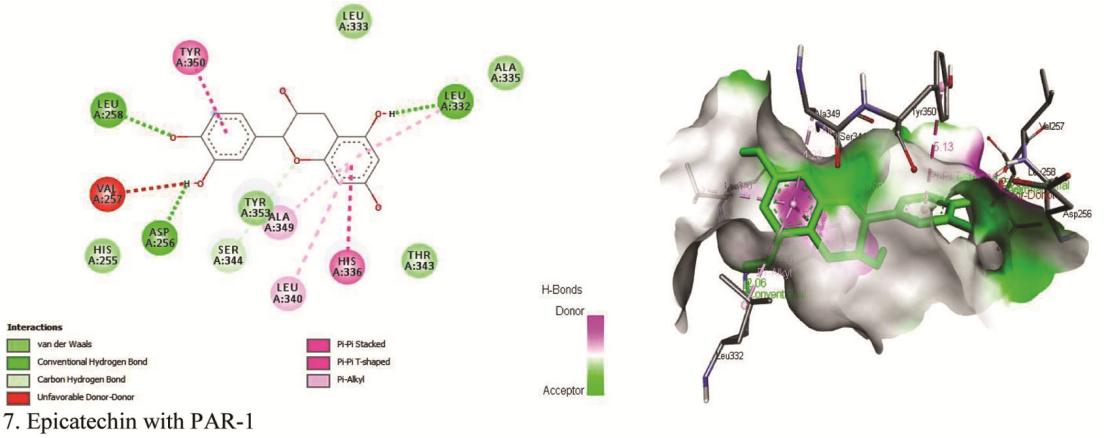
(*Contd. Fig 2)*



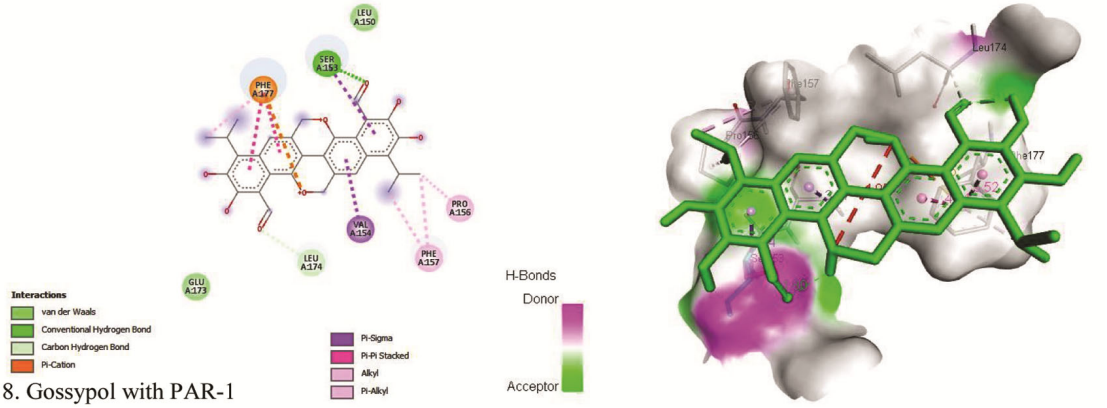
5. DMF with PAR-1



6. Embelin with PAR-1



7. Epicatechin with PAR-1



8. Gossypol with PAR-1

(*Contd Fig .2*)

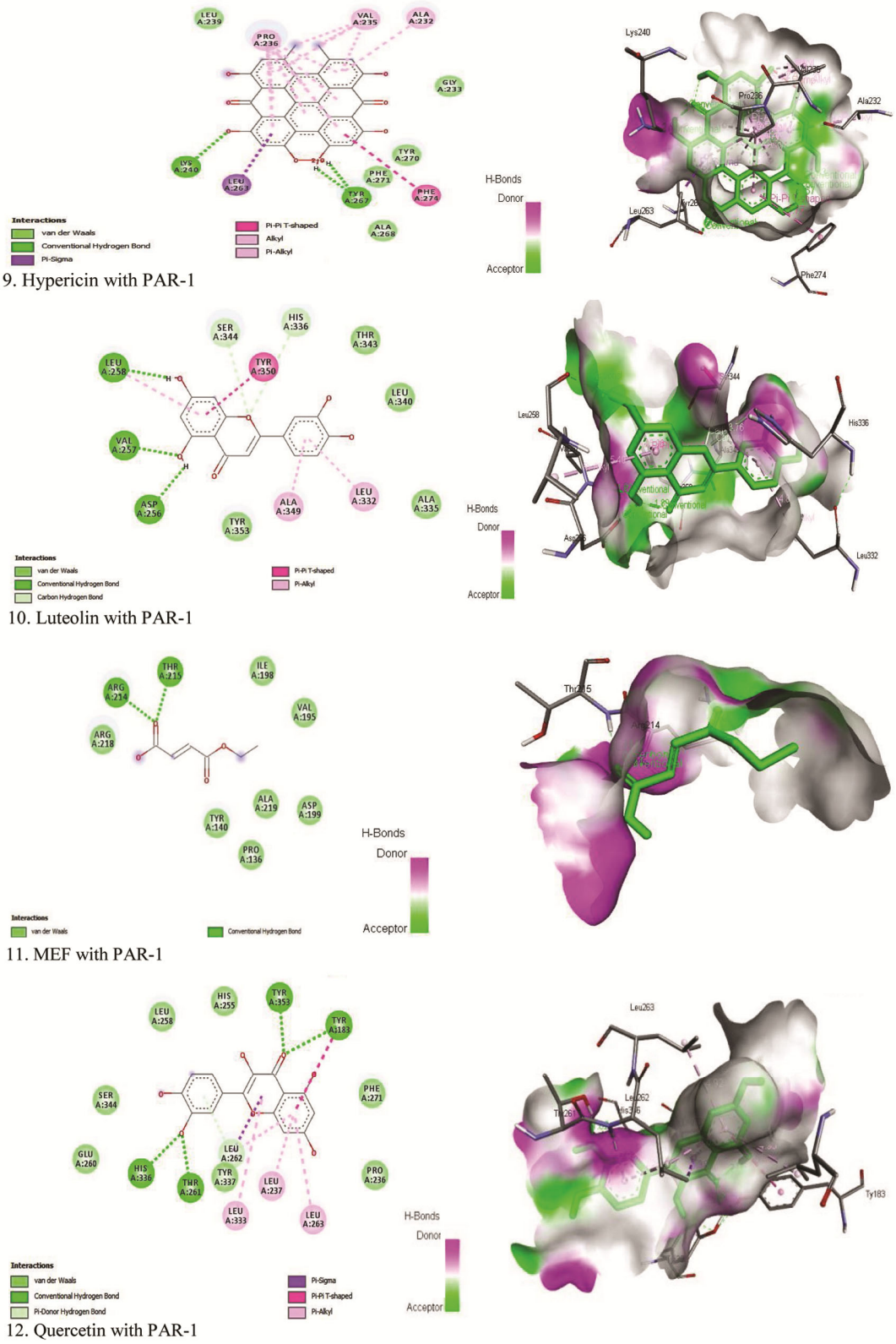
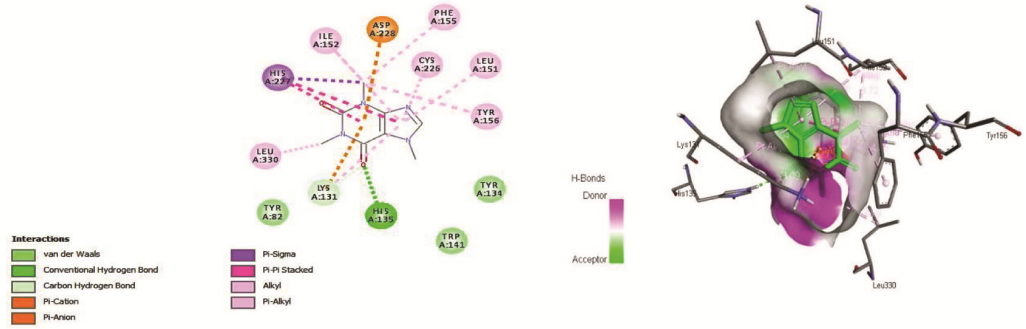
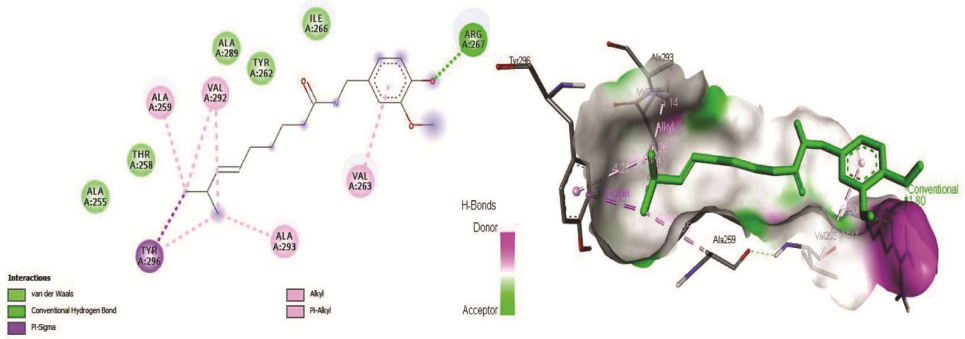


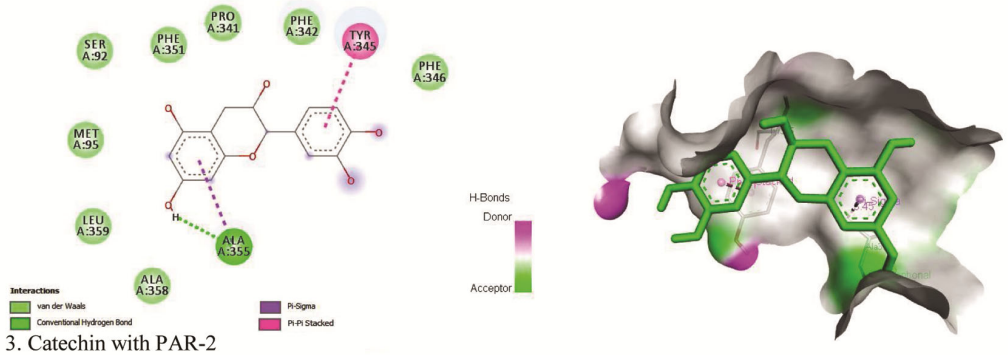
Fig. 2 — 2D and 3D interactions of natural products with PAR-1 receptor.



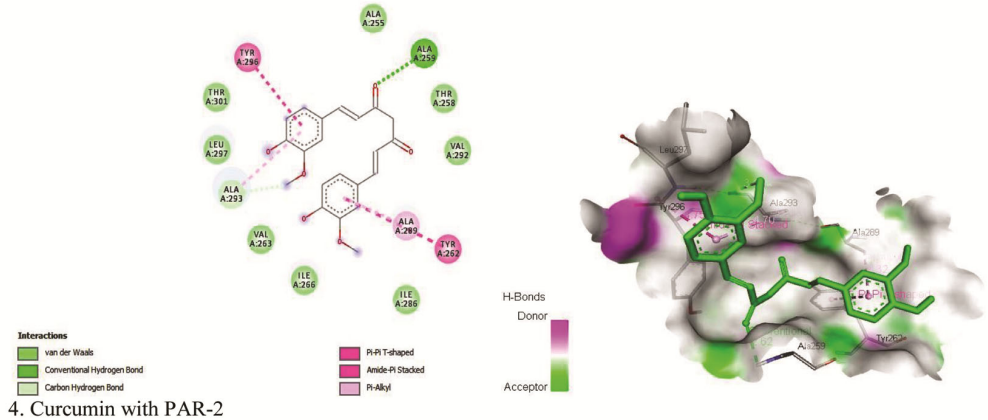
1. Caffeine with PAR-2



2. Capsaicin with PAR-2

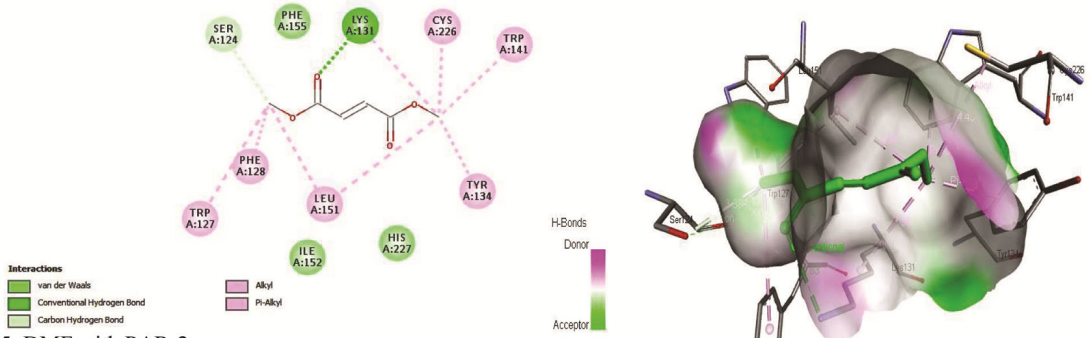


3. Catechin with PAR-2

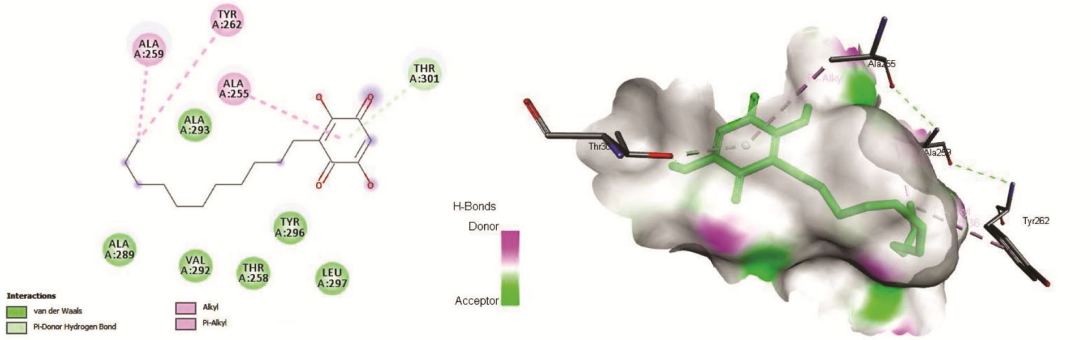


4. Curcumin with PAR-2

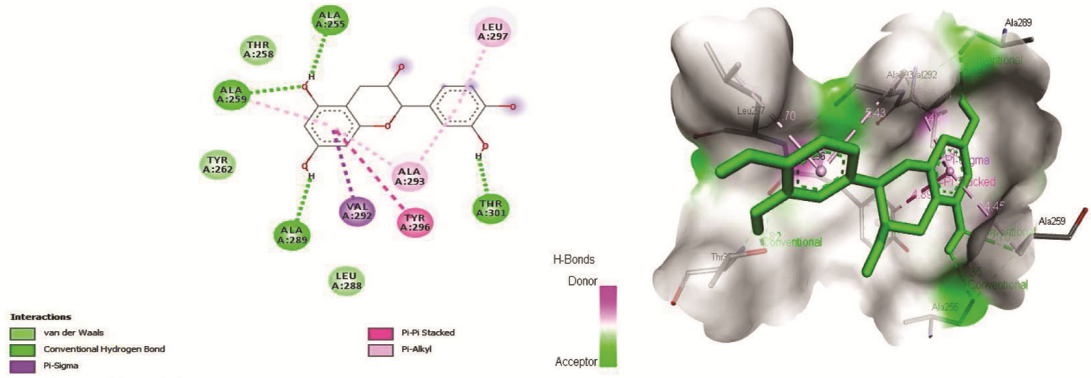
(*Contd Fig 3*)



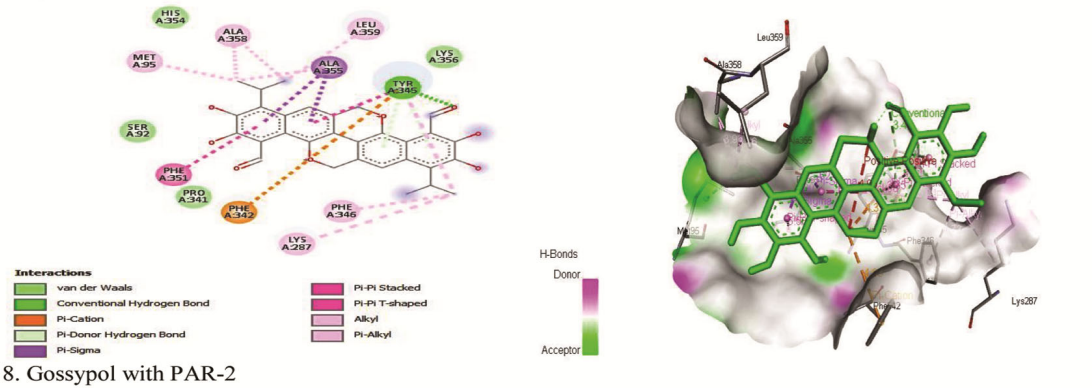
5. DMF with PAR-2



6. Embelin with PAR-2



7. Epicatechin with PAR-2



8. Gossypol with PAR-2

(*Contd Fig 3*)

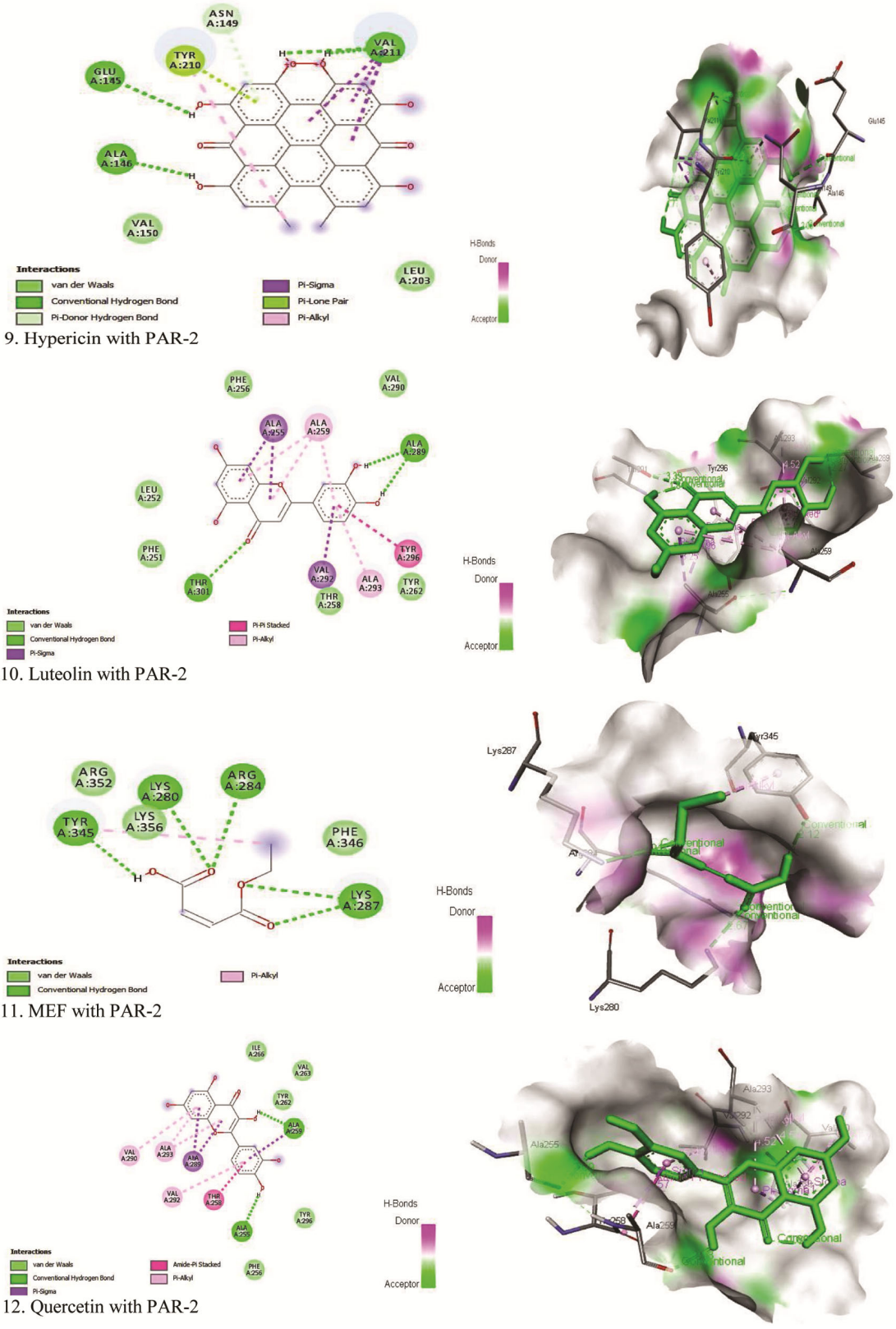


Fig. 3 — 2D and 3D interactions of natural products with PAR-2 receptor.

Molecular dynamics simulation studies

The molecular dynamic results of catechin in complex with human protease-activated receptor 1 (PAR-1) and curcumin in complex with human protease-activated receptor-2 (PAR-2) were carried out for 100 ns to determine the stability of proteins as well as ligand-protein complex.

The root mean square deviation (RMSD) serves as a crucial parameter for evaluating the stability, dynamic characteristics, and behaviour of the protein-ligand complex. Fig. 4 and 5 illustrate the graph depicting the progression of protein RMSD, represented on the left Y-axis. When the protein's RMSD falls within the 1-3 Å range, it indicates the protein's stability and minimal

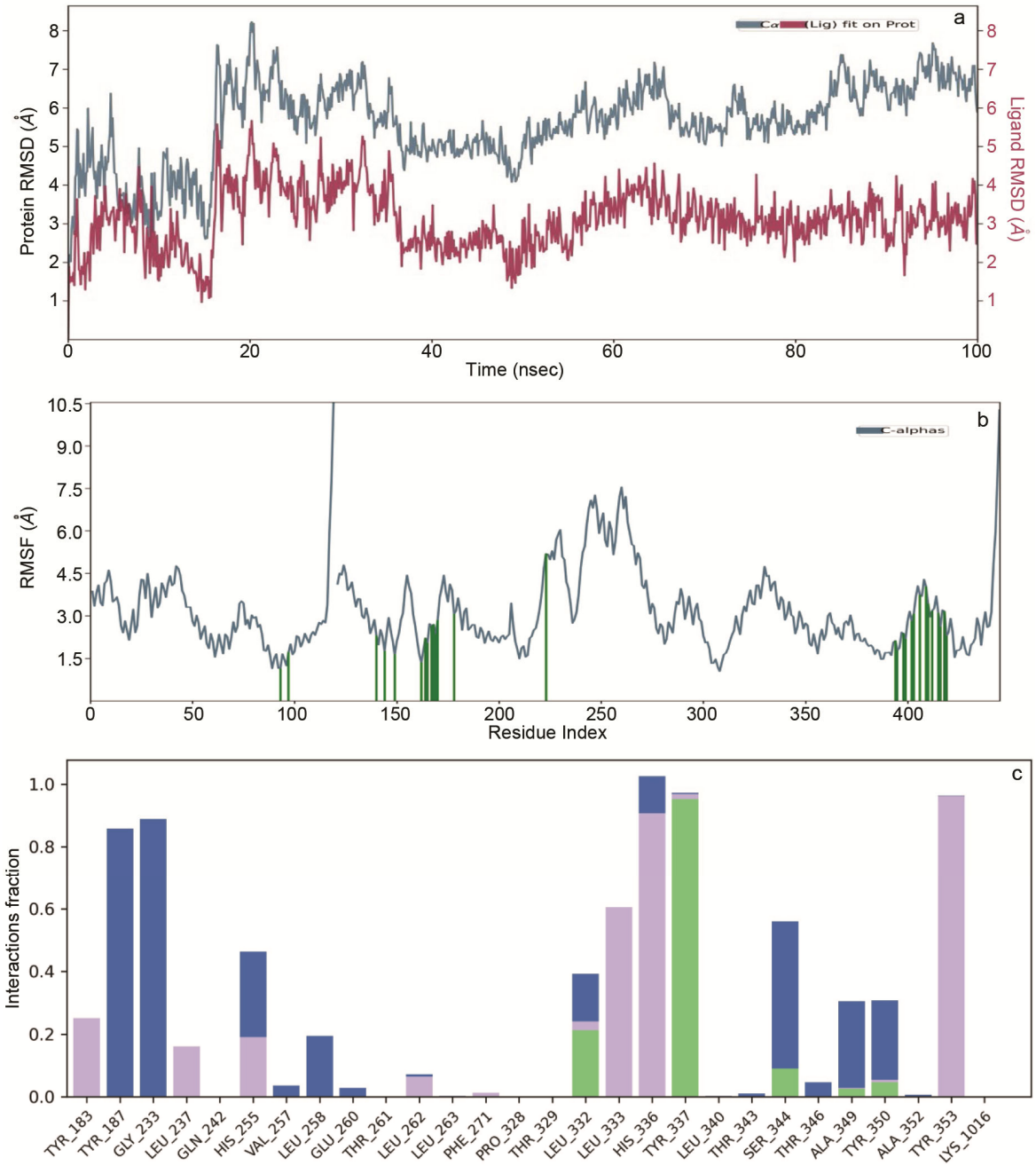


Fig. 4 — a) RMSD of backbone atoms comparative to the original complexes during 100ns MD simulation of catechin in complex with human protease-activated receptor 1 (PAR-1); b) RMSF plot of catechin in complex with human protease-activated receptor 1 (PAR-1); and c) Histogram presentation of per-residues analysis of catechin in complex with human protease-activated receptor 1 (PAR-1) in 100 ns simulations.

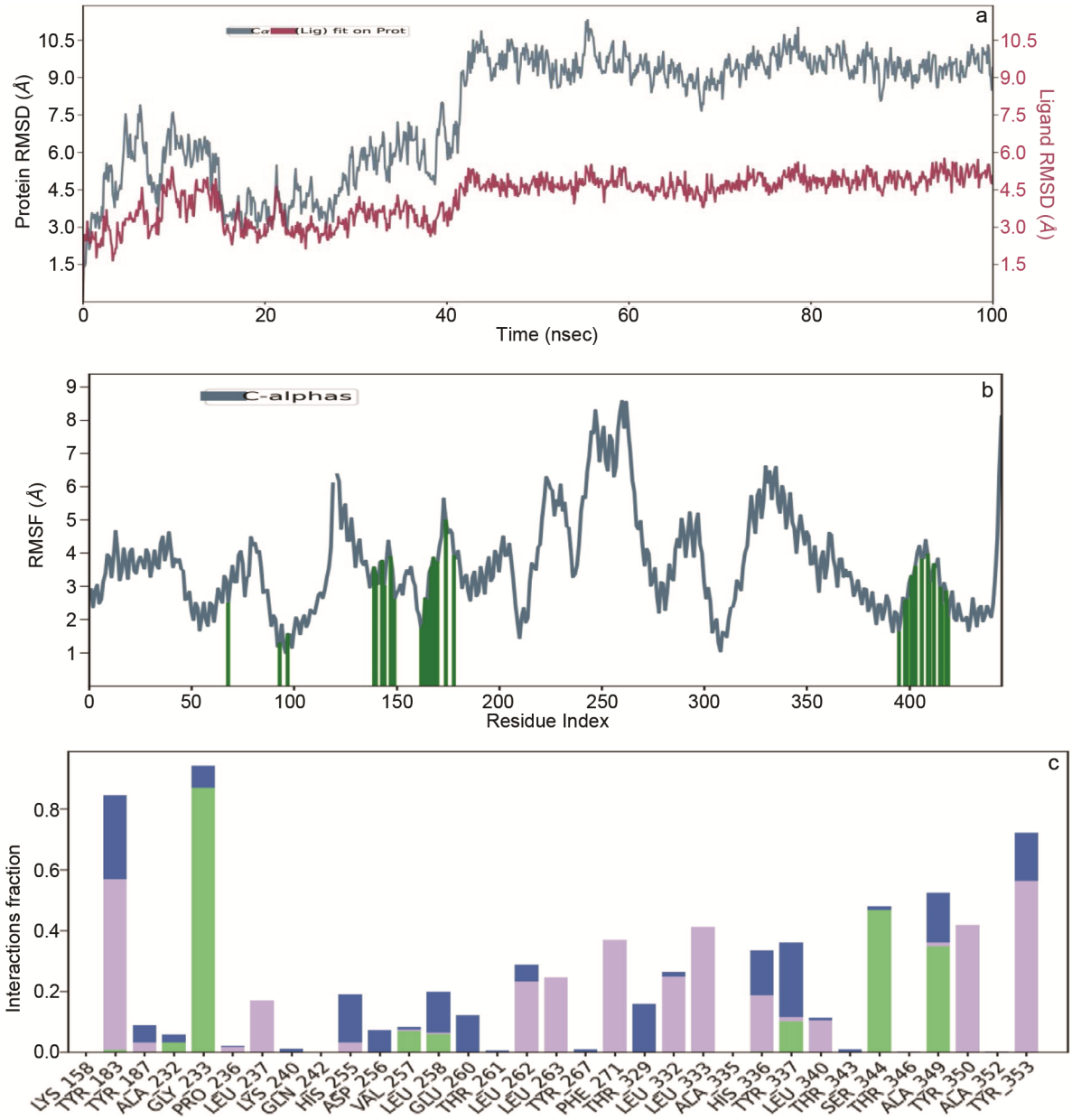


Fig. 5 — a) RMSD of backbone atoms comparative to the original complexes during 100 ns MD simulation of curcumin in complex with human protease-activated receptor-2 (PAR-2); b)RMSF plot of RMSF plot of curcumin in complex with human protease-activated receptor-2 (PAR-2); and c) Histogram presentation of per-residues analysis of curcumin in complex with human protease-activated receptor-2 (PAR-2) in 100 ns simulations.

variations in its backbone structure. The simulated complexes of catechin in complex with human protease-activated receptor 1 (PAR-1) and curcumin in complex with human protease-activated receptor-2 (PAR-2) have shown low stability based on their MD value throughout the simulation, having RMSD values as shown in (Fig. 4a, b, and c) for catechin with protein and Fig. 5a, b, and c respectively. (Fig. 4a and 2a) represent the root mean square deviation values of 7 Å and 10.5 Å for catechin with human protease-activated receptor 1 (PAR-1) and curcumin in complex with human protease-activated receptor-2 (PAR-2), respectively. RMSF results also show poor stability between protein-ligand complexes. In Fig. 4a and 5a, protein ligands have shown great deviation from each other. Overall, MD results concluded that the MD values were not found in an acceptable range, which suggests that the protein-ligand complex was not stable.

Discussion

Protease-activated receptors 1 and 2, once activated by the coagulation cascade molecule thrombin, promote the induction of interleukin-6,8 and GM-CSF in cultured keratinocytes. These mediators are responsible for the proliferation of keratinocytes[19]. Iwakiri *et al.* 2004 demonstrated that recruitment of interleukin-8 is mediated by PAR-2, which is activated by human airway trypsin-like protease in psoriasis vulgaris[20]. Billi *et al.* 2020 demonstrated that KLK (Kallikrein-related peptidase-6) expression in the skin can induce psoriasiform dermatitis. KLK-6 is a type of serine protease that may promote inflammation and autoimmunity via PAR-1 and PAR-2. Levels of KLK6 are supposed to be increased in psoriasis and other skin-related disorders[21].

In the current study, only four natural ligands, i.e. Caffeine, Capsaicin, Catechin, and Dimethyl Fumarate, formed a prerequisite hydrogen bond with Tyr337 to be a selective antagonist as they bound at the carboxy-terminal of the transmembrane-6 of protease-activated receptor-1. Although among all the natural ligands, Hypericin exhibited maximum binding affinity, i.e. -7.69 kcal/mol, it could not form mandatory hydrogen bonds or interact with PAR-1 outside its active pocket. Among four ligands that formed hydrogen bonds with Tyr337, Catechin showed maximum binding affinity, i.e-6.44 kcal/mol. Capsaicin and Quercetin interacted with PAR-1 at the base of the ligand binding pocket, but only Capsaicin formed a hydrogen bond with Tyr 337 to be a selective antagonist of PAR-1. Other ligands viz. Gossypol, MEF, Luteolin, and Epicatechin could not interact with the receptor in its active site. Hence, only Catechin and Capsaicin can be considered selective antagonists towards PAR-1.

Molecular docking interactions with PAR-2 demonstrated that only dimethyl Fumarate could manage to form the required hydrogen bond[19] with amino acids in its active pocket, whereas other ligands could bind with protease-activated receptor-2 in the nearby regions of the active pocket. Dimethyl fumarate exhibited the least binding energy compared to other ligands except Embelin, which neither formed hydrogen bonds nor exhibited appreciable binding energy. Based on the docking results, we can discuss here that dimethyl fumarate was found to be a suitable PAR-2 antagonist in terms of binding interactions. A similar kind of study was carried out by Agrawal *et al.* 2024 on PDE-4B and PDE-4D using the natural ligands and demonstrated the antagonistic activity of these enzyme subunits[22].

Molecular dynamics results have not shown good stability during MD simulation at 100 ns, but when we see the stability of protein and ligand individually, there is not much deviation because the protein-ligand simulation runs in a straight line after 15 ns in (Fig. 4a and 40 ns in Fig. 5a). Hence, docking results have shown good interaction, so there is a possibility that the protein-ligand complex can attain stability after 100 ns. Bhosle *et al.* 2021, screened phytoconstituents of *Andrographis paniculata* against various targets of the Japanese encephalitis virus (JEV). They found that molecular docking studies have shown lower docking scores against different targets of JEV, but *in-vitro* studies demonstrated good activity against JEV[23].

Conclusion

Our studies conclude that Capsaicin and dimethyl Fumarate, in terms of binding interactions, could behave as protease-activated receptor-1 and protease-activated receptor-2 selective antagonists, respectively. Other ligands demonstrated good binding energies with targets but could not form required amino acid interactions with them.

Conflict of interest

The authors declare no conflict of interest.

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