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INSIGHTS INTO THE MOLECULAR DOCKING STUDY OF WEDELOLACTONE TO IMPROVE THE PERFORMANCE OF COMPUTER-AIDED DRUG DESIGN

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ABSTRACT

The structure based docking of a ligand molecule to a biological receptor urge for an efficient sampling of possible docked poses in the binding vicinity of the target molecule to assure the optimal binding. A computational molecular docking is commonly used to study the molecular interactions in drug design. Most of the docking algorithms use receptor as rigid and ligand as flexible molecule which may lead to decrease in an accuracy of predicted docked poses. The MD simulation gives an insight into the dynamics of biological macromolecules. Therefore prior to molecular docking dynamics study of the receptor molecule may result in more accurate docking results. The contribution of MD (Molecular Dynamic) simulation along with docking in a study on human 5-LOX (lipoxygenase), the key player in the inflammatory cascade, was evaluated. Using MD simulation and web based automated molecular docking procedure, it was found that short MD simulations prior to molecular docking significantly improved the docking results. Extensive analysis of the results has revealed that MD simulation generated snapshots have shown the better binding affinity towards wedelolactone in contrast to its crystal structure selected from PDB.

KEY WORDS: Molecular dynamics simulation, Structure-based docking approach, Wedelolactone, Protein flexibility.

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1. INTRODUCTION

Computational docking has added a new paradigm to drug discovery. Molecular docking is the most popular method used in computational docking due to easily available 3D structures of biological targets[1]. Computational methods used in computer aided drug design offers user's defined protocols which influence the performance of docking[2]. Despite of ease of molecular docking in drug discovery it has some drawbacks too. Most of the docking algorithms use rigid receptor/flexible ligand model[3]. Today major challenge in the field of drug designing through rigid receptor docking methods is the misleading ligand binding sites and poor docking scores that results in ineffective drug designing. In nature proteins undergo conformational change during interactions and combine with ligand in an energetically favourable position[4-5]. Such specific changes are of immense importance in drug designing. Studies support the conformational changes in active site of biological molecules while binding with ligands or solvents[6]. Rigid receptors may also influence the ligand sensitivity and selectivity which can be overcome by molecular dynamics approaches which simulate the target biological molecules. In the present work, the molecular docking of wedelolactone with human lipoxygenase (5-LOX) has been assessed. 5-LOX is the key player in the inflammatory cascade contributing towards the angiogenesis, tumor cell invasiveness, and disruption in the pathways of cellular proliferation/apoptosis. Various LOX products have been linked to tumerigenesis in experimental models and consecutive inhibition of LOX metabolism has been targeted for developing anticarcinogenic intervention[7]. *Eclipta prostrata L.* is one of the most important medicinal plants of the traditional medicinal system. It is reported to possess antiseptic, analgesic, antipyretic, antispasmodic, antimalarial and antiviral properties. It has also been reported to be a good hepatoprotective, anti-inflammatory and rejuvenator too. Studies have also reported its inhibitory activity against HIV-1 integrase[8]. Main active principles consist of coumestans like wedelactone, desmethylwedelolactone, furanocoumarins, oleanane & taraxastane glycosides. Wedelactone found commonly in the aerial parts of the plant is the active ingredient used in many medicines. Research on wedelolactone has gained pace during the last decades owing to its multifarious responses on cancer[9-10], hepatitis C virus[11] and as an antioxidant[12]. The molecular dynamics simulation of a total length of 1 ns has been performed to improve the results of molecular docking and then evaluated the performance of the docking on each snapshot with respect to its crystal structure.

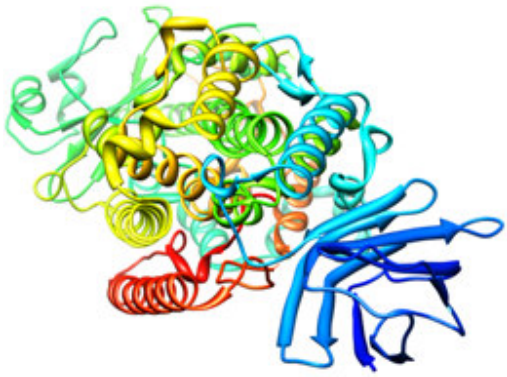
2. MATERIALS AND METHODS

A human lipoxygenase (5-LOX) was used to perform the MD simulation and docking studies. The initial coordinates of the target protein were taken from high-resolution crystal structure, with PDB code 3V99 (at 2.25 Å). All the hetero atoms such as FE2 and ACD were removed and only the proteins atoms were retained. The final model of human lipoxygenase is shown in Fig. 1. Before performing docking studies of the ligand wedelolactone with human 5-LOX, the MD simulation was performed to optimize the molecular-docking.

2.1. Molecular Dynamics set up

The MD simulation was performed with the GROMACS software package[13-14] using Amber force field. The structural model was solvated with TIP3P water model[15] in a cubic periodic box with a 9 Å solute-wall minimum distance. Solvation resulted in 23727 water molecules added in the system during simulation. 21 sodium ions were added to neutralize the overall system charge. The entire system was minimized using a combination of steepest descent followed by conjugate gradient method until no significant energy change could be detected. Particle Mesh Ewald (PME)[16] summation was used for long-range electrostatics. The explicit solvent MD simulation was performed under NPT conditions. A 10 Å Van der Waals cut-off was set and the coupling time constants τ_p and τ_t were kept 0.5 ps for both pressure and temperature. The time step was set at 2 fs and SHAKE[17] algorithm was applied to hydrogen involving bonds. The temperature of the system was increased from 0-100 K during 0-100 ps, 100-200 K during 100-200 ps and 200-300 K in next 100 ps and then kept constant at 300 K using Berendsen algorithm[18]. The trajectories were stored at every 100 ps interval of the simulation. The trajectories of first 500 frames were considered as the equilibration phase and the remaining trajectories were used in our analysis. The snapshots generated were stored at 100 ps interval of the simulation.

Figure.1
The structural model of human lipoxygenase (PDB code: 3V99).



2.2. Molecular docking studies

Since Protein-Ligand interactions play a significant role in structure based drug designing[19] a structure-based blind docking approach was implemented as a computational docking in the present work. All molecular-docking experiments were performed using web based programs (SwissDock[20] and DockingServer[21]. The docking studies were performed between selected snapshots generated through MD simulation and the crystal structure of human LOX-5. Different parameters were used to identify the best mode of the ligand wedelolactone against snapshots and the crystal structure. The physical properties of the ligand are given in binding Table 1.

Table 1
Physical Representations of wedelolactone

H-bond donors	H-bond acceptors	Molecular weight (g/mol)	Rotatable bonds
3	7	314.24	1

2.2.1. Parameter selection for molecular-docking studies

To get an insight into an accurate docking, slow docking was set as a default parameter. To avoid false positive or false negative results blind docking approach and repeats of runs with the same ligand were carried out. Docking Server (http://www.dockingserver.com/)**:** The server uses MMFF94 force field to minimize the energy of ligand molecules. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools[22]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.
SwissDock (http://www.swissdock.ch/)**:** It optimizes the orientation, position, and conformation of a ligand interacting with a protein regarding to a scoring function. It is based on the docking software EADock DSS. The molecular-docking was performed using the default parameters.

3. RESULTS AND DISCUSSION

3.1. Molecular-docking

Results obtained from an automated molecular-docking approach are discussed in Tables 2 & 3 respectively. Based on the docking results obtained from both SwissDock and DockingServer it is clear that wedelolactone has shown a better binding affinity towards all snapshots generated through the simulation than the crystal structure of human 5-LOX. The details of docking studies of wedelolactone with the crystal structure and the 9th snapshots are explained in Figs. 2-4 respectively. The docking study of wedelolactone with the crystal structure has shown hydrophobic and polar type of interactions, while the docking with the snapshot has revealed some hydrogen bond interactions (ARG479) along with polar and hydrophobic interactions. The hydrogen bond interactions can be considered due to the added flexibility to the protein molecule through MD simulation.

Figure.2(a)
Binding interaction of wedelolactone with the crystal structure of human 5-LOX.

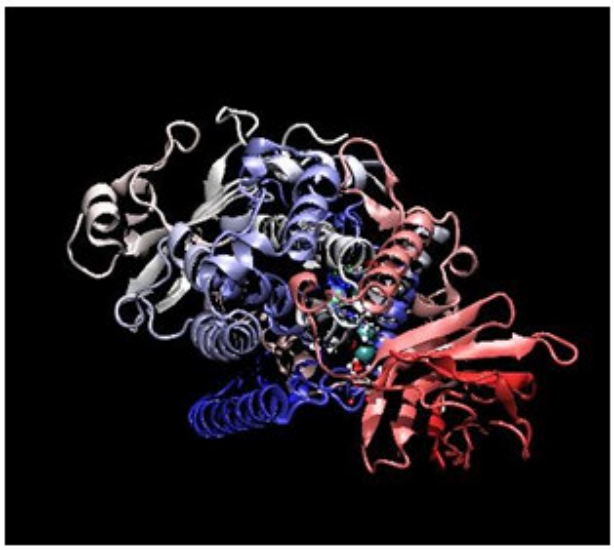


Figure.2 (b)
Binding interaction of wedelolactone with 9th snapshot generated through molecular dynamics simulation of human 5-LOX.

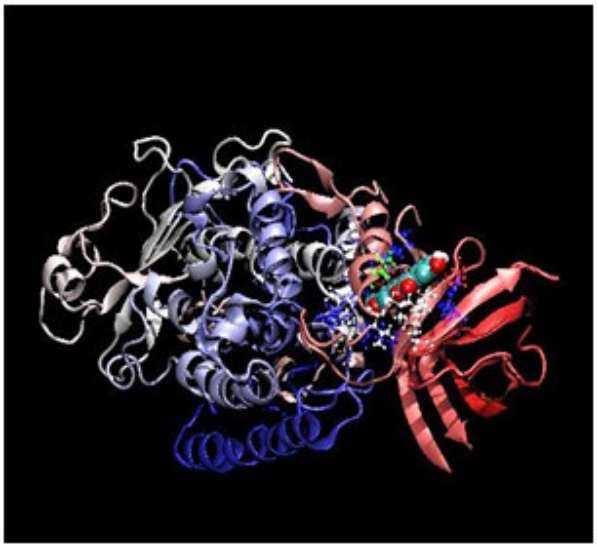


Table 2

Results obtained from DockingServer for wedelolactone with human LOX-5 and its snapshots generated through MD simulation.

Structures	Est. Free Energy of Binding (kcal/mol)	vdW + Hbond + desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Intermol. Energy(kcal/mol)
Crystal structure	-4.19	-4.02	-0.14	-4.16
Snapshot 1	-4.92	-4.60	-0.17	-4.77
2	-5.80	-5.60	-0.25	-5.85
3	-5.19	-4.87	-0.32	-5.19
4	-5.27	-5.13	-0.02	-5.16
5	-5.21	-5.15	+0.00	-5.15
6	-5.40	-5.22	-0.08	-5.30
7	-5.08	-4.85	-0.08	-4.93
8	-4.36	-4.10	-0.27	-4.37
9	**-5.85**	**-5.68**	**-0.05**	**-5.73**
10	-4.94	-4.55	-0.48	-5.02

The observed maximum binding affinity of wedelolactone towards the snapshot is shown in bold.

Table 3

Results obtained from SwissDock for wedelolactone with human LOX-5 and its snapshots generated through MD simulation.

Structures	FullFitness (kcal/mol)	ΔG (kcal/mol)	Energy
Crystal structure	-3479.09	-6.49	33.18
Snapshot 1	-3392.97	-7.50	30.07
2	-3430.36	-6.50	31.43
3	-3471.52	-7.45	24.00
4	-3450.25	-7.58	27.35
5	-3548.59	-7.03	28.43
6	-3474.81	-7.08	31.42
7	-3454.44	-6.78	33.88
8	-3470.47	-7.13	27.39
9	**-3540.62**	**-7.59**	**23.99**
10	-3410.33	-6.87	30.98

The observed maximum binding affinity of wedelolactone towards the snapshot is shown in bold.

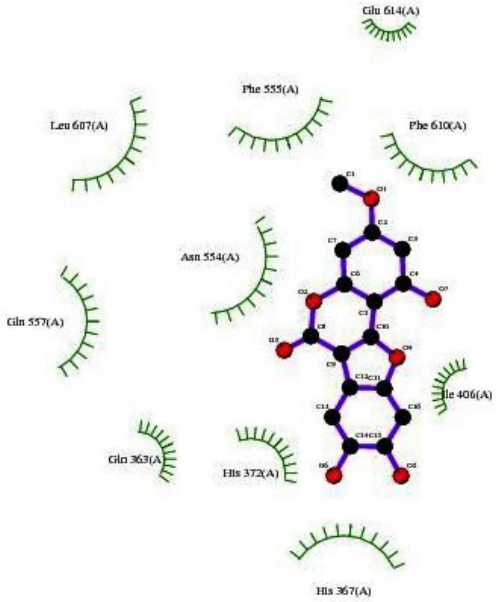


Figure.3

The docking analysis of wedelolactone with the crystal structure of human 5-LOX has shown some polar and hydrophobic type of interactions only.

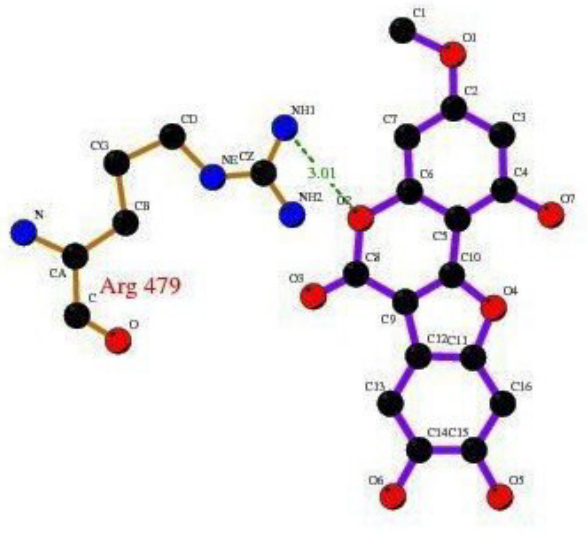


Figure.4

Docking analysis of wedelolactone with the 9th snapshot of human 5-LOX has shown one hydrogen bond between amino acid residue ARG479 and wedelolactone along with some polar and hydrophobic type of interactions.

A comparative analysis of results obtained from both docking servers, shown in Table 4, has shown that binding affinity of wedelolactone was better towards the snapshot stored at 900th ps of the simulation than the crystal structure of human 5-LOX, indicating the vital role of molecular dynamics simulation of biological molecules to get an insight of a better conformation of the biomolecules for docking studies.

Table 4

Comparative analysis of docking results obtained from SwissDock and DockingServer

	SwissDock	DockingServer
Structures	**ΔG (kcal/mol)**	**Est. Free Energy of Binding (kcal/mol)**
Crystal structure	-6.49	-4.19
Snapshots 1	-7.50	-4.92
2	-6.50	-5.80
3	-7.45	-5.19
4	-7.58	-5.27
5	-7.03	-5.21
6	-7.08	-5.40
7	-6.78	-5.08
8	-7.13	-4.36
9	**-7.59**	**-5.85**
10	-6.87	-4.94

Best docking results obtained from the both docking servers are shown in bold. Snapshot 9th stored at 900th ps of the simulation has shown the best results among all the generated snapshots and the crystal structure selected from PDB. It has been also performed the analysis of each type of interaction energies involved in binding of wedelolactone with the crystal structure and the 9th snapshot in detail. The details of decomposed interaction energies involved in binding as shown in Figs. 3 and 4 are described in Tables 5 and 6 respectively

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Table 5
Decomposed Interaction Energies of wedelolactone with the crystal structure of human LOX-5 (PDB ID: 3V99) in kcal/mol

Polar	Hydrophobic	Other
ASN554 (-0.8741)	LEU607 (-0.8568)	ILE406 (-0.2228)
GLN557 (-0.7835)	HIS367 (-0.8352)	
HIS372 (-0.4208)	PHE555 (-0.7665)	
GLN363 (-0.2766)	PHE610 (-0.675)	
GLU614 (-0.1633)		

Values inside the parentheses are interaction energies in kcal/mol.

Table 6
Decomposed Interaction Energies of wedelolactone with the snapshot 9th of human 5-LOX (PDB ID: 3V99) in kcal/mol

Hydrogen bonds	Polar	Hydrophobic	Other
ARG479 (-1.3861)	ARG64 (-0.7726)	VAL106 (-1.3467)	GLU130 (-0.9487)
	ARG108 (-0.7101)		ILE122 (-0.4677)

Values inside the parentheses are interaction energies in kcal/mol.

3.2. Salt bridges

A salt bridge may be defined as an interaction between two groups of opposite charge in which at least one pair of heavy atoms is within hydrogen bonding distance[23]. A cut-off distances have been used to investigate the salt bridge formation between polar atom pairs nitrogen (N) and oxygen (O) of amino acid residues. A cut-off distance of 4.0 Å[24-25] was used to find only “good" salt bridges for analysis. We studied the significance of salt bridges in the binding affinity of wedelolactone towards the crystal structure and snapshots during the course of simulation.

A total no. of identified salt bridges in the crystal structure and in all snapshots were given in Tables 7 and 8. All snapshots generated through simulation have shown the higher no. of salt bridges and networked salt bridges in contrast to the crystal structure of human LOX-5. Among all snapshots, the snapshot stored at 900th ps i.e. 9th snapshot has shown the highest no. of salt bridges and networked salt bridges. Some amino acid residues ARG64 and ARG108 involved in networked salt bridges were also found to be involved in binding interactions of wedelolactone with the simulation generated snapshots

Table 7
The identified salt bridges and networked salt bridges

Structures	No. of Salt bridges	No. of Networked salt bridges
Crystal structure	27	2
Snapshot 1	38	1
2	37	3
3	33	2
4	36	3
5	33	2
6	31	2
7	35	3
8	36	3
9	**38**	**3**
10	33	3

The observed highest number of salt bridges and networked salt bridges for the snapshot is shown in bold.

Table 8
The identified networked salt bridges formed between the amino acid residues.

Networked salt bridges			
Structures	**No.**	**Donor residues**	**Acceptor residues**
Crystal structure	2	Asp122 Asp472	Arg112, Arg131 Arg471, Lys462
Snapshot 1	1	Glu66	Arg68, Lys33
2	3	Asp118 Asp403 Glu66	Arg108, Arg127 Arg229, Arg347 Arg68, Lys33
3	2	Glu66 Asp403	Arg68, Lys33 Arg229, Arg34
4	3	Glu66 Glu104 Asp118	Arg68, Lys33 Arg64, Lys33 Arg108, Arg127
5	2	Glu104 Asp118	Arg64, Lys33 Arg108, Arg127
6	2	Glu104 Asp118	Arg64, Lys33 Arg108, Arg127
7	3	Glu66 Glu104 Asp118	Arg68, Lys33 Arg64, Lys33 Arg108, Arg127
8	3	Glu66 Glu104 Asp118	Arg68, Lys33 Arg64, Lys33 Arg108, Arg127
9	3	Glu66 Glu104 Asp118	Arg68, Lys33 Arg64, Lys33 Arg108, Arg127
10	3	Glu66 Glu104 Asp118	Arg68, Lys33 Arg64, Lys33 Arg108, Arg127

4. CONCLUSION

In the present research,an attempt has been made to study the influence of MD simulation on molecular docking. And the docking of wedelolactone between the crystal structure and snapshots generated through the MD simulation of human 5-LOX has been performed. A comparative analysis of docking results has revealed the better docking performance of MD snapshots than its rigid crystal structure. The MD simulation adds flexibility to the biomolecules. Hence, it has been concluded that the MD simulation prior to molecular docking can significantly improve the best conformation selection strategy of the target which is a vital step in structure based docking approach.

5. CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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