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ORIGINAL RESEARCH

Evaluation of wound healing effect of *Mallotus philippensis* (Lam.) Mull. Arg. by in silico multitargets directed for multiligand approach

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Abstract

The healing of wound is a tightly-regulated cascade of events, involving interplay of enormous factors. Now a days, pain alleviation and faster wound healing have attracted considerable attention. Several natural compounds have played crucial role in this intriguing process. The present study deals with five selected molecules from the plant *Mallotus philippensis* (Lam.) Mull. Arg. targeting the eight essential proteins involved in the wound healing and inflammatory process. Considering that various phytoconstituents of medicinal plant can simultaneously interacts with multiple targets, in current work multiligand and multitarget approach was employed instead of traditional one ligand-multitarget approach. Docking studies were performed using AutoDock Vina and molecular dynamics was performed using GROMACS 2019. The current study revealed the potential interactions of five selected constituents with multiple chronic wound healing targets. The wound healing effect of *Mallotus philippensis* (Lam.) Mull. Arg. fruits may be due to combined effect of all these compounds. Effective interactions with the amino acid residues present in the active site of some of the essential proteins involved in the wound healing process also suggests possible mechanism in the wound healing process. The current work thus provides a meaningful insight that *Mallotus philippensis* (Lam.) Mull. Arg. fruits could be used as potential candidate for faster healing of wound. Also, in silico studies depicting interaction with the targets and receptors provide a meaningful insight that this plant would be used as potential candidate for new drug development.

Keywords Wound · Wound healing · Docking · Inflammation · In silico · *Mallotus philippensis* (Lam.) Mull. Arg.

Abbreviations

MP	*Mallotus philippensis* (Lam.) Mull. Arg.
ROTT	Rottlerin
KC	Kamalachalcones C
KD	Kamalachalcones D
MPA	Mallotophilippen A
MPB	Mallotophilippen B
SDF	System data format
PDB	Protein data bank
IL 6	Human interleukin-6
IL-1β	1Interleukin-1 beta
GSK 3	Glycogen synthase kinase 3
NF-κB	NF-kappa B
TGF1-β	Transforming growth factor-beta type I receptor
IGF 1R	Insulin-like growth factor receptor
FGF-1	Fibroblast growth factor-1
MMP 9	Matrix metalloproteinases
a.a	Amino acid

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Background

Wounds are the loss of structural integrity or normal anatomical architecture of skin. Wound healing is the restorative response by tissue which involves complex cascade of cellular events (Sekar et al. 2018). Normal wound healing process is well regulated, orderly process usually characterized by four sequential but overlapping phases mainly haemostasis, inflammation phase, proliferation phase and remodeling. These phases mainly comprise of events like inflammation, cell proliferation, matrix deposition, tissue modeling, collagenation, and epithelialization (Vidya et al. 2012). These phases not only involve various cells and tissues but there

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is the interplay of various sub-stages, mediators, cytokines, enzymes and growth factors (Varghese and Shinde 2021). The first phase haemostasis begins immediately after injury involving vasoconstriction, platelet aggregation and finally clot formation. The factors such as platelet-derived growth factor (PDGF), transforming growth factors (TGFs), the fibroblast growth factors (FGFs), and vascular endothelial growth factor (VEGF) assist in the wound repair process. The second phase inflammation occurs within 24 h of wounding and lasts for two weeks or more. During this phase neutrophils and macrophages come to the site of action and destroy debris, bacteria through phagocytosis. Various growth factors like PDGF, TGF-β, β-FGF, TNF-α, proinflammatory cytokines such as interleukin 1 (IL-1) and IL-6 secreted by macrophages assist in wound healing. Third phase, proliferation phase (4–21 days) various significant events take place such as fibroblast proliferation, collagen synthesis, extracellular matrix reorganization, angiogenesis, granulation tissue formation and reepithelization. Platelet-Derived Growth Factor (PDGF), Connective Tissue Growth Factor (CTGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, transforming growth factor (TGF) β family, Epithelial Growth Factor (EGF), keratinocyte growth factors (KGFs), insulin like growth factor IGF-1 and TGF-α. Fourth, remodeling phase spans between 3 weeks to up to 1 year after injury. This is maturation stage in which reepithelization occurs, extracellular matrix remodeling takes place, tensile strength of skin increases and wound healing occurs. The matrix metalloproteinases (MMPs) play vital role in tissue repair and remodeling (Barrientos et al. 2008; Bodas and Shinde 2021).

Deregulation of normal wound healing process leads to delay in wound healing (usually more than 6–8 weeks) which is termed as chronic wound. Chronic wounds are the significant burden to not only patients but for overall healthcare systems. Chronic wounds are characterized by prolonged inflammation, hypoxia, biofilm formation, increased phagocytic cells, elevated proteases and reactive oxygen species (ROS) and diminished growth factors, cell migration and proliferation (Bjarnsholt et al. 2016).

It has been shown that the proinflammatory cytokines such as interleukins 1α (IL-1α), 1β (IL-1β), and 6 (IL-6) and Tumour necrosis factor alpha (TNF-α); transcription factor NF-κB, enzymes like Glycogen synthase kinase 3 (GSK-3 β), Matrix metalloproteinases (MMP 9); growth factors like Fibroblast growth factor (FGF-2), Transforming growth factor (TGF-β1), Insulin like growth factor (IGF 1) play pivotal role in wound healing process (Barrientos et al. 2008; Bodas and Shinde 2021). Hence targeting these proteins through molecular docking would be beneficial way to provide new wound healing treatment.

Medicinal plants have been choice of interest for treatment of chronic wounds since ancient time. *Mallotus philippensis* (Lam.) Mull. Arg. commonly known as Kamala, Sindur and Rohini is a perennial shrub or small tree grows at an altitude of 300–1600 m. The plant is widely distributed in India, Sri Lanka, southern China, throughout tropical Southeast Asia, Malesia to Australia, West Pacific and the Philippines. The plant is known for its different pharmacological activities such as antimicrobial, antiviral, immunomodulatory, cytotoxic, purgative, anthelmintic, carminative, anti-inflammatory, antioxidant, antidiabetic, antidiarrheal, analgesic and antifertility activity (Tripathi and Chaudhary 2017). The plant is useful in treatment of respiratory, digestive, psychological, excretory, reproductive, skeletal and skin disorders (Kumar et al. 2020). The large number of ethnic groups in Indian subcontinent and all over the world use *Mallotus philippensis* (Lam.) Mull. Arg. for medicinal, ritual and economic purposes. Among all other plant parts, fruits are the most exclusively used part due to its wide array of therapeutic activities (Kumar et al. 2020). The fruits are the treatment of choice for dermatological disorders especially for non-healing and infected wounds. The fruits of the *Mallotus philippensis* (Lam.) Mull. Arg. are covered with glandular hairs coated with reddish exudates called 'Kamala' which can acts as dye and the drug (Furusawa et al. 2005). The fruit glands and hairs are rich in phloroglucinol derivatives like rottlerin, isorottlerin, isoallorottlerin, mallotophilippen A and B (Hemachandran et al. 2018), chalcone derivatives like kamalachalcones A, B, C and D (Furusawa et al. 2005), mallotophilippens C, D, and E (Gangwar et al. 2014).

These phytoconstituents present in fruit may be responsible for the wound healing activity. The acute wound healing activity of *Mallotus philippensis* (Lam.) Mull. Arg. fruit glandular hair extract has been reported (Gangwar et al. 2015). However, no studies had been reported on interactions of various constituents of *Mallotus philippensis* (Lam.) Mull. Arg. fruit with chronic wound healing mediators.

O.M. Oyedemi et al. have already proved that the rottlerin and the red compound in *Mallotus philippensis* (Lam.) Mull. Arg. showed potent activities against a panel of clinically relevant gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). Also, both rottlerin and the red compound strongly inhibited conjugal transfer of the plasmids pKM101, TP114, pUB307 and R6K amongst *Escherichia coli* at a subinhibitory concentration of 100 mg/L. These results show that rottlerin and other compounds are potential candidates for drug lead development. In the view of theses evidences, current study was undertaken (Oyedemi et al. 2016).

Molecular docking is the in-silico approach to study best interactions between the protein and ligand (drug candidate). It's very significant, cost effective and time saving tool in drug design and discovery. The results of in silico study could be used to find out relevant data before conducting

in vitro and in vivo study (Utami et al. 2020). However, for synthetic drugs in silico docking studies with single target and multiple ligands are more prevalent for prediction of their efficacies but for medicinal plants this approach is inadequate. In medicinal plants variety of phytoconstituents are present acting on multiple targets.

Hence, in present study the attempts are made to utilize this docking tool to predict interactions between various phytoconstituents of *Mallotus philippensis* (Lam.) Mull. Arg. fruit and different chronic wound healing mediators. Based on literature survey five phytoconstituents were selected for docking study. We have carried out docking analysis for their selective binding interactions against eight different targets which play crucial role in chronic wound healing.

Methods

Ligand preparations

Based on literature survey five important phytoconstituents such as Rottlerin (ROTT), Kamalachalcones C (KC), kamalachalcones D (KD), Mallotophilippen A (MPA), Mallotophilippen B (MPB) present in *Mallotus philippensis* fruit were selected for studies. The 3D structures of phytoconstituents were retrieved from Pub Chem https://pubchem.ncbi.nlm.nih.gov database in SDF format. The 2D structures have been displayed in Table 1. The 3D structures of ligand were minimize using open babel by MMFF94 force field (Halgren 1996; Boyle et al. 2011). The PDBQT format required for the AutoDock Vina was obtained by using MGL Tool script Preapare_ligand.py (Sanner 1999; Morris et al. 2004). Obtained PDBQT structures of the ligand were used for the docking studies.

Protein preparation

Coordinates of all protein were obtained from the protein data bank (http://www.rcsb.org). As discussed, earlier targets were selected from each category factors by carefully studying wound healing process. They were proinflammatory cytokines such as human interleukin-6, PDB ID: 1ALU (Somers et al. 1997); interleukin-1 beta, PDB ID: 1ITB (Vigers et al. 1997); Enzymes like Glycogen synthase kinase 3, PDB ID: 1Q5K (Bhat et al. 2003); matrix metalloproteinases 9, PDB ID: 5UE4 (Scannevin et al. 2017); transcription factor like NF-kappa B bound as homodimer to DNA, PDB ID: 1SVC (Muller et al. 1995); growth factors such as transforming growth factor-beta type I receptor (PDB ID: 1VJY) (Gellibert et al. 2004); insulin-like growth factor receptor, PDB ID: 2ZM3 (Mayer et al. 2008); fibroblast growth factor-1, PDB ID:4OEF (Li et al. 2014). Coordinates of the protein structure were downloaded in PDB format. Protein preparation each target was carried out using Auto Dock Tools. All the water molecules were removed. Hydrogens were added and Gasteiger charges were added. Atom types were defined and then structures were saved in PDBQT format. These prepared structures were used for further docking studies.

Docking studies

Docking studies for all molecules were performed using AutoDock Vina (Trott 2010). Molecular docking was carried out after creating a grid box of 40 × 40 × 40 Å centered at—center_x 3–center_y − 19.97–center_z 9, 40 × 40 × 40 Å by taking—center_x 30.92–center_y 13.02–center_z 14.8, 40 × 40 × 40 Å by taking—center_x 24.93–center_y 22.45–center_z 9.24, 40 × 40 × 40 Å by taking—center_x 37.5–center_y 14.5–center_z 38.0, 40 × 40 × 40 Å by taking—center_x 15.44–center_y 67.12–center_z 4.38, 40 × 40 × 40 Å by taking—center_x 36.69–center_y 79.9–center_z 58.31, 40 × 40 × 40 Å by taking—center_x 12.55–center_y-19.97–center_z 21.31 and 40 × 40 × 40 Å by taking—center_x 47.5–center_y 36.73–center_z 42.02 for the human interleukin-6 (1ALU, 1.90 Å, 185 a.a), interleukin-1 beta (1ITB, 2.50 Å, chain A 153 a.a and chain B 310 a.a), Glycogen synthase kinase 3 (1Q5K, 1.94 Å, 414 a.a), NF-kappa B bound as homodimer to DNA (1SVC, 2.6 Å, Chain B with 365 a.a), transforming growth factor-beta type I receptor (1VJY, 2.00 Å, chain A with 303 a.a), Insulin-like Growth Factor Receptor 1 (2ZM3, 2.50 Å, Chain A, B, C, D with 308 a.a), fibroblast growth factor (4OEF, 1.80 Å, chain A with 155 a.a), matrix metalloproteinases 9 (5UE4, 1.80 Å, Chains A, B with 236 a.a) respectively. Docking studies performed at exhaustiveness 100 and docking pose outputs is saved in PDBQT format. Docking pose converted to PDB format using open babel (Boyle et al. 2011) which was used for further analysis.

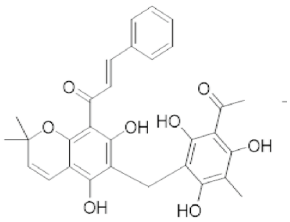
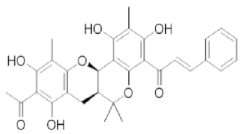
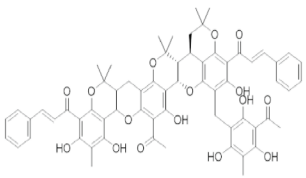
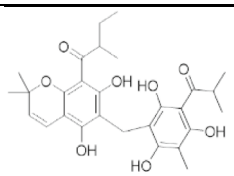
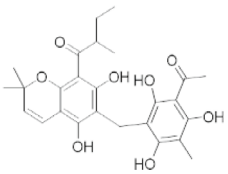
Protein ligand interaction analysis

Docking pose analysis is important part after docking studies. Protein ligand interaction analyzed by the software Protein Ligand Interaction Profiler (PLIP) (Salentin et al. 2015). PLIP analyzed the hydrophobic interactions, hydrogen bonding interaction, pi-pi stacking, cation-pi interactions, halogen bond and salt bridge interaction. It gave output in the text, xml and Pymol session file. Protein ligand interactions were visualized using Pymol session files and from text file all type of interactions were extracted.

Molecular dynamics study

GROMACS 2019 tool was explored for running molecular dynamic simulation (Hess et al. 2008; Abraham et al. 2015).

Table 1 Ligands selected for docking analysis

Compound	PubChem CID & Molecular Formula	Structure
Rottlerin (ROTT)	5281847 $C_{30}H_{28}O_8$	
Kamalachalcones C (KC)	101721039 $C_{31}H_{30}O_8$	
kamalachalcones D (KD)	101721040 $C_{65}H_{62}O_{16}$	
Mallotophilippen A (MPA)	10185281 $C_{28}H_{34}O_8$	
Mallotophilippen B (MPB)	10205431 $C_{26}H_{30}O_8$	

GROMOS 54A7 Force Field was used to develop protein topology (Schmid et al. 2011) and ligand parameters were calculated from automatic topology builder web server (Stroet et al. 2018) for gromacs force field and subsequently, the ligand parameters were appended to protein topology so as to get protein ligand complex file. SPC216 predefined water model was used and to neutralize the protein inhibitor complex, counter ions were added. Further, solvated protein inhibitor complex was subjected for energy minimization using steepest decent algorithm. Thus, minimized solvated structure was heated at 300 K and equilibrated with constant volume and temperature initially and subsequently pressure and temperature. Equilibrated structure was finally subjected for molecular dynamics simulation for 50 ns to explore the stability of the complex. The results of molecular dynamics study trajectories were employed for analysis including radius of gyration, RMSR, structural changes with time function and RMSD. The dynamic study was performed for a docked complex of ROTT with FGF-1 (4OEF) and KD with GSK 3 β (1Q5K).

Results

Docking studies

Binding score of the molecules with the protein is given in Table 2. All the molecules were showing the binding affinity in the range of 6.4–11.1. Binding poses were visualized in Pymol. IL-6 is one of the important targets in wound healing process. KD exhibited maximum interaction with IL-6 as shown in Fig. 1 with binding score − 8.1. In the binding cavity, KD was having three hydrogen bonding interaction. The hydrogen bonds are formed with Lys86, Thr97 and Thr137. KD has formed two salt bridges with Lys70 and Lys86. Moreover, KD was in closed contact with Lys66, Lys66, Glu93, Thr143 and Leu147 where it was having hydrophobic contact. However, Rottlerin forms maximum number hydrogen (09) bonds with receptor 1ALU with amino acid residues such as Arg104, Arg104, Arg104, Glu106, Glu106, Gln156, Gln156, Gln159 and Asp160. Also, maximum hydrophobic interactions (07) were observed with MPA with amino acid residues such as LYS 27, TYR 31, TYR 31, ALA 114, MET 117, MET 117 and PHE 125.

IL-1β is another target which was having good interactions with all molecules but KD has shown the maximum binding with -8.4 binding score as displayed in Fig. 2. It was in close contact with Pro116, Pro116, Val117, Glu202, Glu203, Lys270, Lys270 and Arg271. KD showed hydrogen binding interactions with Phe150, Glu202 and Asn204. However, the numbers of hydrogen bonds are more with Mallotophilippen B involving amino acid residues GLU 202, GLU 202, GLU 256, ALA 268, and LYS 270.

GSK 3 appears to control progression of wound healing and fibrosis by modulating ET-1 level. Among all the eight selected targets GSK 3 was having high binding interactions with all the molecules. GSK 3 was having the highest binding score with KD as depicted in Fig. 3. Among all studies binding of KD with GSK 3 exhibit highest binding score − 11.1. KD was having hydrophobic interactions with Ile62, Ile62, Phe67, Val70, Thr138, Gln185, Leu188, Tyr222 and Pro255. KD was having eight hydrogen bonding interactions with Lys85, Lys183, Asn186, Asp200, Ser203, Arg220, Arg220 and Arg220. Moreover, KD exhibit T type π–π stacking interaction with Tyr140. The extensive hydrogen bonding interactions and π–π stacking interaction may be responsible for highest binding score.

NF-κB activates innate immune reaction, proliferation and cell migration. It also modulates cytokines growth factors and MMP expression indicating potential role in healing process. The KD showed maximum binding score i.e. − 8.8. KD exhibited higher number of hydrogen bonds with NF-κB (PDBID 1SVC) with amino acids like ARG 54, ASP 209, LEU 210, SER 211, TYR 241, ASN 247 and GLN 277. Also, KD was showed hydrophobic interactions with Tyr60, Tyr60, Lys244, Lys147, Lys147, Val150, Leu210, Tyr241 and salt bridge interactions with Lys147, Lys275, Lys278, Arg308 and Arg308. The interaction of KD with NF-κB has been displayed in Fig. 4.

TGF1-β another target which involve in number of stages of wound healing, collagen synthesis, remodeling of new extracellular matrix and scar formation. Chronic and non-healing wounds exhibit less TGF-β1 signaling. Similar to the above result, KD showed maximum binding affinity with TGF1-β with binding score − 10.9 as shown in Fig. 5. KD was having close contact with Lys213, Val219, Val219, Leu340 and Thr375. Also, it was having the hydrogen bonding interactions with Lys213, Arg215, Lys335, Lys337, Asn338, Lys376, Lys376 and Asp435.

IGF 1R has important role in diabetic wound repaired. Surprisingly KC showed highest binding affinity with IGF-1 with binding score − 10.2 as shown in Fig. 6. KC was surrounded by Leu1005, Ala1031, Val1063, Met1079 and Asp1153. Also, KC exhibit two hydrogen bonding interactions with Met1082 and Gly1152. However, MPB exhibited maximum hydrogen bonding with IGF 1R (PDBID 2ZM3)

Table 2 Binding affinities of phytoconstituents of *Mallotus philippensis* (Lam.) Mull. Arg. with different targets

Sr. no.	Targets (with PDBID)	Ligands				
		KD	KC	ROTT	MPA	MPB
1	IL-6 (1ALU)	− 8.1	− 7.5	− 7.3	− 7	− 6.4
2	IL-1β (1ITB)	− 8.4	− 8.1	− 8.2	− 7.9	− 7.4
3	GSK 3 β (1Q5K)	− 11.1	− 9.9	− 8.6	− 8.6	− 7.8
4	NF-κB (1SVC)	− 8.8	− 7.7	− 7.4	− 7.3	− 6.9
5	TGF1-β (1VJY)	− 10.9	− 8.7	− 9.1	− 8.1	− 7.6
6	IGF 1R (2ZM3)	− 8.4	− 10.2	− 8.5	− 7.6	− 7.7
7	FGF-1 (4OEF)	− 7.1	− 7.4	− 7.9	− 6.6	− 6.6
8	MMP 9 (5UE4)	− 8.4	− 8.2	− 7.1	− 6.8	− 6.5

KC, KD, ROTT, MPA, MPB, represents kamalachalcone C, kamalachalcones D, rottlerin, mallotophilippen A, mallotophilippen B

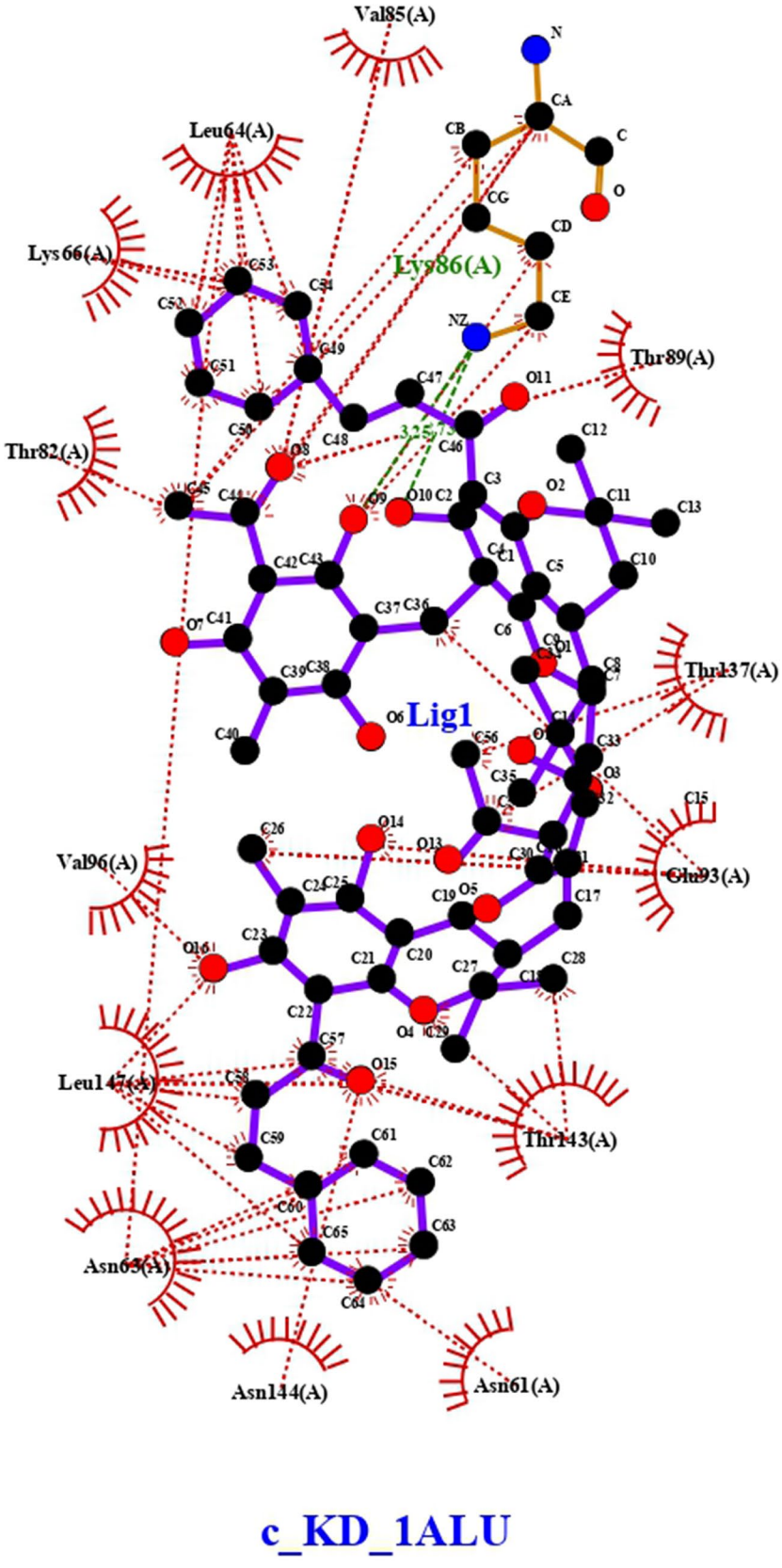


Fig. 1 Interaction of KD with 1ALU

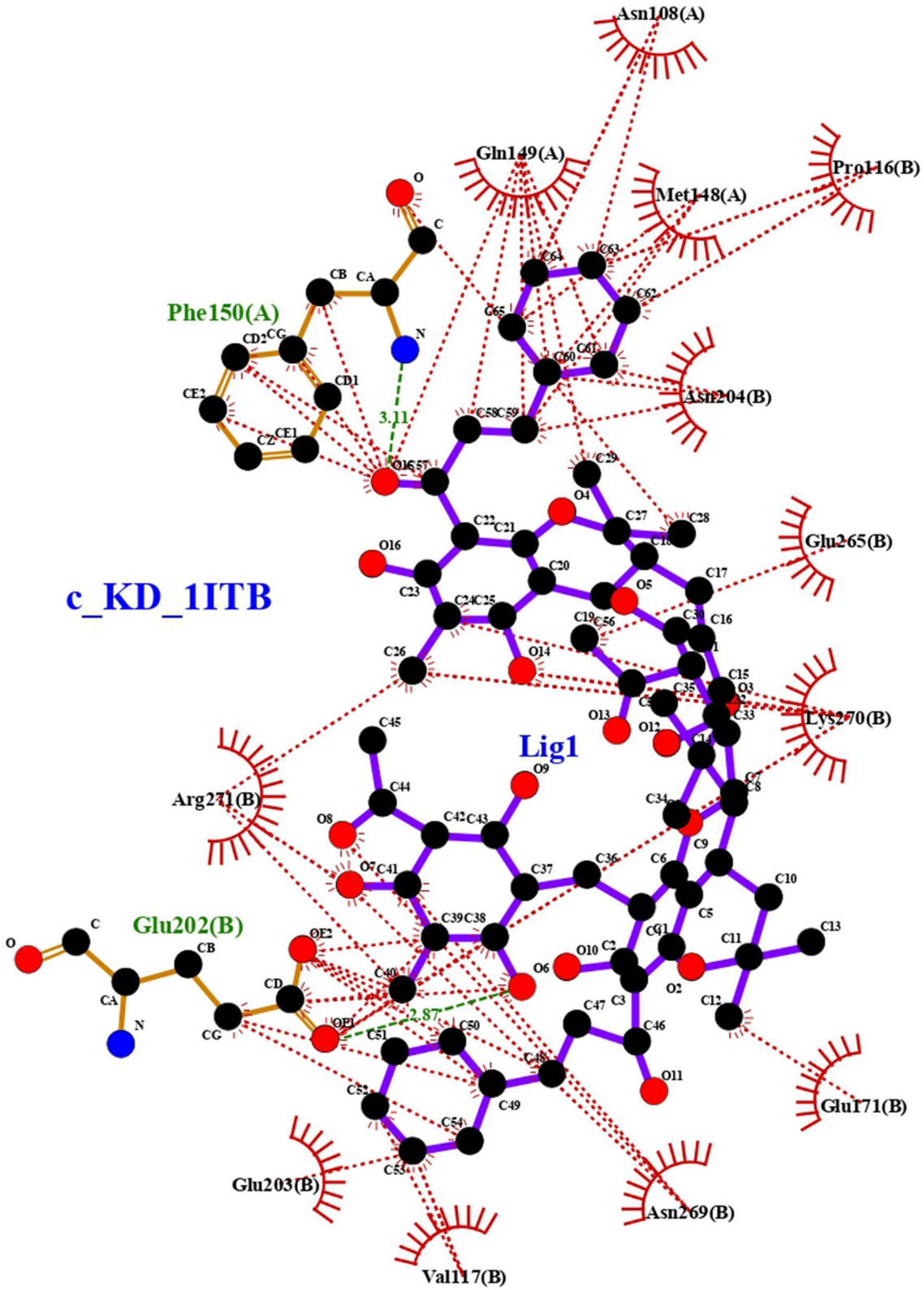


Fig. 2 Interactions of KD with 1ITB

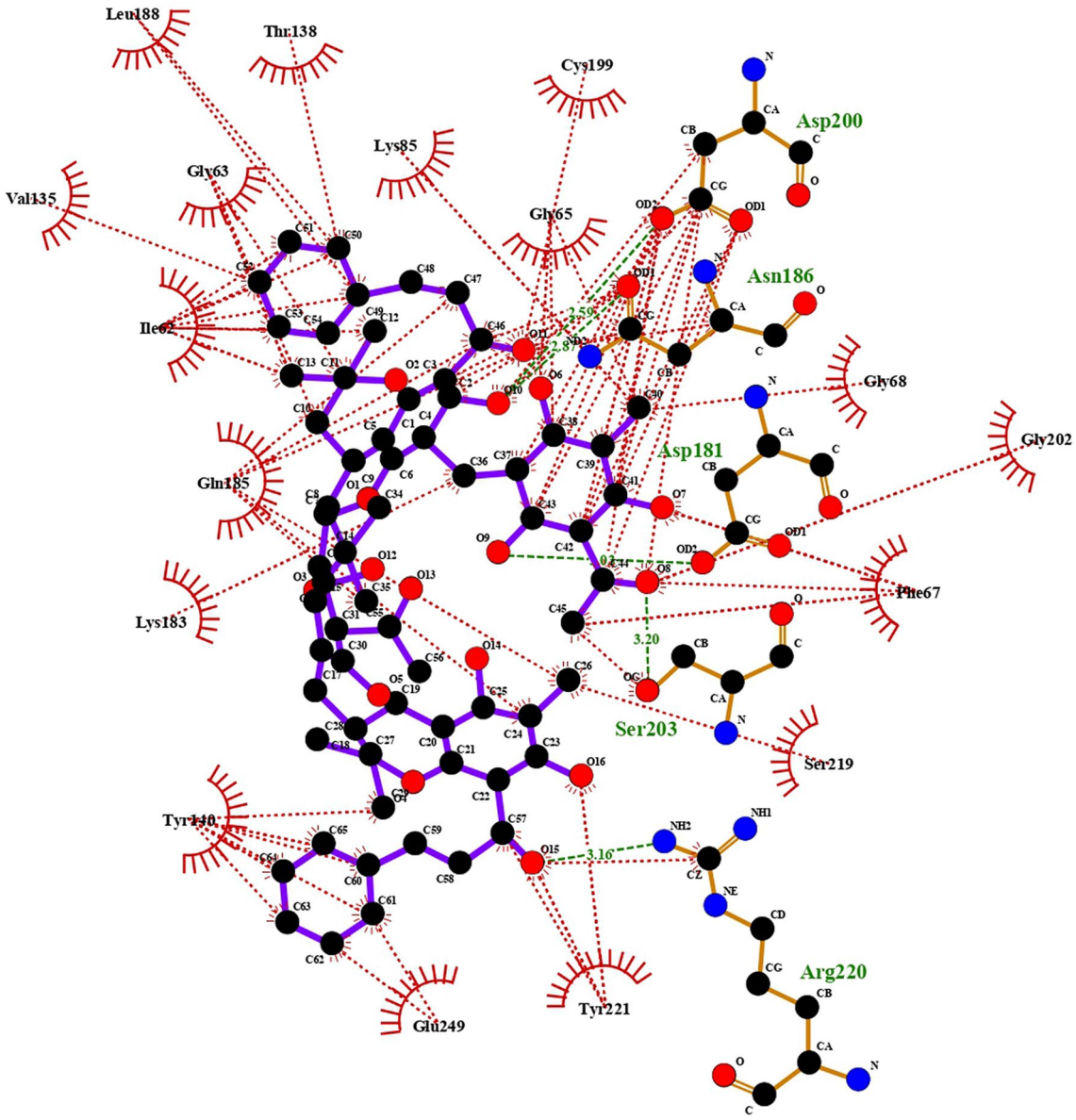


Fig. 3 Interactions of KD with 1Q5K

with amino acid residues such as GLN 1007, SER 1009, SER 1009, ARG 1139, ARG 1139 and ASN 1140.

The FGF-1 has role in tissue repair and regeneration. In average, FGF-1 exhibit lower binding score for all molecules. ROTT has showed highest binding with FGF-1 with binding score − 7.9 as depicted in Fig. 7. ROTT having hydrophobic interactions with Arg39, Arg39, Leu83, Leu83, Gln123, Tyr124, Leu126 and Leu126. ROTT was having hydrogen bonding interactions with Val40, Arg81, Arg81 and Tyr124. Also, ROTT was showing π–π stacking interactions with Tyr124. Mallotophilippen B also showed maximum number of hydrogen bonds with FGF-1 (PDBID 4OEF) involving residues such as VAL 40, VAL 40, ARG 81, ARG 81 and TYR 124.

Up regulation of MMP-9 is detrimental for wound healing process. The MMP-9 helps in wound healing by modulating angiogenesis. KD showed the highest affinity with MMP-9 with binding score − 8.4. KD was having hydrophobic interactions with Gln43, Leu44, Tyr48, Tyr48, Tyr52, Arg98, Tyr179, Asp182 and Leu187. Also, KD was forming π–π stacking interactions with Tyr179. But ROTT showed highest number of hydrogen bonds with MMP-9 with amino acid residues such as GLY 213, GLN 216, PHE 250, LYS 184, LEU 209, TYR 218, TYR 248, PHE 250 and PHE 250 (Fig. 8).

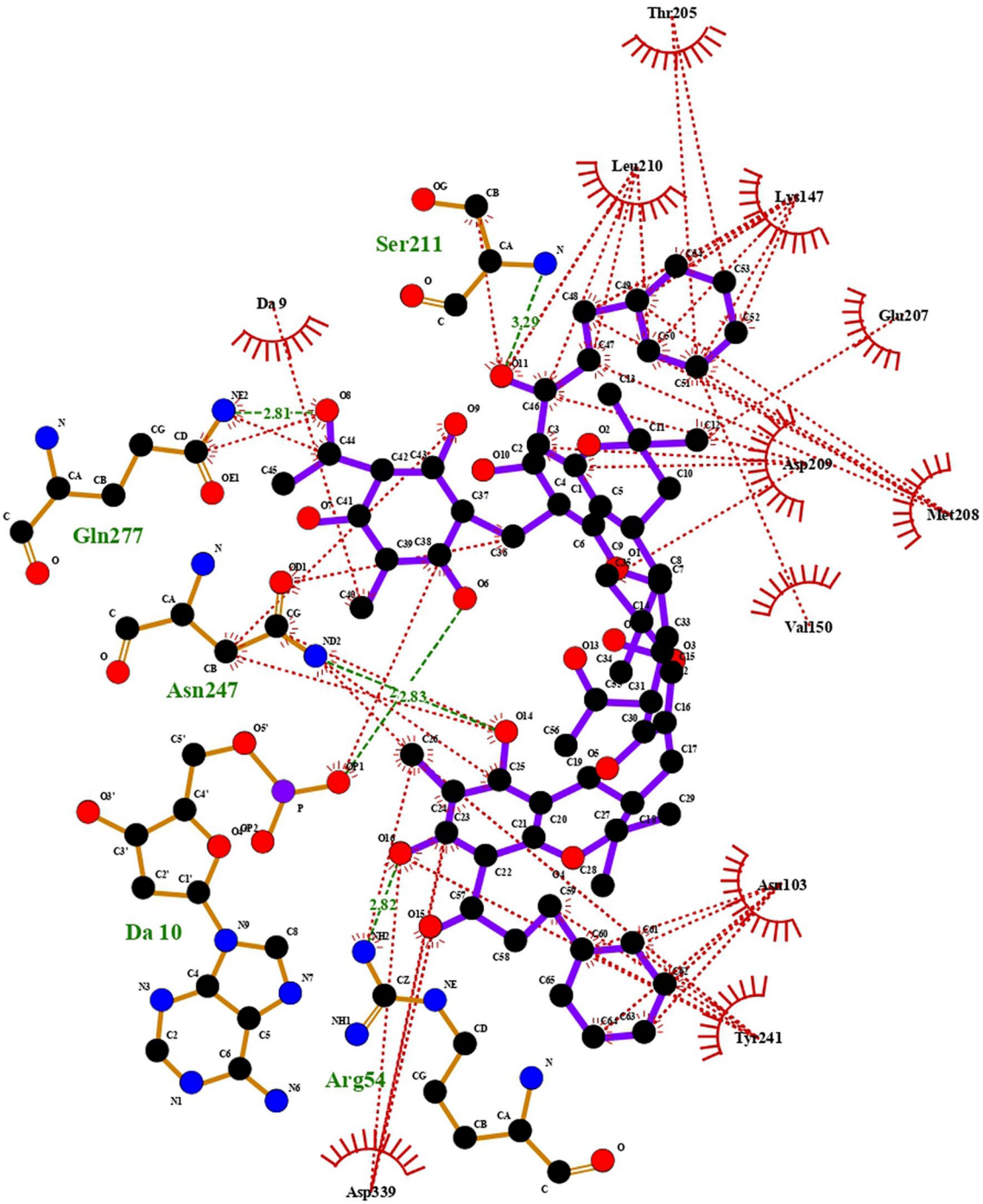


Fig. 4 Interactions of KD with 1SVC

Protein ligand interaction analysis

Protein ligand interactions like hydrophobic interactions, hydrogen bonding, pi–pi stacking, cation-pi interaction, salt bridge and halogen bond were studied. The protein ligand interaction is stronger if the numbers of hydrogen bonds are more. The significant interactions of molecules with different targets have been displayed in Table 3.

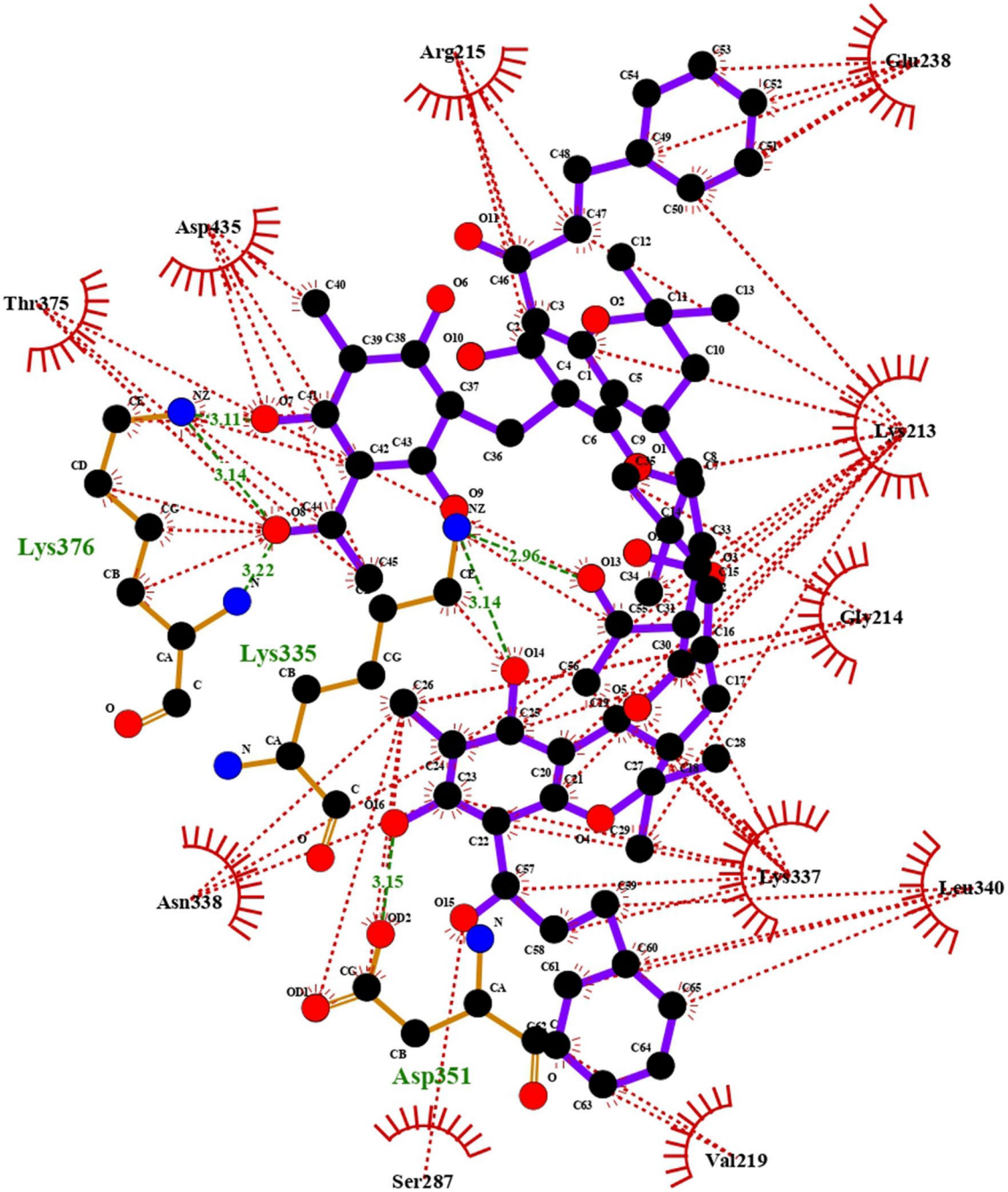


Fig. 5 Interactions of KD with 1VJY

Molecular dynamics study

Molecular dynamics simulation was performed on for docked complex of ROTT with FGF-1 (4OEF) and KD with GSK 3 β (1Q5K) to check the stability. The trajectory of MD simulation thoroughly analyzed to address the stability of the complex by calculating RMSD, RMSF and radius of gyration and manual visualization of protein ligand complex. The RMSD give information of the stability of the complex. The lower RMSD indicates the higher stability of protein ligand complex and higher RMSD indicate the lower stability. In case of ROTT, RMSD of protein backbone was observed in the range of 4–5 Å (Fig. 9). Initially protein backbone RMSD started at 2 Å which was the increase continuously of 5 Å till 15 ns. It remained same up to 30 ns where it decreased to 4.4 ns and remained same till the 50 ns. The ligand RMSD was started at 1.1 Å and remained same throughout the MD simulation. The high RMSD of protein backbone may be

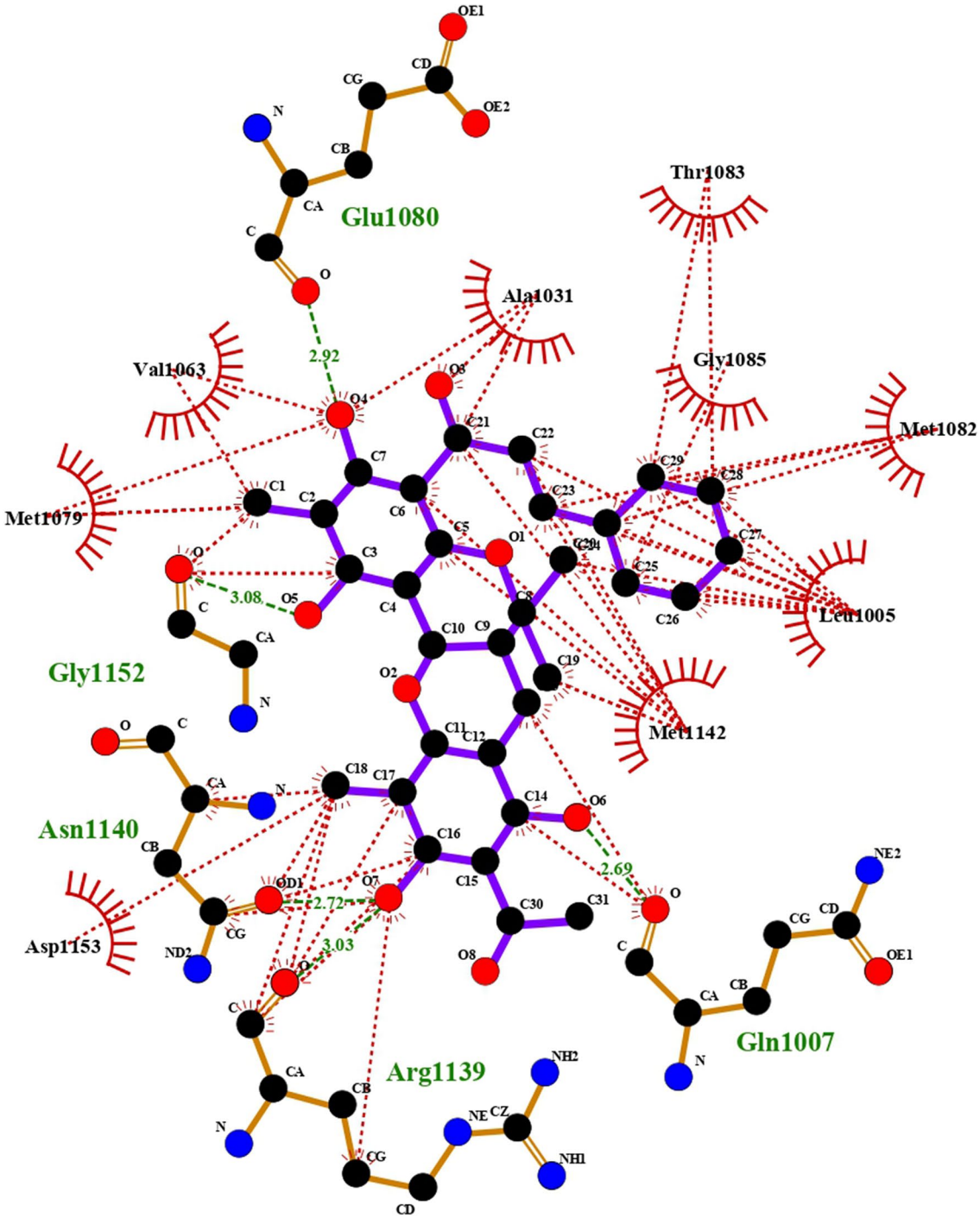


Fig. 6 Interactions of KC with 2ZM3

due to the high flexibility of the N-terminal loop present in the structure. The RMSF is another criterion to measure the stability of protein complex. The RMSF gives the fluctuation of each amino acid during the MD simulation. The RMSF graph show that the fluctuation was within the range. The terminal amino acids were showing the high fluctuation and the binding site amino acids were shown the lower fluctuation. High RMSF was observed for the loop preset at N-terminal. The superimposed pose of the conformation at the starting and end of the simulation showed that there was no much difference (Fig. 10). Overall MD simulation studies supported the stability of docked complex. In case of KD,

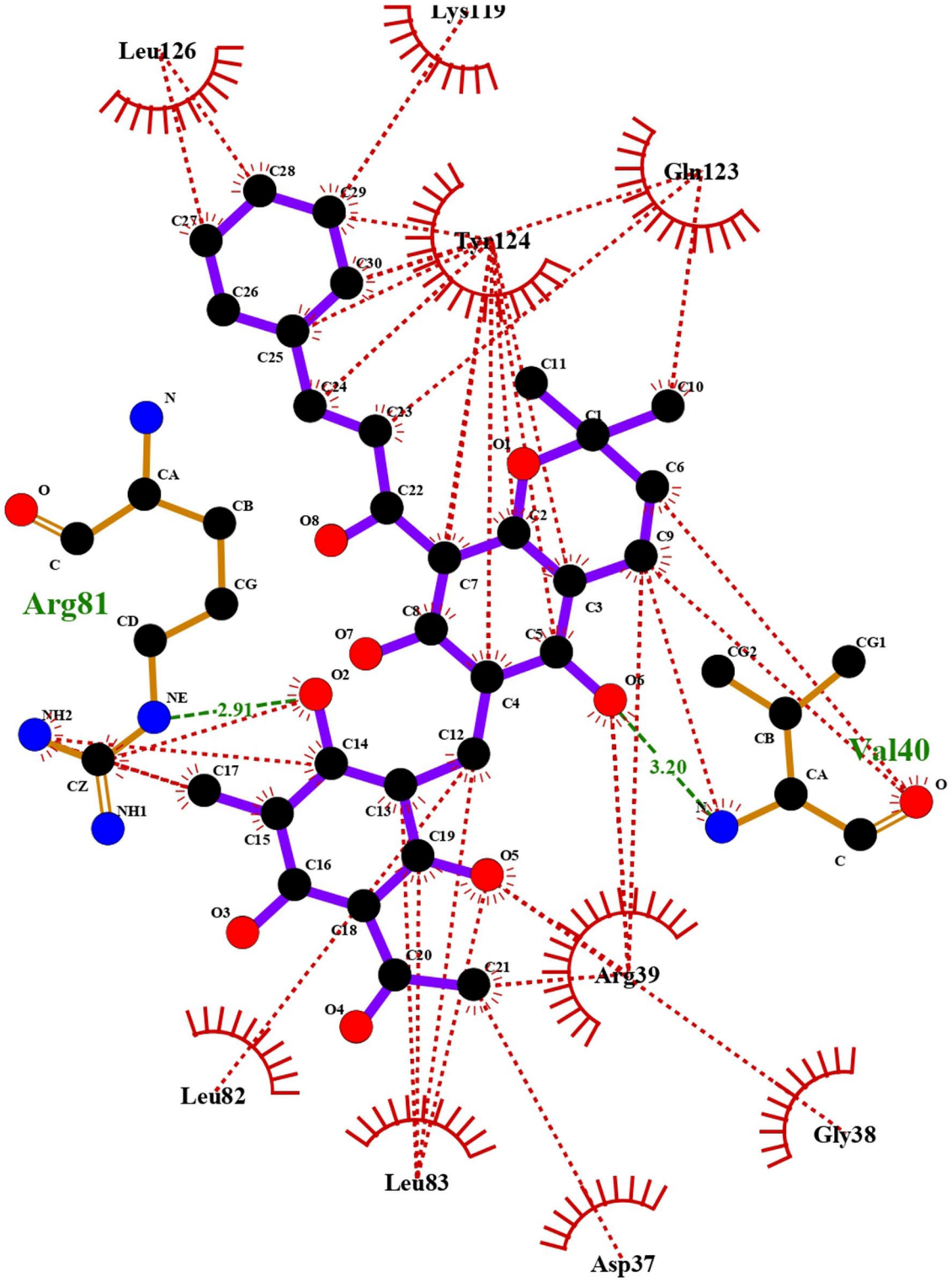


Fig. 7 Interactions of ROTT with 4OEF

RMSD of protein backbone was observed to be higher so it was not the stable complex (Figs. 11 and 12).

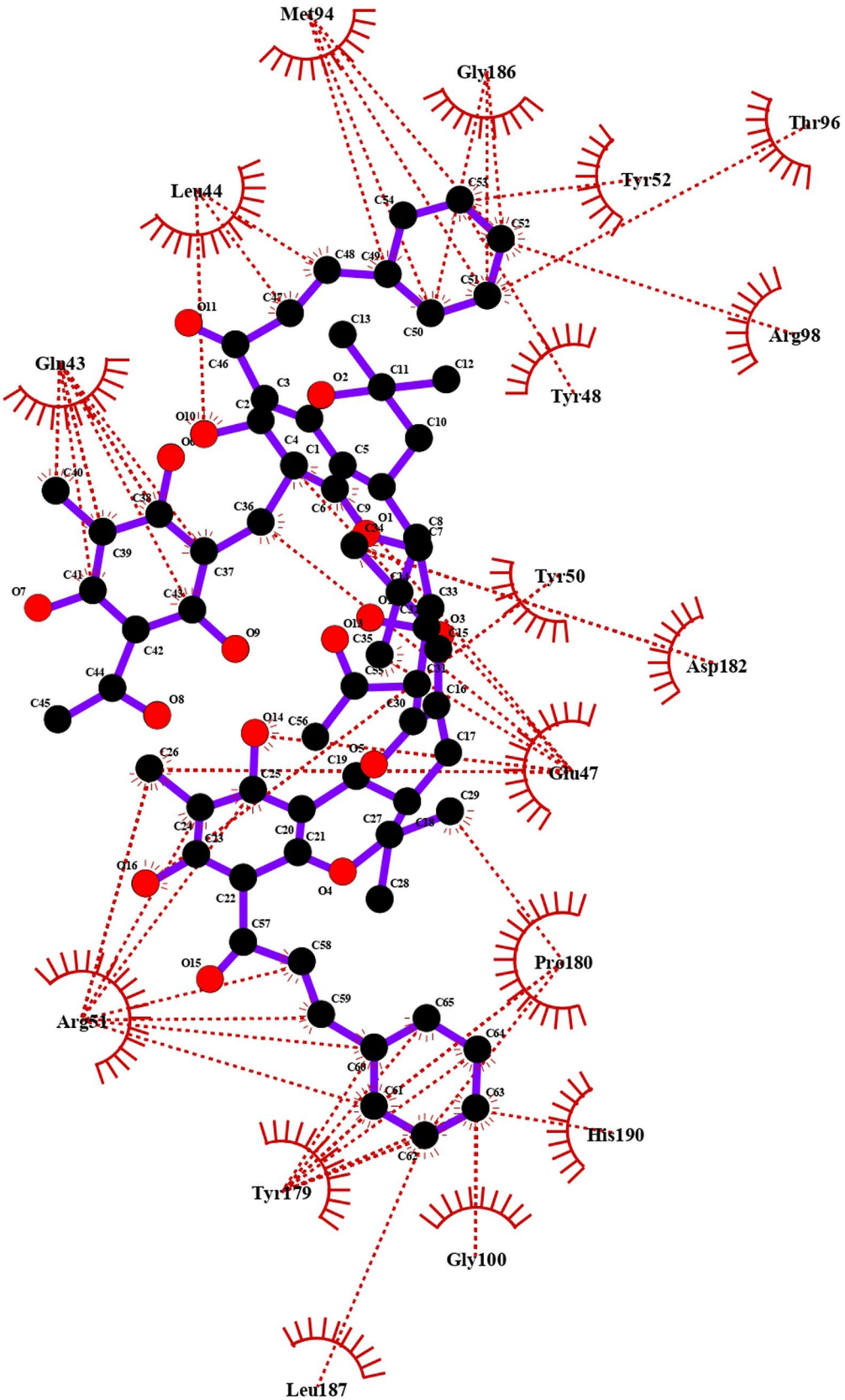


Fig. 8 Interactions of ROTT with 5UE4

Table 3 Docking results revealing significant interactions with target and distance

Name and target	Hydrogen bonding interactions	Hydrophobic interactions	Other type of interactions
KD 1ALU	LYS86: 2.51, TYR97: 3.33, THR137: 2.13	LYS66: 3.75, LYS66: 3.79, GLU93: 3.49, THR143: 3.62, LEU147: 3.57	LYS70: 4.95 (salt bridge), LYS86: 3.31 (salt bridge)
KD 1ITB	PHE150: 2.11, GLU202: 2, ASN204: 2.77	PRO116: 3.44, PRO116: 3.64, VAL117: 3.63, GLU202: 3.6, GLU203: 3.9, LYS270: 3.54, LYS270: 3.61, ARG271: 3.99	–
KD 1Q5K	LYS85: 2.52, LYS183: 2.48, ASN186: 2.47, ASP200: 2.51, SER203: 2.27, ARG220: 3, ARG220: 3.45, ARG220: 2.53	ILE62: 3.77, ILE62: 3.61, PHE67: 3.61, VAL70: 3.93, THR138: 3.88, GLN185: 3.72, LEU188: 3.69, TYR222: 3.97, PRO255: 3.93	TYR140: 4.82 (Pi–Pi)
KD 1SVC	ARG57: 2.91, ARG59: 3.59, TYR60: 1.95, GLU63: 2.37, GLU63: 1.99, GLU63: 3.5, HIS67: 3.33, LYS147: 1.74, LYS244: 2.47, GLN277: 2.03, GLN309: 1.92, ARG54: 2.42, ASP209: 2.5, LEU210: 2.56, SER211: 2.7, TYR241: 2.58, ASN247: 2.07, GLN277: 3.04	TYR60: 3.74, TYR60: 3.93, LYS244: 3.93, LYS147: 3.58, LYS147: 3.63, VAL150: 3.77, LEU210: 3.63, TYR241: 3.48	LYS147: 4.21 (salt bridge), LYS275: 4.42 (salt bridge), LYS278: 4.74 (salt bridge), ARG308: 5.08 (salt bridge), ARG308: 5 (salt bridge)
KD 1VJY	LYS213: 2.7, ARG215: 2.67, LYS335: 2.24, LYS337: 2.96, ASN338: 2.9, LYS376: 2.25, LYS376: 2.1, ASP435: 2	LYS213: 3.8, VAL219: 3.94, VAL219: 3.57, LEU340: 3.58, THR375: 3.73	–
KC 2ZM3	MET1082: 2.51, GLY1152: 2.73	LEU1005: 3.77, ALA1031: 3.75, VAL1063: 3.79, MET1079: 3.81, ASP1153: 3.62	–
ROTT 4OEF	VAL40: 2.32, ARG81: 2.31, ARG81: 1.95, TYR124: 2.29	ARG39: 3.59, ARG39: 3.79, LEU83: 3.78, LEU83: 3.89, GLN123: 3.49, TYR124: 3.98, LEU126: 3.51, LEU126: 3.86	TYR124: 4.8 (Pi–Pi)
ROTT 5UE4	GLY 213: 2.67, GLN 216: 3.23	GLY 213: 2.67, GLN 216: 3.23, PHE 250:2.24, LYS 184:3.61, LEU 209:3.98, TYR 218:3.54, TYR 248: 3.74, PHE 250: 3.81, PHE 250: 3.85	LYS 184: 4.71 (cation Pi)

KC, KD, ROTT, MPA, MPB, represents kamalachalcones C, kamalachalcones D, rottlerin, mallotophilippen A, mallotophilippen B

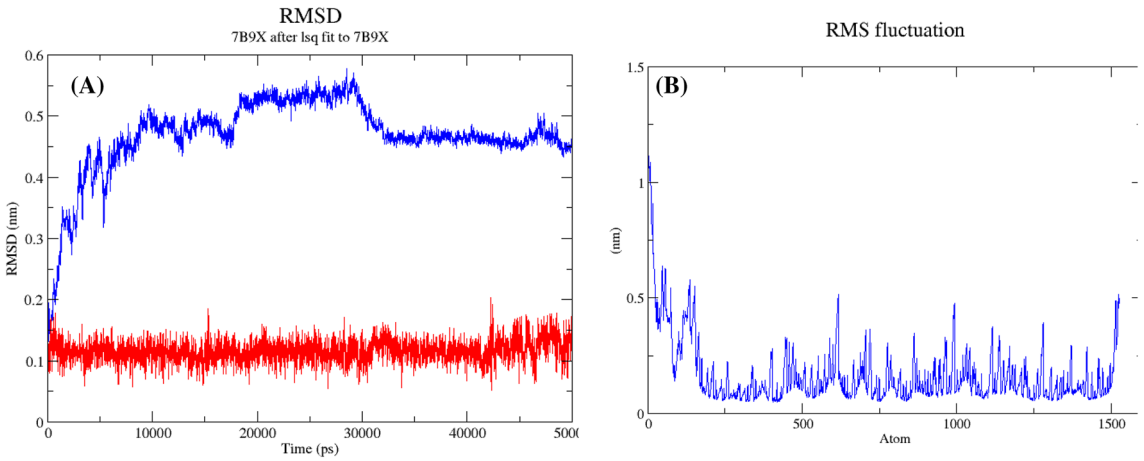


Fig. 9 MD trajectory analysis for a docked complex of ROTT and 4OEF

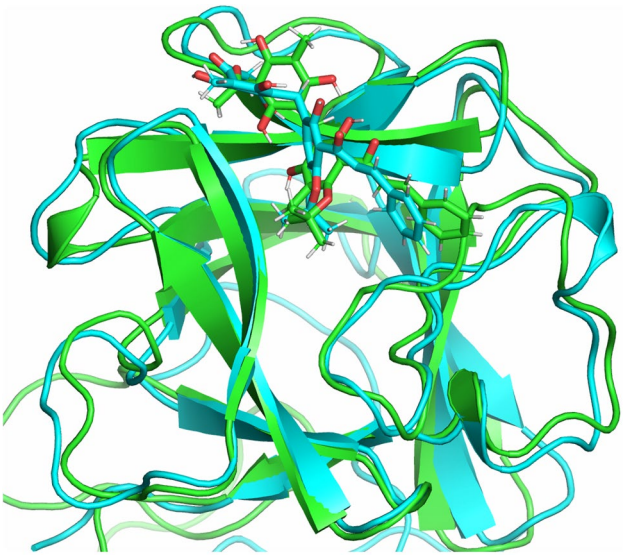


Fig. 10 Superimposed pose of configurations of ROTT and 4OEF

Discussion

All the five selected constituents from *Mallotus philippensis* (Lam.) Mull. Arg. fruit were assessed for their potential wound healing ability by analyzing their interactions with specific proteins playing crucial role in chronic wound healing phases. Medicinal plants comprise of various phytoconstituents which can interact with multiple targets of physiological process. Hence the binding affinity of each of the constituent was studied with each target for predicting their efficacy.

The proinflammatory cytokines such as interleukins 1α (IL-1α), 1β (IL-1β), and 6 (IL-6) and TNF-α are low molecular weight proteins. These play crucial role in wound healing process such as stimulation of keratinocyte, fibroblast proliferation, immune response modulation, synthesis and breakdown of extracellular matrix proteins, and fibroblast recruitment to the wound site (Agyare et al. 2019). The balance between pro and anti-inflammatory cytokines is lost during chronic wounds. It leads to increased levels of 1α (IL-1α), 1β (IL-1β), and 6 (IL-6) and TNF-α causing persistent inflammation (Satish 2015). Over expression of IL-1β and IL-6 inhibits proliferation and migration of fibroblast and keratinocyte, leading to delay the epithelization and granulation process. FGF-2 synthesis decreases due to inhibition of fibroblast proliferation, resulting decline of regulation of TGF-β1 expression. It ultimately hinders the endothelial cell proliferation, neo- angiogenesis, vasculogenesis and the wound healing process (Dharshan 2018). Hence the modulation of these inflammatory mediators could aid the wound healing process.

Docking of molecules with IL-6 indicates the higher binding affinity of KD. For current docking study we have selected two significant cytokines IL-1β and IL-6. Docking analysis of all five constituents of *Mallotus philippensis* (Lam.) Mull. Arg. indicated that KD showed highest binding affinity followed by KC, ROTT, MPA and MPB with IL6 (PDBID1ALU). Rottlerin form highest number of hydrogen bonds while highest number of hydrophobic interactions was observed with MPA. KC showed pi stack interaction with residue PHE.

With IL-1β (PDBID 1ITB) KD exhibited highest binding affinity with dock score − 8.4 kcal/mol followed by rottlerin, KC, MPA and MPB. The numbers of hydrogen bonds were more with MPB and the hydrophobic interactions were more with KD.

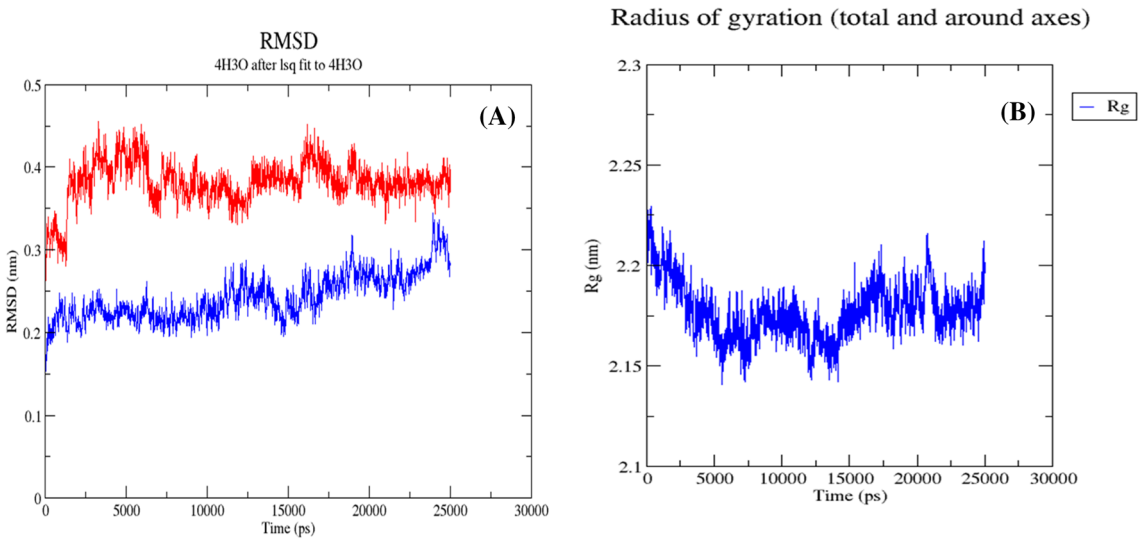


Fig. 11 MD trajectory analysis for a docked complex of KD with GSK 3 β (1Q5K)

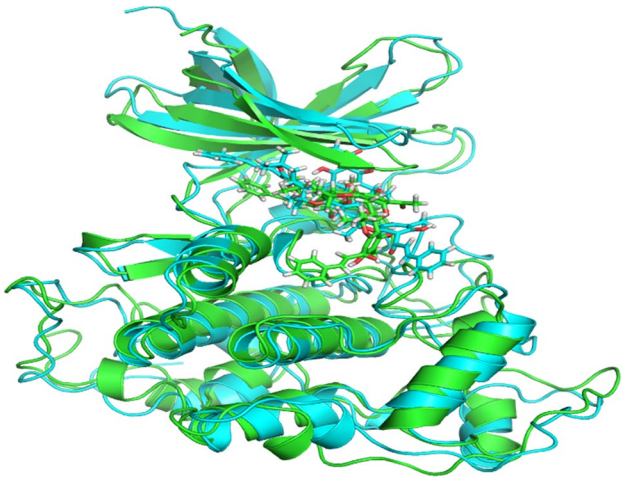


Fig. 12 Superimposed pose of configurations of KD with GSK 3 β

The transcription factor NF-κB plays a pivotal role in wound healing due to their anti-inflammatory, anti-oxidant effects and immune response. Its main function is to monitors gene expression involved in inflammatory process, oxidative stress response and controls differentiation, proliferation, apoptosis, cell adhesion. It regulates expression of matrix metalloproteinases, secretion and stability of cytokines and growth factors. The classical NF-κB pathway gets activated as an innate immune reaction during wound healing. However, overexpression or deactivation of NF-κB leads to impaired wound healing. Hence, targeting the NF-κB signaling pathway would be the attractive approach to treat chronic wounds (Ambrozova et al. 2017). Among the five selected constituents Kamalachalcones D exhibited higher number of hydrogen bonds with NF-κB (PDBID 1SVC).

Wnt are the glycoproteins involved in cell differentiation processes. It is known that genes encoding for Wnt express during skin regeneration process. There are two types of Wnt signaling pathway the canonical pathway or Wnt/b-catenin pathway and the noncanonical pathway. Wnt/b-catenin pathway through inhibition of GSK-3 β, an important regulatory enzyme can promote wound healing (Harish et al. 2008; Vidya et al. 2012); Docking analysis with GSK3β (PDBID 1Q5K) exhibited highest binding affinity with KD followed by KC, ROTT, MPA and MPB.

Matrix metalloproteinases (MMPs) are the class of 24 known matrix degrading and proteolytic enzymes found in extracellular matrix (ECM). Under normal conditions, MMPs are in balanced stage but if deregulated due to oxidative stress at wound site, it leads to degradation of newly formed ECM. It also inhibits cell migration and break down growth factors. It delays wound healing process (Mokhtar et al. 2021). It has been proved that high level of MMP 9 is indicator of inflammation and poor wound healing. Therefore MMP 9 inhibitors could be promising strategy to develop wound healing drugs (Hariono et al. 2018). Docking analysis of MMP 9 (PDBID 5UE4) showed highest number of hydrogen bonds with ROTT and maximum hydrophobic interactions with KD.

Growth factors play a key role in regulation and promotion of wound healing. Variety of growth factors regulating cell migration, proliferation, and synthesis of extracellular matrix (ECM) proteins includes epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin growth factor (IGF), granulocyte macrophage colony stimulating factor (GM-CSF), platelet derived growth factor

(PDGF), Transforming growth factor β (TGF-β), fibroblast growth factor (FGF) and connective tissue growth factor (CTGF). The complex wound healing process involves coordinated efforts of various cells, growth factors, chemokines and cytokines (Barrientos et al. 2008). The proteolytic environments formed during chronic wounds disrupts this balance by degrading growth factors, inhibiting their function or by downregulating their receptors (Zhao et al. 2020). Therefore, targeting these receptors would be the novel strategy of wound healing treatment. We have selected IGF, TGF-β and FGF which play pivotal role in inflammation, proliferation, granulation tissue formation, reepithelialization and remodeling phases of wound repair.

Docking analysis of TGF1-β (PDBID 1VJY) with *Mallotus philippensis* (Lam.) Mull. Arg. phytoconstituents indicated maximum hydrogen bond formation with KD while maximum hydrophobic interactions with MPA.

MPB exhibited maximum hydrogen bonding with IGF 1R (PDBID 2ZM3) followed by ROTT and KD. Hydrophobic interactions were more with MPB followed by MPA and KC.

With FGF-1(PDBID 4OEF) maximum number of hydrogen bonds and hydrophobic interactions was observed with MPB and ROTT respectively.

The MD simulation study predicted stable interaction of ROTT with FGF-1 (4OEF) on the contrary to the interaction of KD with GSK 3 β (1Q5K). So, ROTT was found to be better as per simulation study.

Overall, all the values of binding affinities were negative hence all the phytoconstituents exhibited affinity towards various receptors. All the molecules were showing the binding affinity in the range of 6.4–11.1. Among 5 compounds KD showed highest binding affinities with 6 targets mainly IL-6, IL-1β, GSK 3, NF-κB, TGF1-β and MMP 9. The second-best scored ligand KC showed dock score ranging from − 7.4 to − 10.2. This may be because of complexity of the KD and high number of hydrogen bond donors and acceptors larger surface area. Rottlerin with dock score ranging from − 7.1 to − 9.1. KC exhibited highest binding affinity with IGF 1R while ROTT presented highest score with FGF-1. As compared to aforesaid compounds MPA and MPB displayed lesser binding affinity to the 8 targets with the dock score oscillating from − 6.4 to − 8.6. Among these two compounds MPA showed higher binding affinity for 6 targets as compared to MPB.

The results implied that all the compounds had some of the binding affinity and interactions towards each of the selected target. Also, the available literature clearly suggested the antibacterial activity of *Mallotus philippensis* (Lam.) Mull. Arg. Antimicrobial activity and inflammation are two important sides of wound healing process. Current study clearly suggested its potential of modulation of inflammation and other factors involved in wound healing process.

Hence, according to the predict of multitargets directed for multiligand approach, the wound healing effect of *Mallotus philippensis* (Lam.) Mull. Arg. fruits may be due to combined effect of all the compounds.

Conclusion

Current in silico study clearly demonstrated that phytoconstituents of *Mallotus philippensis* (Lam.) Mull. Arg. interacted with multitargets of chronic wound healing process indicating their significant propensity as wound healer and possible additive effect. This also necessitate further study to support our predict efficacy for better wound healing. The present study also proposed the new multitargets directed for multiligand approach to predict efficacy of herbal drugs against various pathological circumstances.

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