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Original Article

COMPUTATIONAL DOCKING STUDIES REVEAL THE MOST POTENTIAL INHIBITORY BINDING MODE OF DEMETHOXYLATED AND REDUCED CURCUMIN COMPOUNDS ON LUNG RESISTANCE PROTEIN

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ABSTRACT

Objective: Drug resistance in anti-cancer treatment continues as a foremost hurdle preventing the success of the treatment regime. The proteins which are involved in drug resistance are known as multi-drug resistance (MDR) proteins which includes mainly P-glycoprotein (P-gp), multi-drug resistance protein (MRP), lung resistance protein (LRP) and breast cancer resistance protein (BCRP). The LRP, which is the major component of complex ribonucleoprotein particles called vaults, is found to be over-expressed in many neoplastic tissue and cell lines indicating a poor response to chemotherapy. Therefore, targeting LRP can be considered as one of the promising approaches for the treatment of cancer. Curcumin, a natural polyphenolic compound is well-reported as an anti-cancer and chemopreventive agent. Though it is experimentally proved that curcumin inhibits lung resistance related protein (LRP), the binding mode of curcumin on LRP is yet to be elucidated due the non-availability of LRP crystal structure. Hence, by this study, we have attempted to model the 3D structure of Major Vault Protein (MVP) repeats of LRP and predicted the probable binding mode of Curcumin and its analogues implementing *in silico* strategies.

Methods: Homology modeling of MVP repeats of LRP was performed using Modeller9v11 and the model was further refined by Molecular Dynamics simulation using GROMACS 4.5 package. Further, molecular docking simulation of curcumin and its analogues with modeled MVP repeats were performed using AutoDock 4.2.

Results: Our results suggest that curcumin compounds which have demethoxylation at the phenol ring along with reduced C7 linker double bond may be efficient in modulating LRP than native curcumin.

Conclusion: The output of this study will aid in better understanding of molecular interactions between LRP and curcumin analogues leading towards potential drug prioritization.

Keywords: Multi-drug resistance; Lung resistance protein; Curcumin analogues; Docking; Molecular Dynamics.

INTRODUCTION

Lung Resistance Protein (LRP) is a drug resistance protein which is highly expressed in tumours and drug resistance cell lines and it does not belong to the ATP binding cassette (ABC) transporter family as other drug resistance proteins like Pg-P, MRP and BCRP. LRP is identified as a major vault protein (MVP), the main components of the vaults are barrel shaped in structure [1]. The MVP was initially described in non-small cell lung carcinoma (NSCLC) that lack P-glycoprotein (P-gp). These vaults form central plugs of the nuclear pore complexes and function to block the transport of drugs from cytoplasm to nucleus. The number of vaults was shown to be elevated in drug resistant cell lines, thereby mediating MDR by the compartmentalization of drugs away from intracellular drug targets [2].

The expression of LRP is present in variety of human cancer cell lines that have not been previously exposed to drugs and these cell lines showed correlation of intrinsic resistance to doxorubicin, vincristine and platinum compounds [3]. LRP over-expression has also been observed in variety of cancers like NSCLC, B-cell lymphoma, Acute Myeloid Leukemia (AML), astrocytic brain tumour, Chronic Myeloid Leukemia (CML) and retinoblastoma (RB) [1,4]. Our *in vitro* study also showed that curcumin inhibited the expression of LRP in RB cell line [5]. However, no information on the site of interaction is reported yet. Curcumin (diferuloylmethane) is the principal curcuminoid found in popular Indian spice turmeric, which is obtained from the rhizomes of the perennial herb *Curcuma longa* [6]. These are naturally occurring polyphenolic compounds containing two ortho-methoxylated phenols linked with a β-diketone function, and they all are conjugated. It's rigid and electron rich structures make it an interesting candidate as a lead compound for further development of new inhibitors. It is extensively used as a spice, food preservative and colouring material in India and South East Asia. Extensive work has been done to establish the biological activities and pharmacological actions of turmeric [7]. Curcumin has a wide variety of biological and pharmacological activities, including anti-carcinogenic, antioxidant, anti-inflammatory, anti-viral, and anti-bacterial.

The chemopreventive effects of curcumin appear to be multi-functional, suggesting the importance of curcumin as a therapeutic agent against various human malignancies. Many synthetic and natural curcumin analogues have additionally been documented to have therapeutic value, due to the instability and poor bioavailability of the parent curcumin compound, the therapeutic effects are limited when administered orally [8]. As our *in vitro* studies showed the inhibition of LRP protein by curcumin [5], and similarly our *in silico* docking studies on P-gp and MRP1 also concurrently infer the binding of curcumin into the nucleotide binding of P-gp and MRP1 functional domain [9,10], we were further interested in finding the binding affinity between curcumin and LRP. Molecular interaction studies are commonly used to calculate the binding mode of drug molecule to their protein targets, which have been widely performed by employing computational docking methods [11]. Thus, the objective of the present study is to compare the binding interaction of curcumin and its different analogues with LRP using molecular docking simulation. Hence, understanding the binding mode of curcumin and its analogues on LRP shall aid in design of more potential anti-cancer agents.

MATERIALS AND METHODS

Homology modeling of MVP repeats

Major vault protein (UniProt: Q14764) has nine MVP repeats at its N-terminal and these tightly packed repeats facilitate as interface for interactions with "vault poly(adenosine diphosphate–ribose) polymerase (VPARP)" and other proteins [12]. Recent studies have shown that Thr52 and S864 (hydrophobic residues) in LRP stabilize the conformation of MVP. Binding of 14-3-3ε on these two phosphorylation sites has found to inhibit the MDR action, thereby exerting bleomycin induced DNA damage in cancer cells [13]. Thus, in this study, we took the first four repeats of the MVP for modeling by homology modeling approaches. BlastP search of these repeats against PDB returned N-terminal domain of murine major vault protein (3GF5)[14]as the best hit with the sequence homology of 93% and was used as the template for modelling. A total of 100 models were generated using modeller9v11 [15]. DOPE score was used for the assessment of these models [16]. The generated models were evaluated for the stereochemical aspects using PROCHECK [17] and for the energy profile using ProSA II [18] server. The structural quality of the protein was further evaluated by comparing the topology of the protein through structural superimposition [9]. TM-score was generated using TM align program, so as to check the probability of proteins sharing the same fold at the structural level. TM-Score > 0.17 is suggestive of a model and template sharing the similar topology [19].

Molecular dynamics Simulation

Molecular Dynamics (MD) studies were performed for the modeled MVP repeats towards obtaining a refined structure with least potential energy. All the MD calculations were performed using OPLS-AA force field in Gromacs [20] 4.5 package. The protein was solvated in cubic box with SPC (Simple Point Charge) water and the protein was centred to 0.1nm from the edge of the box. Further the system was minimized for 1000 steps using steepest descent algorithm. Minimized system was then equilibrated with NPT and NVT ensembles. Particle Mesh Ewald was used for calculating long order electrostatics. The production run was carried out for 1 nanosecond. The MD trajectory was analysed to plot the evolution of Potential energy in terms of timescale. The conformation with the lowest potential energy was extracted and was utilized for docking studies [21].

Compounds collection and preparation

The compound dataset comprise of 9 compounds which includes 5 natural analogues apart from one native curcumin and three synthetic curcumin analogues. All the analogues of this study were selected based on the documented literature, wherein, the chosen analogues were experimentally proven to have enhanced anticarcinogenic efficacy and metabolic stability in comparison with native curcumin [22]. The natural compounds comprises of two types of curcumin analogues, the first set which includes the Bisdemethoxycurcumin (BDMC) and Demethoxycurcumin (DMC) which are known as curcuminiods. The BDMC and DMC differ from the native curcumin in methoxy substitution on aromatic ring. The other set of analogues are the reduced analogue of curcumin which are formed by the hydrogenation of c7 linker double bonds. The reduced analogues include Dihydrocurcumin (DMC), Tetrahydrocurcumin (THC), and Hexahydrocurcumin (HHC) [23]. The structural coordinates of curcumin (Pubchem Accession number: CID 969516) and its natural analogues were retrieved from NCBI-Pubchem database [24]. Further, the synthetic analogues of curcumin were built using Marvin sketch [25]. Geometries of all the compounds were optimized using PRODRG2 server [26].

Protein ligand interaction using AutoDock 4.2

The optimized LRP structure was docked to all compounds using semi-flexible docking option in autodock4.2, in which, the protein is treated as rigid molecule and the ligand as flexible. Further, the ligands were optimized by adding Gasteiger charges. The flexibility of the ligands were assigned based on respective torsional degrees of freedom through AutoTors.

The grid box was set enclosing the cavity between first two repeats of LRP with Thr52 set as centre atom, as this residue is documented to be a key residue involved in the stability of LRP [13]. Further, the grid was generated using AutoGrid4. Finally, Molecular Docking simulation was carried out using AutoDock 4.2 which implements Lamarckian genetic algorithm (LGA) for choosing optimal drug binding pose [27-29]. The post docking analysis was done using LigPlot+ [30] and the docked poses were visualized using pymol. The ligand conformation that has least binding energy and also interacting with THR52 were selected as the best conformation.

Results and Discussion

The present docking study gives an insight into the probable modulation of LRP function by curcumin and its analogues predicted through plausible binding mode. The molecular docking simulation was performed using Autodock 4.2 implementing Lamarckian Genetic Algorithm. The curcumin and its different analogues were selected based on the literature (**Table 1)**.

LRP homology model

With MVP of *murin* (PDBID: 3GF5) as template, three dimensional structure of LRP is modeled (**Fig. 1**) by homology modeling through Modeller9v11 tool. Further, the predicted model was geometry optimized through energy minimization. The modeled human LRP protein showed MVP repeats as beta-sheets, as similar to the template considered.

The overall stereo chemical property of the model was evaluated based on Ramachandran plot analysis, which showed 93.9% of the residues in the most favoured region with no residues spanning the disallowed region **(Fig. 2)**. Furthermore, structural aspect of the model in comparison with the experimentally determined structures as calculated using ProSA [18] also showed a Z-score of -6.52. Moreover, Fold level assembly of the human LRP with experimentally determined MVP of *murine* showed TM-score of 0.7 [19] and the Root Mean Square Deviation (RMSD) of superimposed backbones of the model and the template was found to be 0.682Å, suggestive of predicted model sharing the same topology with high probability. All these analysis confirm the stability and the plausibility of the structure predicted.

Table1: Showing the list of Curcumin and its Analogues with respective IUPAC names

Compounds	IUPAC NAME
Synthetic-1	1,7-bis(3-4-dihydroxyphenyl)-5-hydrohepta-1,4-6,-trien-3-one
Synthetic-2	5-hydroxy-1,7-bis(4-hydroxyphenl)hepta-1,4,6-triene-3-one
Synthetic-3	5-hydroxy-1,7-bis(2-hydroxy-3-hydroxyphenyl)hepta-1,4,6-triene-3-one
Curcumin	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
Demethoxycurcumin (DMC)	(1E,6E)-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
Bisdemethoxycurcumin (BDMC)	(1E,6E)-1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
Dihydrocurcumin (DHC)	(E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hept-1-ene-3,5-dione
Hexahydrocurcumin (HHC)	5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one
Tetrahydrocurcumin (THC)	1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-dione

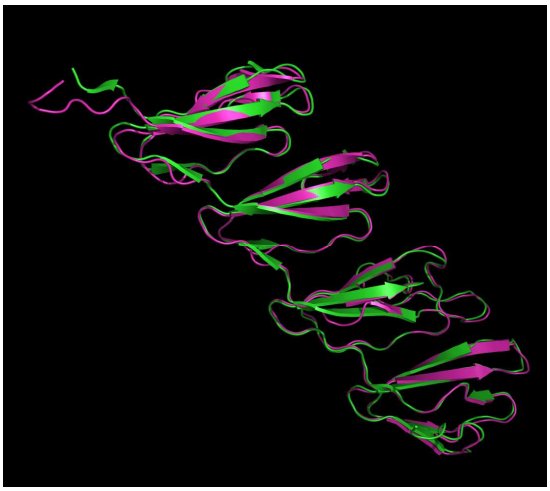

Fig. 1: It shows predicted 3-D model of LRP protein (magenta) superimposed to the MVP of *murin* (3GF5)(green)

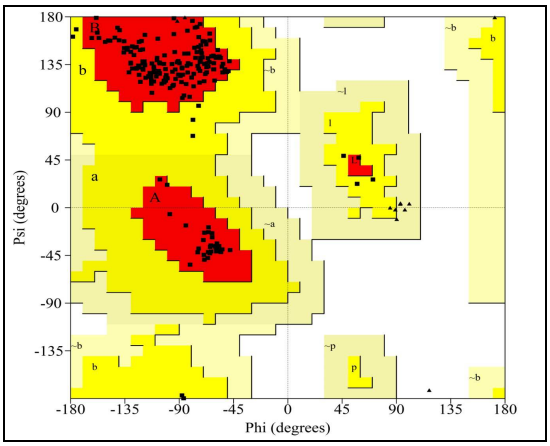

Fig.2: It shows Ramachandran plot for the modeled human LRP protein showing 93.9% of residues in the allowed regions.

Molecular Dynamics Simulation

The modeled structure was further subjected to molecular dynamics simulation for assessing stability and also to further refine the structure. The Root Mean Square Deviation (RMSD) of protein backbone and RMSF (Root Mean Square Fluctuation) of individual residues sampled at periodic intervals during the MD simulation were plotted against the time scale so as to assess the stability of the model.

RMSD plot showed displacement within a range of 1 to 2.5 Å (**Fig. 3**) which is suggestive of good stability of the predicted model. Moreover, the potential energy of protein at all frames were calculated and plotted using g_energy option in gromacs and the conformation with least potential energy was further utilized for docking studies.

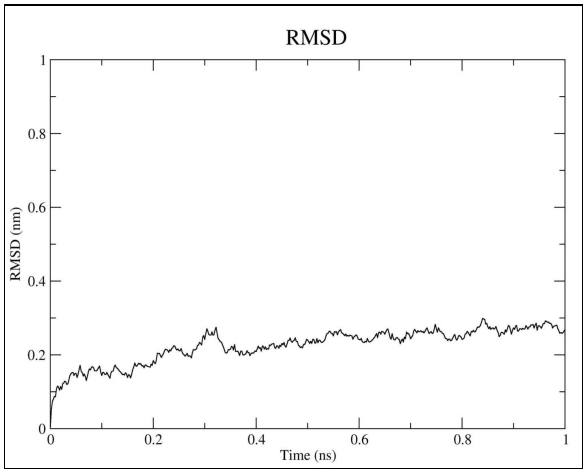

Fig. 3: It shows RMSD plot showing the system evolved in the course of 1ns simulation and remained stable

Docking

Molecular docking studies were performed for all the optimized curcumin and its analogues against the LRP structure with least potential energy as derived from molecular dynamics simulation. All the small molecules used were processed using PRODRG server and docked sequentially to LRP using Autodock 4.2. The docking run produced 10 conformations for each ligand. Docking analysis of LRP-curcuminiods complex showed binding energy **(Table2)** of -4.51, -5.28 and -4.54 kcal/mol for curcumin, DMC and BDMC, respectively, thereby suggesting that DMC is efficient among curcuminiods in binding with LRP, whilst, the Reduced analogues DMC, THC and HHC showed binding energy of -5.18,-3.49 and -5.09 kcal/mol, respectively. Further three synthetic compounds, synthetic 1, synthetic 2 and synthetic 3 (refer Table 1) showed binding energy of -4.16,-5.69 and -5.93, respectively. Binding energy analysis showed that the synthetic compound 5-hydroxy-1, 7-bis (2-hydroxyphenyl) hepta 1,4,6-triene-3-one (synthetic 3) to have lowest binding energy of -5.93 kcal/mol and inhibitory constant of 45.28nM [32] among all the natural and synthetic compounds (**Fig 4(A-F) & Fig 5(A-C)**).

Table2: It shows the details of molecular interactions observed in the docked complexes of LRP and Curcumin analogues

Compounds docked with LRP	Binding energy.kcal/mol	Inhibitory constant (um=unimolar)	Hydrogen Bond forming Residues	Residues showing Hydrophobic contacts
Curcumin	-4.51	493.71 um	Thr52, Arg49	Tyr13, Met50, Pro96, Val51, Pro54, Ile109
Bisdemethoxy curcumin	-4.54	466.62 um	Arg49,Thr52	Tyr13, Met50, Pro96, Val51, Pro54, Ile109
Demethoxy curcumin	-5.28	133.82 um	Thr52, Val53	Ile109, Val51, Arg49, Met50, Pro96
Dihydrocurcumin	-5.18	158.97 um	Thr52, val53	Pro54, val51, Met50, Arg49,Pro96,Gln94,Asp95
Hexahydrocurcumin	-3.49	2.78 mm	Thr52, Arg49	Ile109, Val51, Met50, Pro96, Val53, Pro54
Tetrahydrocurcumin	-5.09	186.41 um	Thr52	Asp95, Cln94, Val53, Pro54, Pro96, Val51, Met50
Synthetic-1	-4.02	888.12 um	Thr52, Arg49	Met50, val51, Pro54, Val53, Pro96
Synthetic-2	-5.69	67 um	Arg49, Thr52, Met52	Pro96, Pro54, Val53, Ile109, Val51
Synthetic-3	-5.93	45.28 um	Thr52, Met50, Arg49	Tyr13, Pro94, Val51, Ile109

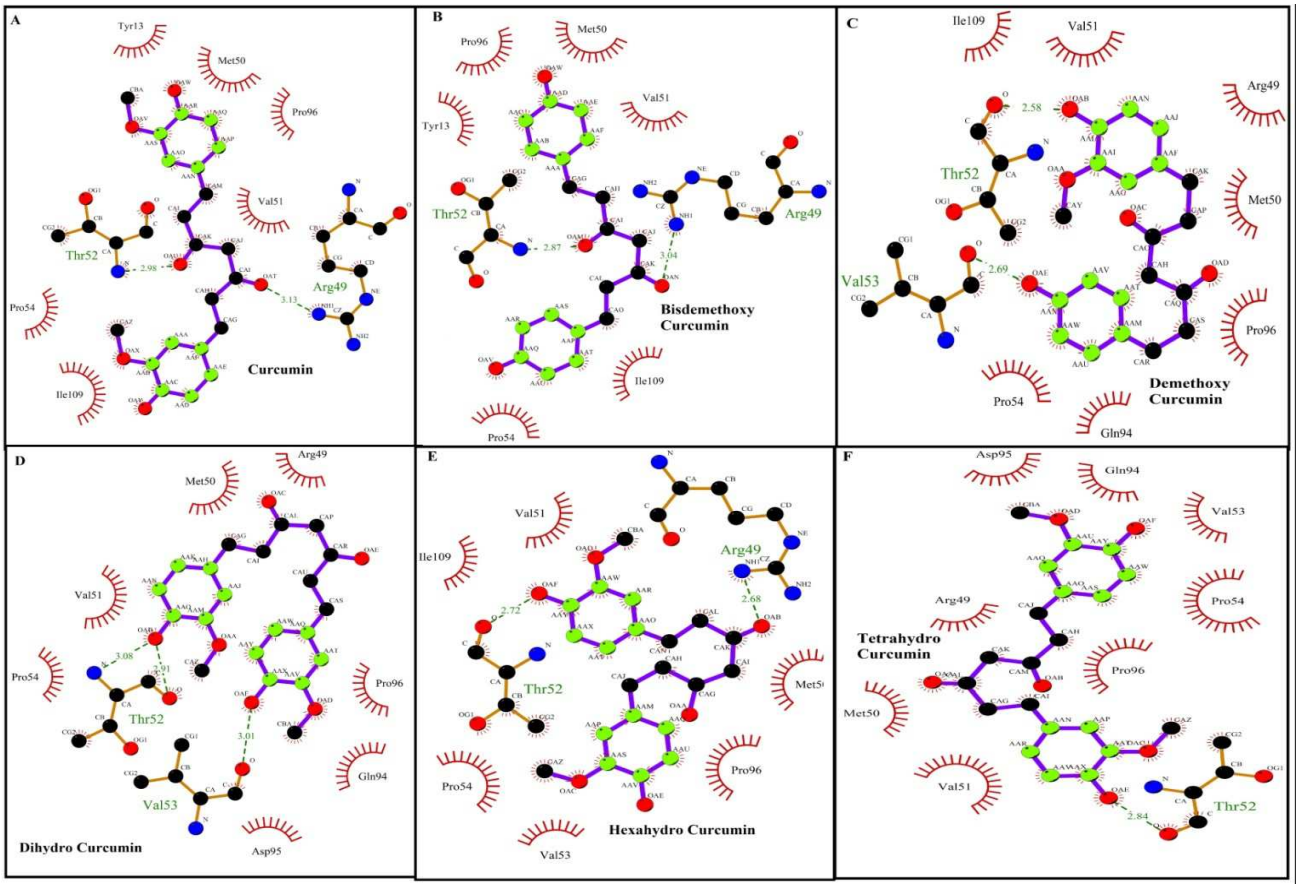

Fig. 4: A-F: It shows bonded and non-bonded interactions of curcumin and its natural analogues against LRP protein.

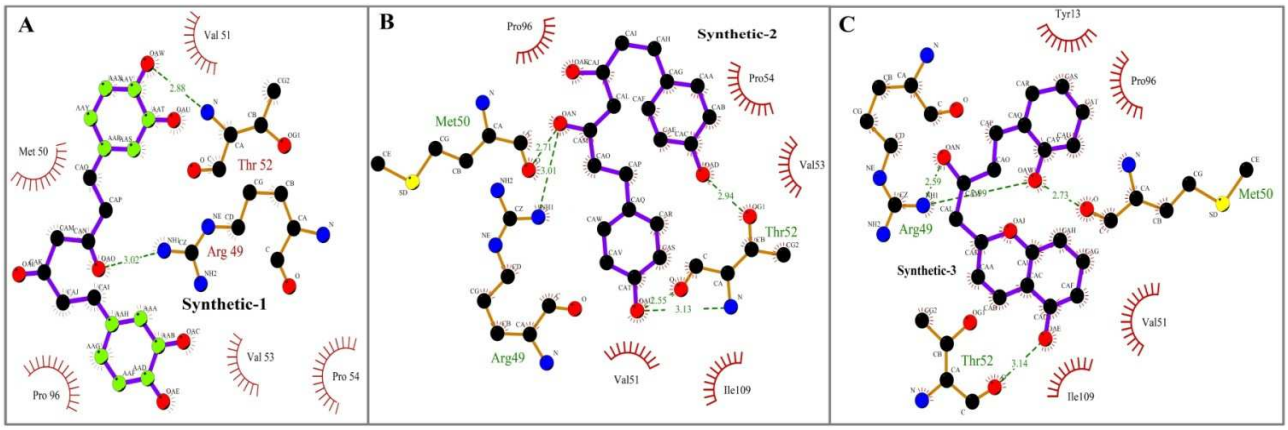

Fig.5: A-C: It shows bonded and non-bonded interactions of synthetic analogues of curcumin against LRP protein.

CONCLUSION

In conclusion, molecular docking of curcumin and its various synthetic analogues was performed to evaluate the efficiency of curcumin and its analogues in binding to active residue (THR52) of LRP. Binding energy of compounds suggests that the curcumin analogues are efficient than native curcumin in binding with LRP. It was also observed that the Synthetic compound that lacks the methoxy group and has reduction in C-7 linker bonds showed more efficiency in binding MVP. The other compounds that showed efficiency are Demethoxycurcumin and Dihydrocurcumin. While the efficiency of reduced analogues had already shown efficient in modulating drug resistance [31], our results suggest that compounds that are methoxylated and as well as reduced may be efficient modulators of LRP. The present study shows that curcumin analogues may serve as a good lead compound to target drug resistance protein (LRP) in tumour cells. Further experimental studies of these interaction can bring in more clarity on how curcumin analogues modulate LRP in drug resistant cells.

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