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De novo design based pharmacophore query generation and virtual screening for the discovery of Hsp-47 inhibitors

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ABSTRACT

Heat shock protein-47 (Hsp-47) is exclusive collagen specific molecular chaperone involved in the maturation, processing and secretion of procollagen. Hsp-47 is consistently upregulated in several fibrotic diseases. Till date there is no potential antifibrotic small molecule drug available and Hsp-47 is known to be potential therapeutic target for fibrotic disorder and drug designing. We used the *de novo* drug design approach followed by pharmacophore generation and virtual screening to propose Hsp-47 based antifibrotic molecules. We used e-LEAD server for *de novo* drug design and ZINCPharmer for 3D pharmacophore generation and virtual screening. The virtually screened molecule may inhibit direct recruitment of collagen triple helix to interact with Hsp-47 and act as antifibrotic drug.

1. Introduction

Collagen folding, processing and assembling is tightly regulated in eukaryotic cell. Collagen is secreted through exocytosis, enters endoplasmic reticulum (ER) co-translationally to ensure correct folding of collagen before secreting into extracellular matrix (ECM) [1]. The progressive nature of fibrotic disorder (FD) and acute accumulation of collagen leads to expansion of fibrotic mass by gradual destroying the architecture of normal tissues leading to organ failure [2,3]. Organ fibrosis deals with morbidity and mortality in several FD such as oral submucous fibrosis (OSF) [4], liver cirrhosis [5], kidney fibrosis [6] and idiopathic pulmonary fibrosis [7]. Besides, molecular mechanism behind the FD is still elusive, overexpression and deposition of collagen was the ultimate cause of FD despite of differential primary stimulus.

Heat shock protein 47 (Hsp-47) has been recognized as a collagen-specific chaperon and has important role in the development of fibrosis. Heat Shock Protein 47 (Hsp-47, SERPINH1, 47-kDa), an ER localized collagen binding protein was identified as "colligin" in mice and "gp46" in rats [8,9]. Hsp-47 is essential molecular chaperone required exclusively for intracellular proper assembly, folding and maturation of triple-helical procollagen and its transport across the Golgi apparatus to ECM [1,9]. Hsp-47 is expressed in all collagen-synthesizing cells, and constitutive expression levels correlate strictly with the amounts of collagen being synthesized in the corresponding cell which have seen in several fibrosis models [10]. Hsp-47 known to interact with many types of collagen such as type I, IV, V which comprised of different α-chains assembled to form triple helix both *in vivo* and *in vitro* [11] (5–7). The recognition of Arg residues at the Yaa position of the collagen Gly-Xaa-Yaa triplet is the critical requirement for Hsp-47 binding [12,13]. Hsp-47, in contrast, recognized only triple-helix conformation but not unfolded procollagen α-monomer in ER [12–16]. Hsp-47−/− mice causes aberrant procollagen folding in the ER, which resulted in early embryonic lethality at embryonic day 11.5 due to impaired basement membranes formation and defective collagen fibers formation in the embryo [17,18]. Type I collagen secreted from Hsp-47−/− fibroblast showed abnormal fibrillar structures with thin and branched fibrils due to impaired N-propeptide processing, triple-helix formation, secretion and accumulation of collagen fibers in the ECM [19]. These results entail the essential role of Hsp-47 in the maturation of type I and type IV collagen and attenuation of fibrinogenesis.

In the present study we have screened the Hsp-47-collagen binding site for the potential antifibrotic drugs by using novel approach based on *de novo* drug designing and virtual screening.

2. Materials and methods

2.1. Hsp-47 coordinate

In the present study, crystal structure Hsp-47 SERPINH1 bound to triple helix collagen (PDB ID 3ZHA) was retrieved from RCSB

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protein data bank (http://www.rcsb.org/). 3ZHA chain A was further used for *de novo* drug design and docking study.

2.2. De novo drug design

e-LEA3D: Chem-Informatic Tools and Databases server (http://chemoinfo.ipmc.cnrs.fr/) was used for the *de novo* drug design. 3D-Coordinates of 3ZHA_A was given as input along with PLANTS docking parameter was defined as coordinates of the center of the binding site ($x = -37.560$, $y = -106.295$, $z = -183.127$), Binding site radius (10 Å) and Weight in final score (1).

2.3. 3D pharmacophore feature generation

The ZINCPharmer (http://ZINCPharmer.csb.pitt.edu/) server was used to 3D pharmacophore feature generation from molecular structure designated as "query" [20]. Hydrogen bond donor (D), hydrogen bond acceptor (A), aromatic ring (R) feature were used to generate the pharmacophore model (Query). The molecular structure at its dock conformation obtained from the *de novo* design, the structure file that was uploaded to ZINCPharmer. The ZINCPharmer automatically extract the molecular pharmacophore features. All recognized features of the molecule are enabled as pharmacophore query features. By enabling specific pharmacophore feature pharmacophore query were generated, as it is derived from a single ligand structure broadly considered as a 3D similarity search [20].

2.4. Virtual screening

To identify the novel hit compounds, the pharmacophore "query" models were used as 3D structural search query to screen chemical databases. Virtual screening was carried out using ZINCPharmer (http://ZINCPharmer.csb.pitt.edu/) which efficiently search the ZINC database of fixed conformers. We also put the constraints that included maximum of 0.7 Root Mean Square Deviation (RMSD), 10 rotatable bond cut-off and molecular weight range of 180–500 Dalton to get the best similarity hits from ZINC database. The hits identified through database screening were further filtered using Lipinski's rule of five and toxicity estimation of filtered Lipinski filtered compounds [21]. A Lipinski-positive compound was filtered by using prepare ligand module in Discovery Studio (DS) has molecular weight <500, <5 hydrogen bond donor groups, <10 hydrogen bond acceptor groups and an octanol/water partition coefficient (Log*P*) value <5 [21]. Toxtree v1.60 software was used for estimation of toxic hazards associated with structure, here we apply structural alert for *in vivo* micronucleus assay and, carcinogenicity and mutagenicity by Benigni/Bossa rule base decision tree methods.

2.5. Docking using AutoDock Vina

Protein and ligands pdbqt files and grid box preparation were completed by using Graphical User Interface program AutoDock Tools 1.5.6 (ADT). ADT assigned polar hydrogens, united atom Kollman charges, solvation parameters and fragmental volumes to the protein. AutoGrid was used for the preparation of the grid map using a grid box. The docking grid with a size of $30 \times 30 \times 30$ Å was used around the active site of 3ZHA_A and centered on the coordinate $x = -37.560$, $y = -106.295$, $z = -183.127$ was used. AutoDock Vina was employed for docking with protein, ligand and grid box information in the configuration file. The pose with lowest energy of binding or binding affinity was extracted and aligned with receptor structure for further analysis. The highest binding energy values corresponding to the RMSD value of zero were considered as the binding affinity value of the ligands for each docking. All structures, docking poses visualization and images were generated using PYMOL Software. The Hydrogen bond interactions were obtained using Molegro molecular viewer 2.5.

3. Result

3.1. Drug designing methodology

The schematic representation of *de novo* drug designing methodology of Hsp-47 is shown in the Supplemental Fig. 1. Upto date

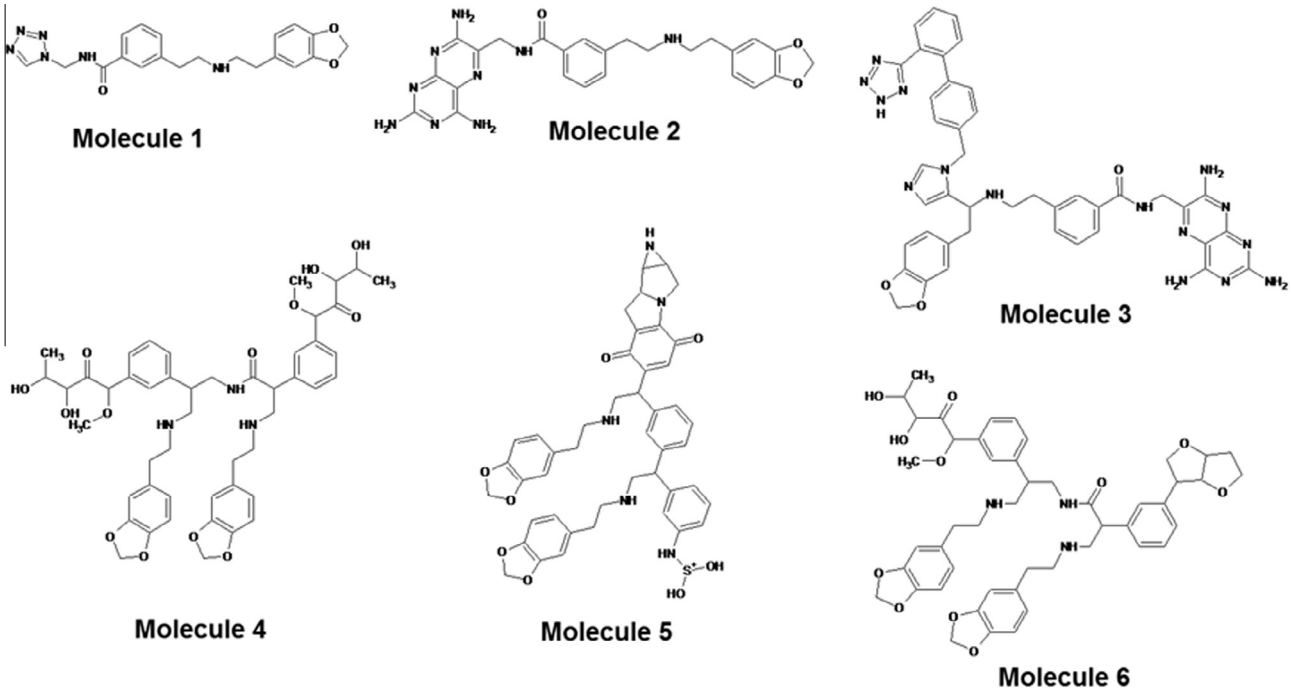

Fig. 1. Structure of *de novo* design molecules retrieved from e-LEA3D server.

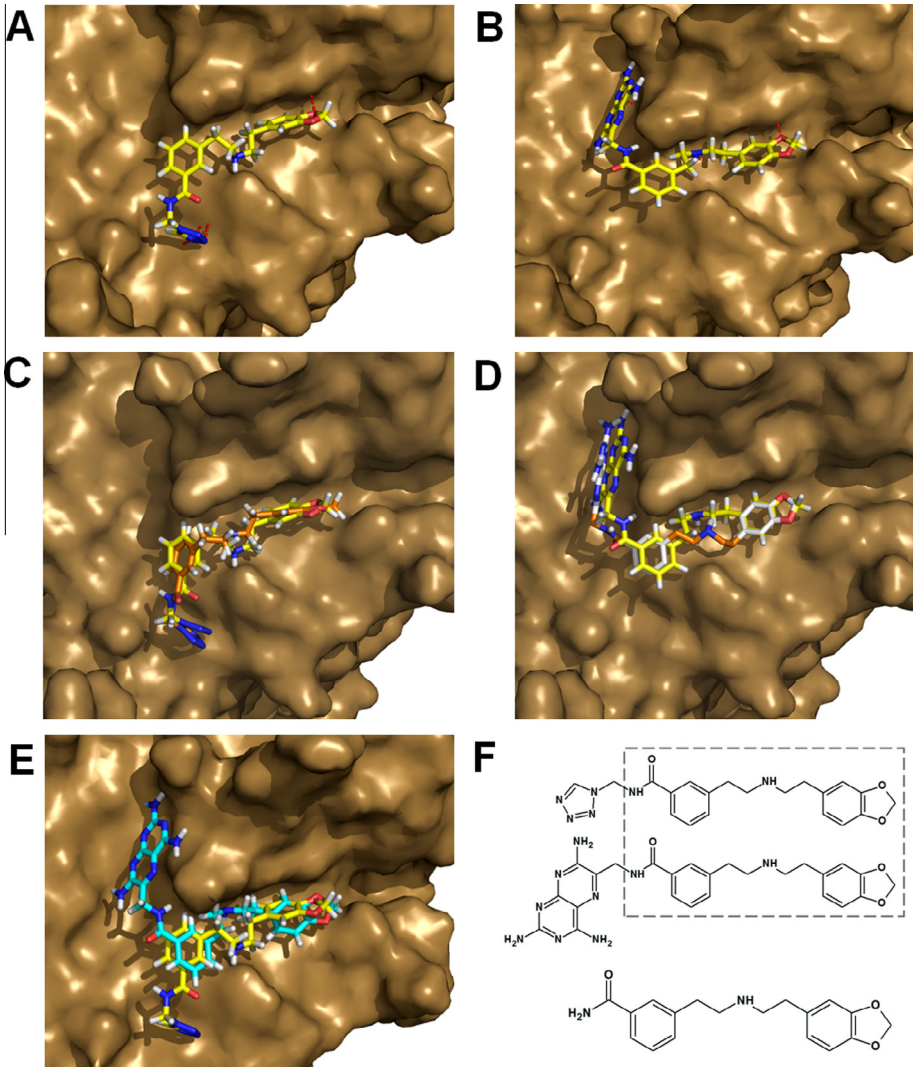

Fig. 2. Docking of *de novo* design molecule in the active site of Hsp-47. Docking pose of (A) molecule 1 (yellow element) in the active site of Hsp-47 (brown), (B) molecule 2 (yellow element) in the active site of Hsp-47 (brown) retrieved from e-LEA3D server. Comparative docking poses of (C) molecule 1 (yellow element) in the active site of Hsp-47 (brown), (D) molecule 2 (yellow element) in the active site of Hsp-47 (brown) retrieved from e-LEA3D server and AutoDock Vina (orange element). (E) Superimposition of molecule 1 (yellow element) and 2 (cyan element) in the active site of Hsp-47 (brown). (F) Common molecular scaffold extracted from molecule 1 and molecule 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

no drug or molecule were reported which has the direct binding with Hsp-47 at the collagen binding site. Recently Hsp-47 3D-structure along with the collagen triple helix peptide was reported with detailed molecular interaction [22]. Active site of Hsp-47 which involved in the collagen binding was used for *de novo* drug designing. The leads obtained from *de novo* drug designing were further subjected to pharmacophore modeling and virtual screening to obtain Hsp-47 inhibitors.

3.2. Hsp-47-complex with collagen, active site and molecular interaction

Crystal structures of Hsp-47 in complex with collagen model peptides was shown to contain the collagen recognizing motif an arginine at the Yaa position of a Xaa-Yaa-Gly triplet (Supplemental Fig. 2). Two Hsp-47 protein molecules bind to one triple helix. The complex is formed by two HSP47 protein molecules that interact in a head-to-head fashion via β-sheet C to two strands of one collagen trimer (Supplemental Fig. 2A). The amino acid residue which formed the active site to dock or interact with Hsp-47 protein was shown in Supplemental Fig. 2B. Hsp-47 interact with collagen and recognizes all three leading and trailing strands via a salt bridge between arginine at the position termed "yaa^{0}" in the Pro-Arg-Gly and Asp385 of Hsp-47. Moreover, His238 was involved in interaction with Asp220 and Ser305 via salt bridge of main-chain carbonyl oxygen atoms of Pro5 and Gly6. In addition, there are a number of hydrophobic interactions particularly involving Leu381 and Tyr383 further strengthened the Hsp-47-collagen complex (Supplemental Fig. 2C). The active site of Hsp-47 was mainly constituted of central hydrophobic residue surrounded by the hydrophilic residue (Supplemental Fig. 2D).

3.3. De novo drug designing

e-LEA3D server provides a *de novo* drug design engine to create novel molecules from lead-hopping. In the present study Hsp-47 protein's three dimensional coordinates were uploaded and active site residues of Hsp-47 (Asp220, Arg222, His238, Ser305 Tyr383,

Asp385 and His388) interacting with Collagen, taking as centroid coordinate was given to perform search around 10 Å space to generate *de novo* leads. We have retrieved six *de novo* molecules from e-LEA3D server docked into the active site of the Hsp-47. The novelty of molecules was cross-checked by performing search on Scifinder and pubchem server. e-LEA3D build *de novo* molecules by using generic algorithm to fit into the active site of the protein from the various 3D fragments extracted from bioactive molecules (FDA approved drug fragments). Their structures, molecular properties and PLANTS score is given in Fig. 1 and Supplemental Table 1. These six *de novo* design molecules further docked into the active site of Hsp-47 using AutoDock Vina to check the goodness and reproducibility of docking pose obtained by e-LEA3D server. Also we filtered the *de novo* design molecules for Lipinski rule. The molecule 1 and 2 docked precisely in the active site of the Hsp-47 and followed the Lipinski rule (Fig. 2A,B and Supplemental Table 1). Molecule 1 and 2- Hsp-47 complex have −7.3 and −7.7 kcal/mol binding affinity respectively. 0.48 Å and 0.73 Å root mean square deviation (RMSD) were observed between docking pose obtained by e-LEA3D server and AutoDock Vina for molecule 1 and 2 respectively. This suggested that molecule 1 and 2 bound with Hsp-47 active site accurately and have similar binding pose that was obtained by e-LEAD server (Fig. 2C and D). Furthermore, molecule 1 and 2 were superimposed on the active site of the Hsp-47 (Fig. 2E) and common molecular scaffold was obtained (Fig. 2F).

3.4. 3D Pharmacophore feature generation, Zinc database search and virtual screening

The molecule 1, 2 and common scaffold were uploaded on the ZINCPharmer as of same conformation dock with the active site of Hsp-47 as obtained from the e-LEA3D or *de novo* drug design server. The ZINCPharmer is capable to extract pharmacophore feature. The specificity was further improved for 3D molecular search with minimum pharmacophore feature which is essential for optimal interaction with Hsp-47 active site. The pharmacophore feature was further refined by editing the properties of the query. We have obtained three query from the ZINCPharmer as per shown in the Fig. 3. Each query were further allowed to explore Zinc database and results were returned as a hit if all the matching features are within the specified search tolerances of the query when the conformer is aligned to minimize the weighted RMSD. Query 1, 2 and 3 retrieved 13, 54 and 156 molecules (collectively 223 molecules) from Zinc database. These entire molecules were further screened for Lipinski rule of 5, and retrieved 9, 12 and 49 molecules (collectively 70) from query 1, 2 and 3, respectively. Furthermore, Lipinski filtered molecules were assessed for toxicity analysis using Toxtree software. Mechanisms of toxic action were identified by Verhaar scheme and Benigni-Bossa method. The molecules with negative for genotoxic carcinogenicity were selected. Collectively 22 molecules were obtained (4, 7 and 11 from query 1, 2 and 3, respectively). Finally these entire 22 molecules were allowed to dock on Hsp-47 active site by using AutoDock Tools. 15 molecules (1, 6 and 8 from query 1, 2 and 3, respectively) showed precise binding with the active site of Hsp-47 and their docking pose and binding energy are shown in the Supplemental Fig. 3 and Supplemental Table 2. The hydrogen bond interaction further reveled the striking and more profound binding of virtually screened molecules from ZINC database compared to *de novo* design molecules. *De novo* design molecule 1 and 2 shows the prudential binding with Thr240, Ser305, Leu381 and Tyr383 (Fig. 4A and B). Whereas virtually screened molecule shows more hydrogen bond interactions which may help to strongly accommodate and improve specific binding of virtually screened molecules into the collagen binding site (Fig. 4C and D). More specifically, ZINC15085064

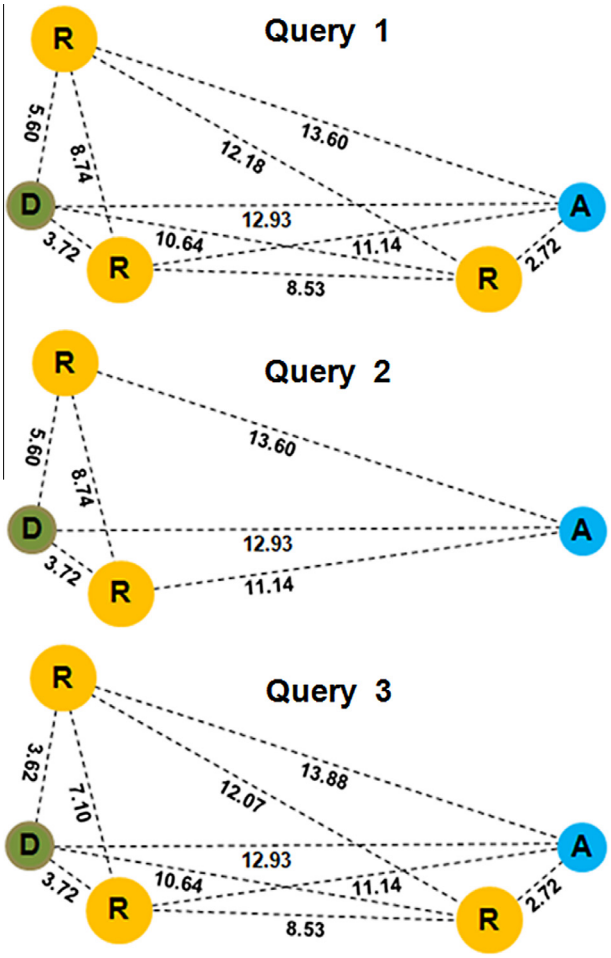

Fig. 3. The schematic representation 3D pharmacophore query generated from ZINCPharmer. The dash line represents the inter-descriptor distance in angstrom, descriptors A; hydrogen bond acceptor (blue), D; hydrogen bond donor (green), R; aromatic ring (orange). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

shows five hydrogen bonds with Arg222, Tyr245, Asp247, Gln254 and Asp385, and ZINC65001938 shows two hydrogen bonds with Asp385 and Ser305. Arg222 and Asp385 was essential residue for recognition of collagen motif. Hence, direct interaction of virtually screened molecule with these residues may further inhibit direct recruitment of collagen triple helix to interact with Hsp-47.

4. Discussion

Collagen accumulation is the hallmark of fibrotic diseases. Hsp-47 has largely been used as a marker of fibrotic disorder [23]. Several studies had correlated the increased Hsp-47 expression with collagen deposition in various human fibrotic diseases suggesting that Hsp-47 could serve as both a biomarker and a therapeutic target in fibrogenesis [24]. The formation and turnover of the ECM depends upon fibroblast cells residing in the connective tissue and is regulated by bioactive molecules acting in the local tissue environment [25].

Presently, no effective anti-fibrotic treatment has been available for patients with fibrosis. However, there were several evidences suggesting inhibition of Hsp-47 would be great therapeutic strategy. siRNA delivery against Hsp-47 using cationized gelatin microspheres in a Unilateral Ureteral Obstruction (UUO) model of kidney fibrosis shows inhibitory effect on Hsp-47 expression

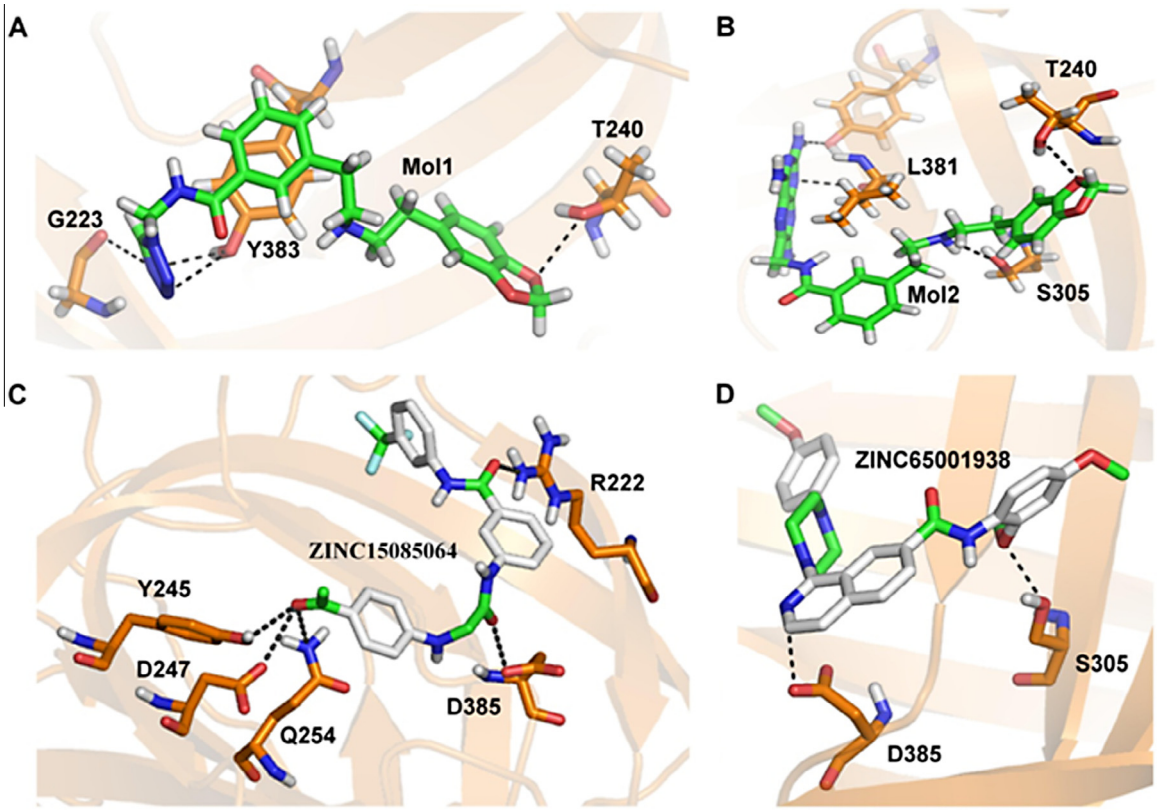

Fig. 4. The hydrogen bond (black) interaction of Hsp-47 active site residue (orange element) with (A) molecule 1 (green element) (B), molecule 2 (green element), (C) ZINC15085064 (white and green element) (D) ZINC65001938 (white and green element). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and subsequently reduced type IV, III and I collagen expression and accumulation compared to classical siRNA administration (14 days instead of 7 days) [26]. Similarly, siRNA against Hsp-47 encapsulated in vitamin A-coupled liposomes (VA-lip-siRNAgp46) resolved fibrosis in a model of liver cirrhosis [27]. Hsp-47 siRNA suppressed collagen and TGF-β1 expression restricted to development of peritoneal fibrosis. Chen et al. demonstrated Hsp-47 inhibition by Hsp-47 siRNA in keloid fibroblast cells drastically reduced collagen deposition and improved intestinal fibrosis [28]. Hagiwara et al. reported a protective effect of Hsp-47 inhibition by reducing lung injury, collagen deposition, improved lung morphology and function in rats following Hsp-47 antisense oligonucleotide administration [29–31]. Also, the suppression of Hsp-47 expression by the administration of antisense oligodeoxynucleotides against Hsp-47 delayed the progression of glomerular sclerotic process by reducing accumulation of collagens in the glomeruli [32]. Chlorhexidine gluconate induced peritoneal fibrosis in rats, treating these rats with antisense phosphorothioate markedly reduced Hsp-47 and deposition of collagens and eventually reduced the extent of peritoneal fibrosis [33]. Moreover, certain drugs seem to exert their indirect anti-fibrotic effects by suppressing the Hsp-47 expression. Pirfenidone stabilized pulmonary functions and improved prognosis of patients with idiopathic pulmonary fibrosis [34]. Recently, administration of pirfenidone was reported to reduce the accumulation of collagens in bleomycin-induced pulmonary fibrosis possibly by suppressing the expression of Hsp-47 [35]. This clearly suggested the Hsp-47 is suitable target for anti-fibrotic therapy to prevent or delay fibrosis. However, the biomedical outcomes of siRNA are high but there are certain arguments that are impeding their practical applications in human. Like, high molecular weight, polyanionic and hydrophilic nature, fail to enter cells by passive mechanisms, endosomal trapping mostly results into null effect, getting active "off-target" as well as inducing the immune response, released of siRNA from the endosome to cytoplasm while avoiding entrapment and degradation [36]. Also major challenges includes quick renal elimination, enzymatic degradation in plasma, half life less than 5 min in plasma, nonspecific uptake by reticulo endothelial system upon systemic administration [36]. Conversely, till date no direct inhibitor of Hsp-47 has been reported, which showed to have direct binding interaction with Hsp-47. In present study attempt was made to find out the virtual screening based Hsp-47 inhibitor which directly interact with active site of Hsp-47. In order to perform virtual screening for Hsp-47 we have used the *de novo* design based approach as there was no reported lead molecule available to screen the chemical database based on the similarity search or structure based drug designing tends to pharmacophore generation and lead optimization.

The identification of novel lead structures is a critical task in early drug discovery. *De novo* drug design attempts was mainly useful for those proteins whose active site information was available but lacks ligand and mode of interaction or orientation of ligand molecule may fit into the active site. *De novo* designing generate the novel chemical structure which can bind to essential active site residue. e-LEAD server used the fragment based *de novo* designing where most ligands that bind strongly to a proteins active site can be considered as a number of smaller fragments or functionalities [37]. Fragments are identified by screening a relatively small library of molecule by X-ray crystallography, NMR spectroscopy. These structures of the fragment binding to the protein can be used to design new ligands by adding functionality to the fragments or by incorporating features of the fragment into existing ligands and enable scaffold-hopping. e-LEAD for *de novo* designing used

genetic algorithm and PLANTS docking program which used empirical scoring functions for advanced protein–ligand docking, balance pose prediction reliability and search speed [37,38].

De novo design methods have confronted challenges that limited their application to experimental drug discovery [39]. Firstly, synthetic difficulties, secondly lack of an efficient search strategy to identify likely fragments to interact within the "druggable space" (binding site) and thirdly, the proposed compounds with less drug-like properties. Hence to enhance drug-likeness of *de novo* design molecule, the structure motifs (scaffold) allow to search with a good strategy to identify leads which possess drug-like properties. The pharmacophore mapping of *de novo* design molecule and virtual screening in available compound databases could improve the hits and lead optimization process for target protein [40]. Hence in the present study we have used similar approach for the discovery of Hsp-47 inhibitor. The *de novo* design molecular scaffold was allowed to 3D pharmacophore feature generation and virtual screening in ZINCPharmer. In conclusion, 223 druglike molecules were screened by ZINCPharmer and 22 molecules successfully pass the Lipinski rule of 5 and toxicity test, out of which 15 molecules show the best binding to the Hsp-47 active site. More specifically, ZINC15085064 and ZINC65001938 have shown interaction with essential residue of Hsp-47 active site required for the recognition of collagen motif. However, the optimized lead molecules need to be validated experimentally for future application as potential Hsp-47 inhibitor.

Conflict of interest

None declared.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.12.051.

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