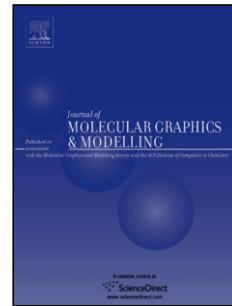


# Accepted Manuscript

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**Molecular docking and dynamic simulation evaluation of Rohinitib - Cantharidin based  
novel HSF1 inhibitors for cancer therapy**

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Short title: RHT-CLA based novel HSF1 inhibitors

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Highlights

- Cantharidin and Rohinitib based hybrid ligands had higher affinity for HSF1
- Hybrid ligands formed stable complex and showed greater interaction with HSF1
- Most of the hybrid ligands were non carcinogenic, non mutagenic and biodegradable
- Hybrid ligands diminished binding of HSF1 to the Hsp70 promoter sequence

### Abstract

Recent developments in the target based cancer therapies have identified HSF1 as a novel non oncogenic drug target. The present study delineates the design and molecular docking evaluation of Rohinitib (RHT) - Cantharidin (CLA) based novel HSF1 inhibitors for target-based cancer therapy. Here, we exploited the pharmacophoric features of both the parent ligands for the design of novel hybrid HSF1 inhibitors. The RHT-CLA ligands were designed and characterized for ADME/Tox features, interaction with HSF1 DNA binding domain and their pharmacophoric features essential for interaction. From the results, amino acid residues Ala17, Phe61, His63, Asn65, Ser68, Arg71 and Gln72 were found crucial for HSF1 interaction with the Heat shock elements (HSE). The hybrid ligands had better affinity towards the HSF1 DNA binding domain, in comparison to RHT or CLA and interacted with most of the active site residues. Additionally, the HSF1-ligand complex had a reduced affinity towards HSE in comparison to native HSF1. Based on the results, ligand RC15 and RC17 were non carcinogenic, non mutagenic, completely biodegradable under aerobic conditions, had better affinity for HSF1 (1.132 and 1.129 folds increase respectively) and diminished the interaction of HSF1 with HSE (1.203 and 1.239 folds decrease respectively). The simulation analysis also suggested that the ligands formed a stable complex with HSF1, restraining the movement of active site residues. In conclusion, RHT-CLA hybrid ligands can be used as a potential inhibitor of HSF1 for non-oncogene target based cancer therapy.

## Keywords

Non-oncogene target based cancer therapy; Heat Shock factor 1 (HSF1); Rohinitib; Cantharidin; molecular dynamic simulation

### 1. Introduction

Cancer is one of the most major cause of death worldwide and a serious socio-economic concern. In India, prevalence of around 2.5 million cases has been estimated, with an annual increase of 8 lacks new cases and 5.5lacks death [1]. The conventional chemotherapies available for cancer treatment are over laden with multiple disadvantages including the lack of aqueous solubility, results in selectivity and multidrug resistance [1]. The lack of selectivity leads to undesirable systemic toxicity to the patients being exposed to them. Recent focus in the field of cancer treatment has been concentrated on target-based cancer therapy. Such strategies involve targeting the protein product of oncogenes or non-oncogenes. The oncogenes are crucial for tumor initiation and maintenance while non-oncogenes are essential for the survival of the transformed cells. A significant amount of efforts have already been made for development of small drug molecules (Iressa, Bombesin, Octreotide, Gleevec and Tarceva) and therapeutic antibodies (Herceptin, Avastin and Erbutix) targeting the oncogenic product for cancer treatment [2, 3]. However, not much attention has been paid on non-oncogene targeted therapies. Targeting non-oncogenes may have atleast equal if not better therapeutic impact than that of oncogene pathways [4].

The Heat shock factor 1 (HSF1) is known as the master regulator of the heat shock response in the cells [5, 6]. Under normal condition, the HSF1 is localized in the cytoplasm as an inactive monomer lacking the ability to bind to the heat shock elements (HSE) in Hsp promoters. The inactive monomeric state of HSF1 is assured by its binding to the molecular chaperone system consisting of Hsp90, Hsp70 and Hsp40. Under cellular stress, Hsp90, due to their preferential binding to the misfolded protein, release the HSF1. The HSF1 forms an

inactive homotrimeric complex and migrates to the nucleus wherein it gets activated by subsequent hyperphosphorylation. In turn, the activated trimeric form of HSF1 has a high affinity for consensus DNA sequence present in the heat-inducible promoters including the stress proteins. This sequence is termed as heat shock element (HSE) and consists of repeating units of nucleotide sequence ‘nGAAn’ in head-to-tail orientation [7]. The binding of active HSF1 to HSE induces the transcription of Hsp40, Hsp70, Hsp90, BAG3 and other chaperones, thus, initiating the cytoprotective molecular mechanisms (Fig. 1) [4, 5, 6, 8].

The cancerous cells have been associated with high level of DNA damage, reactive oxygen species and aneuploidy and thus require stress response pathways for their survival [8, 9]. The presence of aberrantly high levels of HSF1 in the cancer cells has already been reported [6, 9, 10]. Moreover, knockdown of HSF1 in the cancer cells have shown cellular death, suggesting crucial role of HSF1 for their survival [10, 11, 12]. Also, targeting HSF1 also sensitizes the cancerous cells more to HSP90 inhibitors (known anticancer agents) [12]. Thus, the pharmaceutical HSF1 inhibition provides an attractive opportunity for development of target-based cancer therapeutics [6, 12].

In the recent reports, Cantharidin (CLA) and Rohinitib (RHT) have been shown to possess the anticancer effect by selectively diminishing the promoter binding activity of HSF1 and expression of its target genes [8, 13, 14]. We herein hypothesized that a hybrid ligand of RHT and CLA might have higher HSF1 inhibiting potential. Keeping this perspective in mind, we modeled and dynamically simulated DNA binding domain of HSF1 to gain its equilibrated and stable conformation. Further, we designed novel HSF1 inhibitors based on RHT and CLA. Herein, the hybrid ligands were made completely on random basis with an aim to design ligands with better inhibitory potential of HSF1-HSE interaction. The pharmacophoric elements of RHT (acceptors: O9, O25, O26, O34 and O35; donors: O25 and O26; Aromatic: C1-C6, C13-C18 and C19-C23; Hydrophobic: C1-C6, C13-C18, C19-C23 and C37) and

CLA (acceptors: O14; Hydrophobic: C1-C6) were majorly kept untouched. The rest of the elements were modified in order to enhance the ligand efficacy for the protein and its ADMET/Tox features (Supplementary Fig. 1). The ligands were then subjected to ADME/Tox evaluation, molecular docking with modeled protein and subsequent simulation of the protein-ligand complex. Furthermore, the docking of the HSF1 was carried out with the heat shock element (HSE) of Hsp70 promoter in the presence or absence of the ligands.

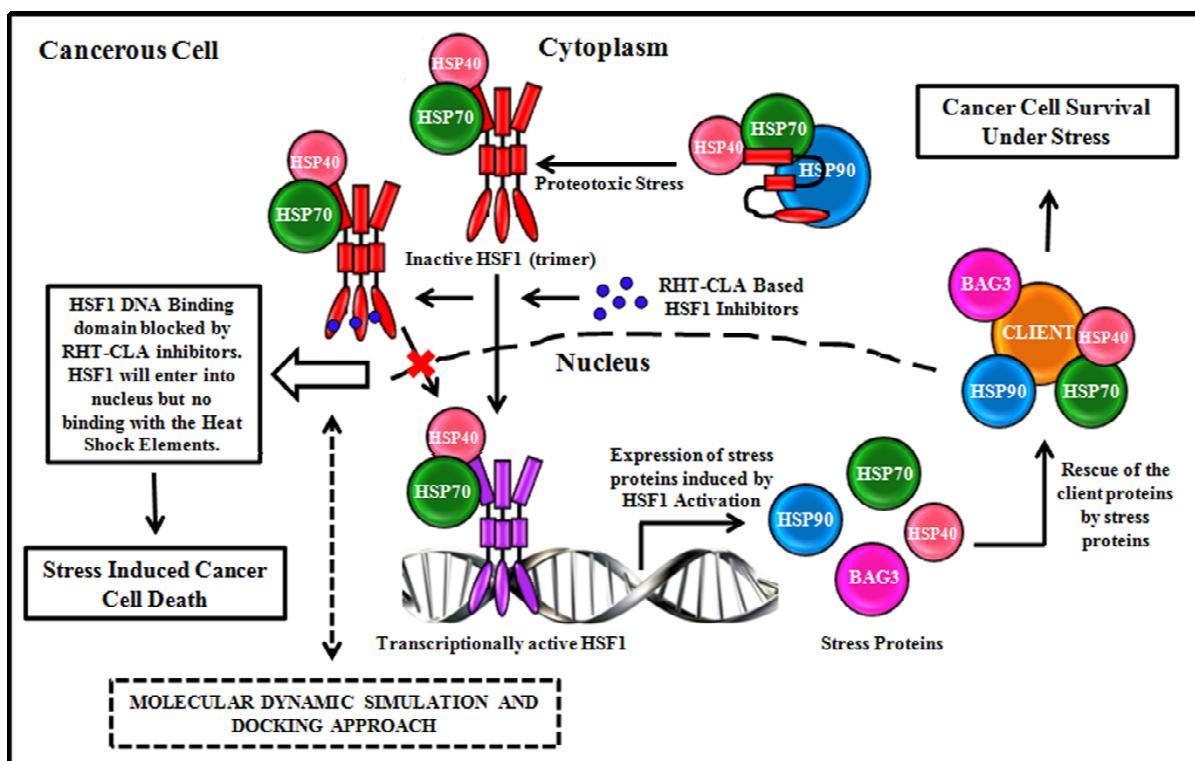


Fig. 1 Role of HSF1 protein in the cancer progression. Under normal condition, HSF1 is present in cytoplasm as an inactive monomer bound by Hsp90, Hsp70 and Hsp40. Under proteotoxic stress (usually observed in cancerous cells), Hsp90 is released from the HSF1-chaperone complex. The HSF1 thereby forms an inactive homotrimeric complex and migrates to the nucleus where it gets activated by subsequent hyperphosphorylation. The activated trimeric form of HSF1 binds to heat shock element (HSE) present in stress proteins, inducing the transcription of Hsp40, Hsp70, Hsp90, BAG3 and other chaperones, thus,

promoting cancer cell survivability. Thus, herein we hypothesized that RHT-CLA based hybrid inhibitors would block HSF1-HSE interaction, resulting in death of cancerous cells.

## 2. Materials and Methods

### 2.1. Homology modelling and structural validation of protein

The protein sequence of DNA binding domain of HSF1 (HSF1D) was obtained from UniProt database (UniProt entry Q00613) (<http://www.uniprot.org/uniprot/Q00613>). The structure of HSF1D was modelled using Modeller v9.12. The best amongst predicted models was selected based on DOPE score and validated using Structural Analysis and Verification Server (<http://nihserver.mbi.ucla.edu/SAVES/>) [15, 16, 17]. Prior to docking application, the HSF1D was simulated under GROMOS96 53a6 force field for 10ns using Gromacs 4.5.6 package [18] (for details, refer supplementary S1). In addition, the structure was also evaluated for the cation–π interactions and stabilizing amino acid residues using CaPTURE (<http://capture.caltech.edu/>) [19] and SRide (<http://sride.enzim.hu/>) [20] servers respectively.

### 2.2. Prediction of HSF1D active site residues

The potency of HSF1 to interact and bind with the DNA was targeted for the analysis. In this regard, it becomes essential to know about the amino acid residues that interact directly with the DNA. The active site residues were predicted using three different methodologies. First, using COACH server which is the meta-server based approach to predict the protein ligand binding site (<http://zhanglab.ccmb.med.umich.edu/COACH/>) [21]. Secondly, by determining the consensus DNA binding domain signature using the ExPASy PROSITE (<http://prosite.expasy.org/>) [22] and thirdly, through blind docking of known inhibitors of HSF1 such as RHT and CLA using Hex 6.3 software.

### 2.3. Preparation of RHT-CLA hybrid ligands

The RHT and CLA based novel hybrid analogues were prepared using ACD ChemSketch

(ver. 12.01, Freeware) and their three dimensional structures were deduced using ProDRG server (<http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg>) [23]. Prior to docking, the hybrid ligands were energy minimized under MMF96 force field using Ligand Scout (demo, version 3.12) [24]. The analogue preparation was done completely on random basis with an aim to design a hybrid ligand with better inhibitory potential. Furthermore, the ligands were analyzed for their ADME/Tox features using Discovery Studio 3.1 Client (study carried out at AMDI, Malaysia).

#### **2.4. Docking and Molecular Dynamic Simulation of HSF1D-ligand complex**

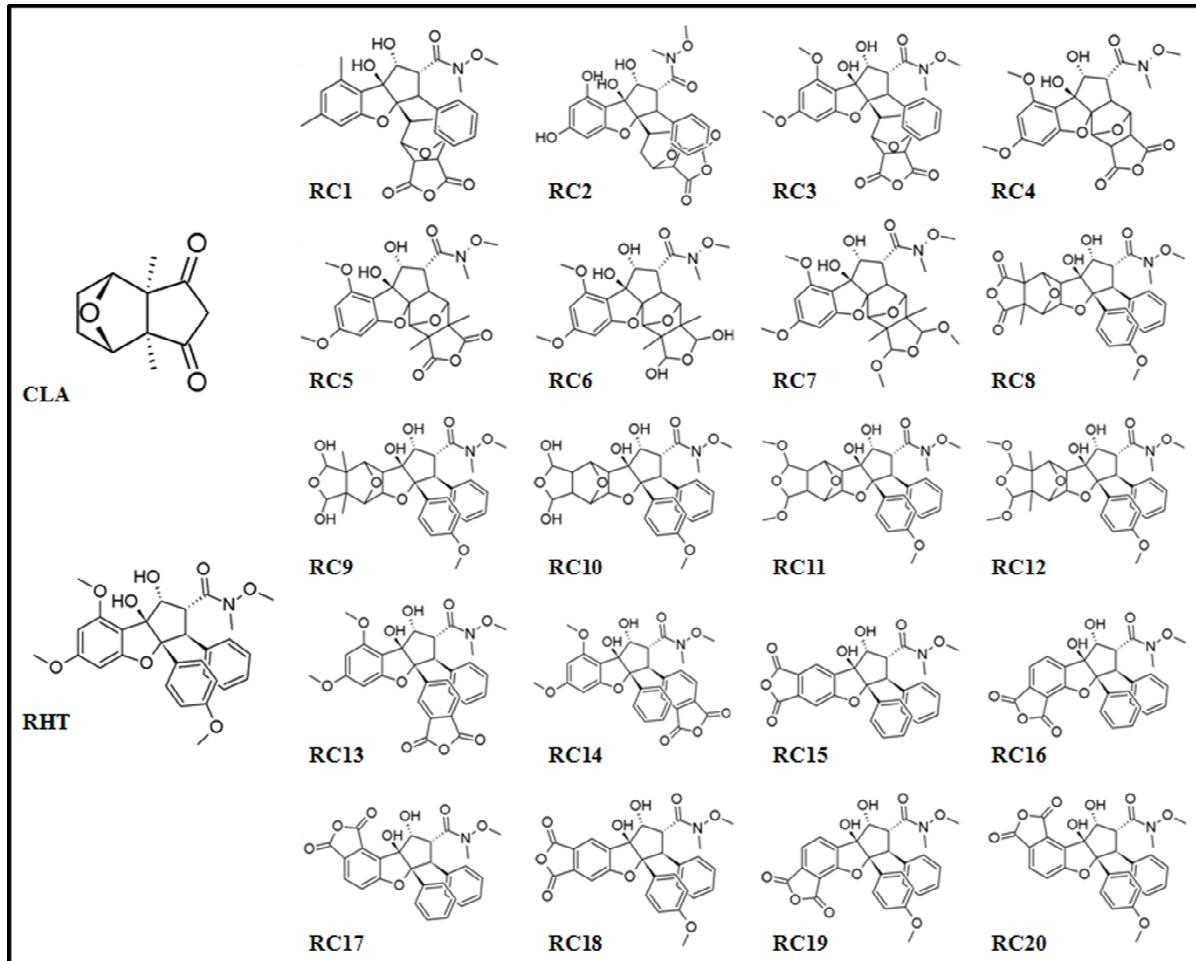
The docking of the hybrid ligands with HSF1D was carried out using Hex 6.3 (blind docking) and Autodock 4.2 (site targeted docking) [25]. The interaction profile of the RHT-CLA based hybrid inhibitors with HSF1D was estimated using LigPlot<sup>+</sup> and Discovery Studio Visualizer 4.1 Client. In addition, the pharmacophoric features essential for the interaction were analyzed manually using Discovery Studio Visualizer 4.1 Client. Furthermore, the molecular dynamics simulation of HSF1D-RC15 and HSF1D-RC17 was performed in aqueous environment implementing GROMOS96 53a6 force field using Gromacs 4.5.6 package (for details, refer supplementary S1).

#### **2.5. Analysis of DNA and protein-ligand complex interaction pattern**

The binding pattern of the HSF1D-ligand complex and DNA was evaluated using Hex online server ([http://hexserver.loria.fr/hex\\_server.php](http://hexserver.loria.fr/hex_server.php)) [26]. For this, the nucleotide sequence of Heat shock element (HSE) located upstream of the human Hsp70 gene from -107 to -94 was modelled using 3D DART service present at HADDOCK server (<http://haddock.science.uu.nl/services/3DDART/>) [27]. The HSE in Hsp70 promoter region is conserved sequence of 17 nucleotides 5' CTGGAATATTCCCGAAT 3' [28]. The docking of HSF1D and HSP70 promoter HSE was taken as control for the analysis. The visualization and interaction analysis was carried out using Discovery Studio Visualizer 4.1 Client.

### 3. Results and Discussions

In the present investigation, an integrated computational approach was implemented to model the DNA binding domain of HSF1 (HSF1D) protein, to design novel RHT-CLA based hybrid inhibitors and evaluate their efficacy against it (Fig. 2).

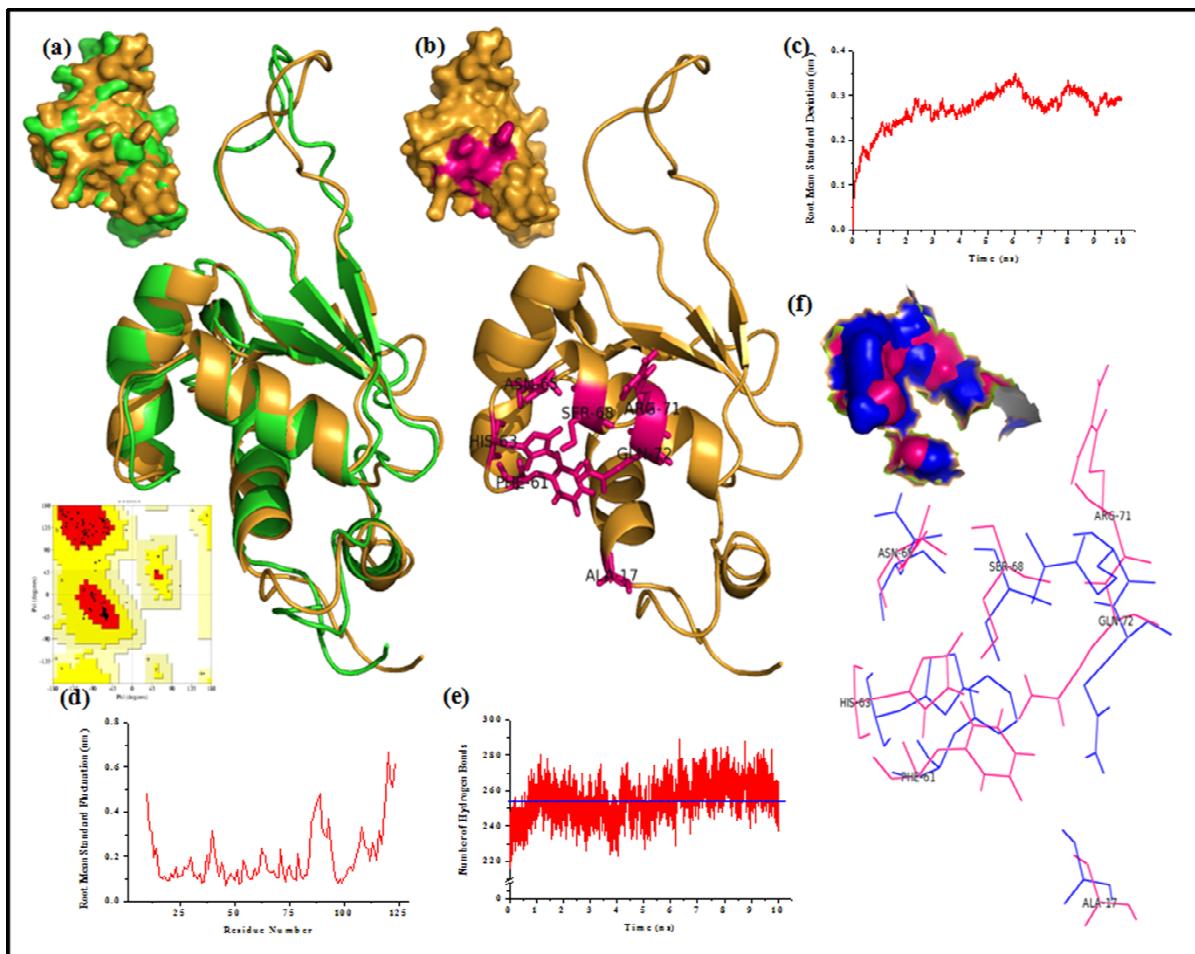


**Fig. 2** Chemical structures of the Rohinitib (RHT) and Cantharidin (CLA) based hybrid ligands targeted towards Heat Shock Factor 1

#### 3.1. Modelling and evaluation of structural aspects of HSF1D

The HSF1D structure was modelled based on sequence alignment with all the structures present at RCSB Protein Databank (PDB) using NCBI BLASTp. The maximum similarity was demonstrated with the NMR structure of Heat Shock Factor protein 1 DNA binding domain from *Homo sapiens* (PDB Id: 2LUD) with an identity of 99.1%. The structural

validation of the refined protein using SAVES showed that 85.7%, 14.3% and 0.0% of amino acid residues in core, allowed and disallowed regions (Ramachandran Plot), 94.78% of amino acid residues had an average 3D-1D score  $> 0.2$  (Verify 3D) and an overall quality factor of 98.05 (ERRAT). The stability and conformational changes associated with the native HSF1D without the ligand were evaluated using molecular dynamic simulation analysis as a function of time (Fig. 3). The root mean standard deviation (RMSD) of C $\alpha$  atoms was found to be fluctuating around 2.84 $\text{\AA}$  at the end of simulation run. The root mean standard fluctuation (RMSF) calculations identified regions containing amino acid residues 10-14, 37-41, 84-95 and 106-123 possessed high flexibility. It was observed that extreme residues 87-89 and C terminal residues possessed major peaks with 4.8 $\text{\AA}$  and 6.6 $\text{\AA}$  fluctuations respectively. The hydrogen bonds formed between the protein and the surrounding aqueous environment accounts for one of the major factor for maintaining its conformational stability. An increase from  $\sim$ 240 to  $\sim$ 254 hydrogen bonds was observed during the 10ns simulation run, suggesting the variations in the HSF1D conformation. Further, the cation- $\pi$  interactions of HSF1D were evaluated before and after the MD simulation. Prior to simulation run, the native HSF1D formed two energetically significant cation- $\pi$  interactions including Arg94-Phe47 (-2.42kcal/mol) and Arg106-Trp37 (-5.61kcal/mol). The simulated HSF1D also possessed two energetically significant cation- $\pi$  interactions with significantly higher energy; Arg117-Phe76 (-6.03kcal/mol) and Arg106-Trp23 (-9.58kcal/mol). The variation in these interactions also supports that HSF1D underwent the structural and conformational variations during the simulation run. The SRIDE server results confirmed Gln100 as the only stabilizing residue HSF1D.



**Fig. 3** Structural Analysis of Heat Shock Factor 1 DNA Binding Domain (HSF1D). (a) Superimposition of the HSF1D before (green) and after (orange) simulation of 10ns and the Ramachandran plot of the protein after simulation; (b) The active site residues of HSF1D after simulation run (nucleic acid binding residues are shown in pink); (c) Root mean standard deviation (RMSD) plot of the HSF1D protein backbone as a function of time; (d) Root mean standard fluctuation (RMSF) plot of HSF1D amino acid residues of during the simulation run; (e) Variation in number of hydrogen bonds formed between HSF1D and surrounding solvent molecules; (f) Superimposition of active site residues before (blue) and after (pink) simulation of 10ns.

### 3.2. Identification of nucleic acid binding site of HSF1D

Under stress condition, once the HSF1 is activated, it binds to the heat shock element of the

target genes. CLA and RHT selectively diminished the HSF1 binding to the promoter region. Thus, it is important to first analyze the amino acid residues involved in the nucleic acid binding. From the COACH server analysis, Ala17, Phe61, His63, Asn65, Ser68, Arg71 and Gln72 were predicted to be involved in its interaction with the nucleic acid. The PROSITE results showed that residues 57-81 had HSF type DNA binding domain signature (PROSITE entry PS004343) with a consensus pattern of L-x(3)-[FY]-K-H-x-N-x-[STAN]-S-F-[LIVM]-R-Q-L-[NH]-x-Y-x-[FYW]-[RKH]-K-[LIVM]. Herein, the HSF1D had a consensus sequence of LPKYFKHNNMASFVRQLNMYGFRKV. The blind docking of HSF1D with CLA and RHT also demonstrated the interaction with predicted active site residues. CLA interacted with Ala17, Phe18, Phe61, Lys62, His63 and Gln72; while RHT interacted with Ala17, Phe18, Lys21, Tyr60, Phe61, Lys62, His63, Ser68, Gln72 and Arg117. This confirms a critical role of Ala17, Phe61, His63, Asn65, Ser68, Arg71 and Gln72 in nucleic acid interaction.

### **3.3. Docking of HSF1D with RHT-CLA based hybrid ligands**

Both the blind and site specific docking analysis demonstrated that RHT-CLA based hybrid ligands had a better affinity towards HSF1D in comparison to either RHT or CLA. Comparative analysis of the binding energy obtained using Hex 6.3 software showed that RC12 ligand possessed the highest affinity towards the HSF1D. The affinity of RC12 was found to be 1.29 and 2.38 folds higher than RHT and CLA respectively ( $p < 0.05$ ). It is important to mention that out of twenty hybrid ligands, 16 ligands possessed a greater affinity than RHT (Binding energy: -272.9kcal/mol); while all 20 ligands had a greater affinity than CLA (Binding energy: -147.98kcal/mol). Further, for site-specific docking, the ligands were docked into the HSF1 nucleic acid binding site analyzed earlier using Autodock 4.2. The results obtained, were found to be quite varied in comparison to those gained through blind docking algorithm using Hex 6.3. Out of 20 ligands, only 10 ligands demonstrated a greater

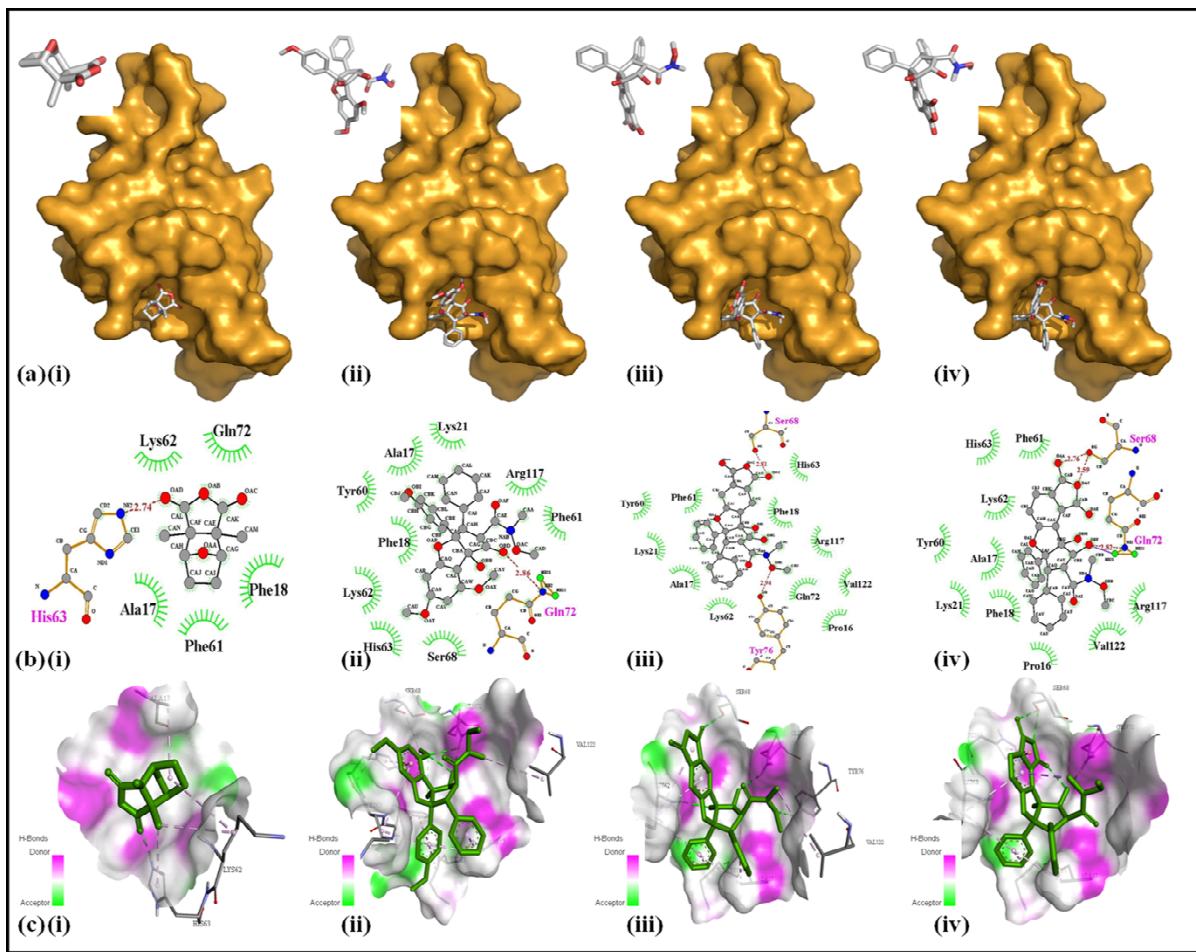
affinity than RHT (Binding energy: -8.20kcal/mol) and all still had greater affinity than CLA (Binding energy: -4.84kcal/mol). The ligand RC15 possessed the highest binding affinity for HSF1D and was 1.13 and 1.91 folds higher than that of RHT and CLA respectively. The ligand RC17 also demonstrated a binding energy of -9.27kcal/mol towards HSF1D. The ligand RC12 showed a binding energy of -8.3kcal/mol during site-specific docking which was comparable to that of RHT. However, RC12 showed around 1.71 folds higher affinity in comparison to CLA (Table 1). Herein, a huge variation was observed in the binding energy of the ligands to HSF1 obtained from Hex6.3 and Autodock4.2. This may be due to the variations in the algorithms; both follow for the binding energy calculations. Furthermore, it is also important to mention that the docking posses (showing highest negative binding energy) were found similar, irrespective of the docking algorithm followed. The amino acid residues interacting with the ligands showed consistency in both the cases.

In addition to the affinity of the ligands towards the protein target, its molecular and ADME/Tox features are also crucial deciding factor for its use as a drug. In this regard, the hybrid ligands were evaluated for the same using Discovery Studio 3.1 Client. Most of the ligands were found to have good human intestinal adsorption, good drug-likeness and less plasma protein binding capability. The results also suggested that the ligands were non carcinogenic, non mutagenic, mild skin and ocular irritant and biodegradable under aerobic conditions (Supplementary Table 1).

### **3.4. Interaction profile of the RHT-CLA hybrid ligands with HSF1D**

The interaction analysis demonstrated that the RHT-CLA hybrid ligands shared a similar binding pattern to that of parent ligands (Fig. 4). Most of the hybrid ligands interacted with the nucleic acid binding site residues including Ala17, Phe18, Lys21, Tyr60, Phe61, Lys62, His63, Ser68, Gln72 and Arg117 as demonstrated by RHT (Supplementary Table 2). The

LigPlot+ results showed that ligand RC19 made a maximum of 4 hydrogen bonds with Lys62, His63, Ser68 and Gln72. Apart from it, ligand RC2, RC 14, RC16 made 3 hydrogen bonds either with Ala17, Lys62, His63, Ser68, Gln72 or Ser121. The ligands RC15 and RC17 (possessing highest binding affinity) formed only 2 hydrogen bonds either with Ser68, Gln72 or Tyr76. A much closer understanding of the interaction pattern was gained through Discovery Studio Visualizer 4.1 Client. The results demonstrated that ligand RC2 formed a maximum number of hydrogen bonds including conventional, carbon and pi-donor hydrogen bonds with Lys62, Ser68, Gln72, Arg117, Ser121, Tyr60 and Phe18. RC15 formed a total of 4 conventional hydrogen bonds with Lys62, Ser68, Gln72 and Tyr76 while RC17 formed 3 of them with Ser68 and Gln72. Ligands RHT, RC2, RC3, RC17 and RC18 were involved in electrostatic interactions. The hydrophobic interaction between the HSF1D and the ligands were majorly of alkyl or pi-alkyl type. In addition, pi-sigma and pi-pi stacked interactions were also observed in many cases (Supplementary Table 3A, 3B and 3C). Furthermore, the pharmacophoric features established for efficient binding with the HSF1D followed HHAAAAA pattern (H: hydrophobic centre and A: acceptor group) (Supplementary Fig. 2).



**Fig. 4** Molecular docking and interaction pattern analysis of the ligands with HSF1D protein

(a) The best binding posses (corresponding to highest binding affinity) of the ligands with HSF1D; (b) Interaction analysis of the ligands with HSF1D active site residues using LigPlot<sup>+</sup>; (c) Interaction analysis of the ligands with HSF1D active site residues using Discovery Studio Visualizer 4.1 Client. Here in the ligands corresponds to (i) CLA, (ii) RHT, (iii) RC15 and (iv) RC17

### **3.5. Molecular Dynamic Simulation analysis**

MD simulations were also performed for HSF1D in complex with RC15 and RC17 (showing highest affinity) for gaining a better understanding of the effect of ligands binding with HSF1D active site (Fig. 5). For the molecular dynamic simulation, the docking poses gained from Autodock4.2 having the highest negative binding energy were chosen. The simulation

results showed that the protein ligand complex had RMSD value of ~5Å. The HSF1D-RC15 and HSF1D-RC17 had RMSD value of 4.02Å and 5.05Å respectively at the end of the simulation run. This clearly indicates that the presence of ligands induced structural variations in the protein conformation. The RMSD of both the ligands was ~2Å (data not shown), suggesting their stability in the protein's active site. When the HSF1D fluctuations were analyzed in the presence of ligands, it was observed that the movements of majority of the active residue were reduced. A greater reduction was observed in the case if RC15. These results support the inhibiting and stabilizing potential of the two ligands. The RMSD of the active site residues (Ala17, Phe18, Phe61, His63, Asn65, Ser68, Arg71, Gln72, Arg117 and Ser121) was 2.23Å and 2.76Å in case of RC15 and RC17 respectively at the end of the simulation run. The RMSD of the same for only protein system was 2.97Å (data not shown). The hydrogen bond analysis of HSF1D-ligand complex showed the presence of hydrogen bonds throughout the simulation run. For ligand RC15, the hydrogen bond occupancy with His63, Ser68, Gln72 and Arg117 was 20.9, 19.7, 61.5 and 5.8% respectively. The same for RC17 was 7.9, 34.8, 22.8 and 29.74% respectively during the simulation run. The interaction analysis between HSF1D and the ligands in the simulated complex demonstrated greater interaction between them. In case of RC15 simulated complex, the ligand formed a total of 5 hydrogen bonds with HSF1D amino acid residues (Asn65, Phe69 and Gln72). On the other hand, RC17 formed 3 hydrogen bonds with Asn65, Gln72 and Phe61 residues. In comparison, the initial RC15 and RC17 complexes (0ns) formed only 3 and 1 hydrogen bonds with HSF1D respectively. Increase in number of hydrophobic and hydrogen bond interaction suggests deeper entry of the ligands in the HSF1 active site pocket (Supplementary Table 4). Furthermore, the analysis of the binding energy of HSF1D-RC15 and HSF1D-RC17 association carried out using g\_mmpbsa tool demonstrated a higher average affinity of RC15 (binding energy: -40.43kcal/mol) in comparision to RC17 (binding

energy: -35.05kcal/mol) throughout the simulation run. These results directly correlated with the results gained through Autodock 4.2 (refer Table 1). A critical analysis showed HSF1 amino acid residues Phe18, Phe61, Ser68 and Gln72 had maximum contribution in the binding energy with the ligands. Tyr60, Lys62, His63 and Gln72 were more crucial for interaction with RC15; while the same for RC17 were Phe61, Ser68 and Arg71 (Supplementary Fig. 3).

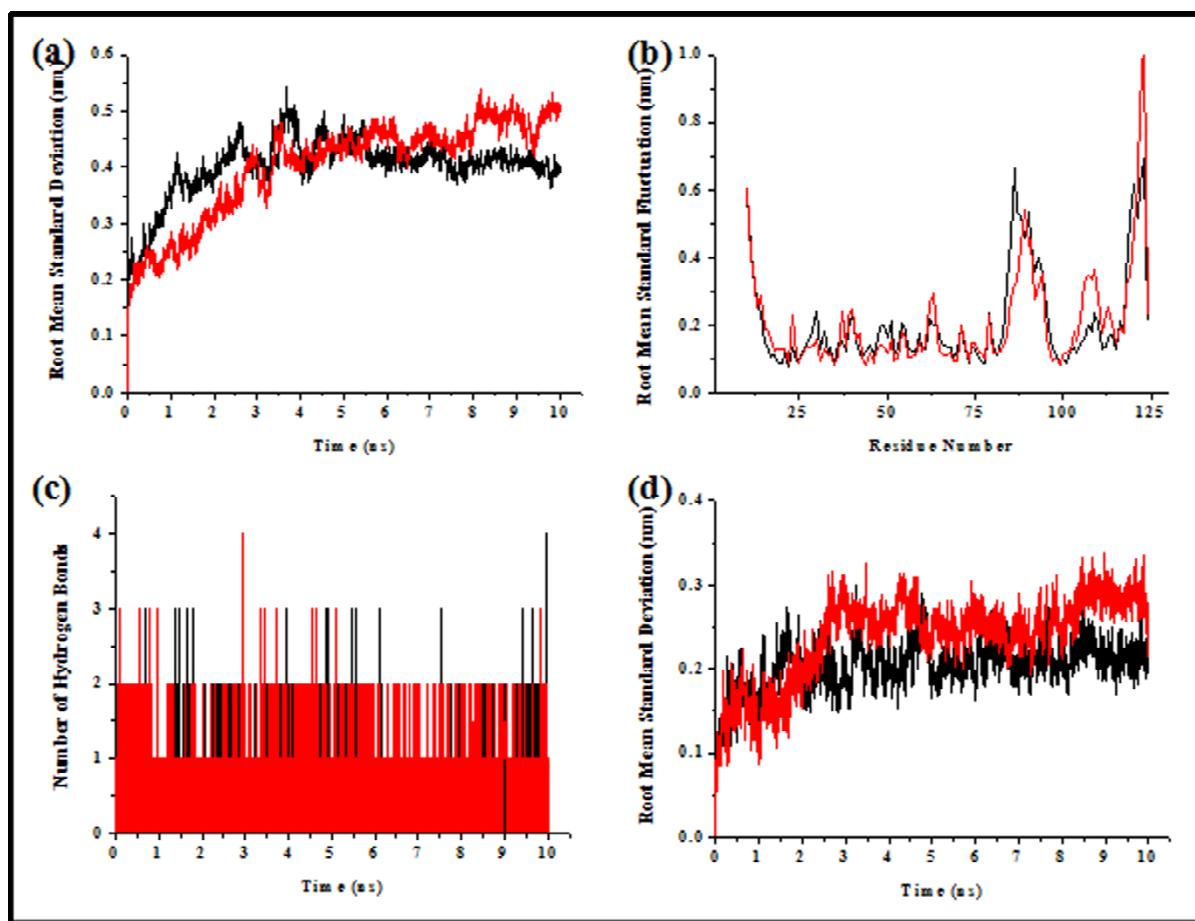


Fig. 5 (a) Root mean standard deviation (RMSD) plot of the protein backbone as a function of time; (b) Root mean standard fluctuation (RMSF) plot of amino acid residues in the complex during the simulation run; (c) Variation in number of hydrogen bonds between protein and ligand. (d) Root mean standard deviation (RMSD) plot of the active site residues as a function of time. All the analysis was carried out as a function of time at 300K. The

black and red plot designates the ligand RC15 and RC17 in complex with HSF1D protein respectively.

### 3.6. Interaction of the HSF1D-ligand complex with the HSP70 heat shock element

The docking of HSF1 with the HSE was carried out both in the presence and absence of ligands. For Protein DNA docking, all the dynamically simulated structures were taken into consideration. The results demonstrated a significant decrease in the affinity of HSF1D towards HSE consensus sequence of HSP70 was observed in presence of inhibitor. In the absence of inhibitor, the binding energy of HSF1D was found to be -749.92kcal/mol. In comparison, the presence of RHT and CLA inhibitors decreased the binding affinity of HSF1D by 1.145 and 1.106 folds respectively. In comparison, the interaction of RHT-CLA hybrid ligand bound HSF1D with HSE was much weaker. It was observed that many of the ligands including RC2, RC9, RC10, RC14, RC15, RC16 and RC17 resulted in a decrease >1.20 folds than the control (without drug). Ligand RC16 showed the highest decrease of 1.29 folds in affinity towards the Hsp70 HSE. However, the ligands RC12, RC15 and RC17 which showed higher affinity towards HSF1D and better interaction pattern resulted in decrease of 1.19, 1.20 and 1.23 folds respectively (Fig. 6). A closure analysis demonstrated that HSF1D amino acid residues; Ala17, Phe18, Ser68, Arg71, Gln72, Arg117 and Thr120 were involved in hydrogen bonding interaction with Hsp70 HSE (G104, A103, A102, T101 and T84 nucleotide residues). Presence of any inhibitor bound to HSF1D reduced its affinity for HSE and also changed the interaction pattern. RHT bound HSF1D interacted through His63, Asn64, Asn65, Ser68 and Arg71 only with A87 nucleotide residue of HSE. In case of RC15, the hydrogen bond interaction of His63, Ser68 and Arg71 occurred with A87 nucleotide residue. The same for RC17 occurred between His63, Asn65, Ser68, Arg71, Lys116, A87 and G105. The presence of CLA in HSF1D active site residue did not change

the interaction pattern much, however decreased its affinity for HSE.

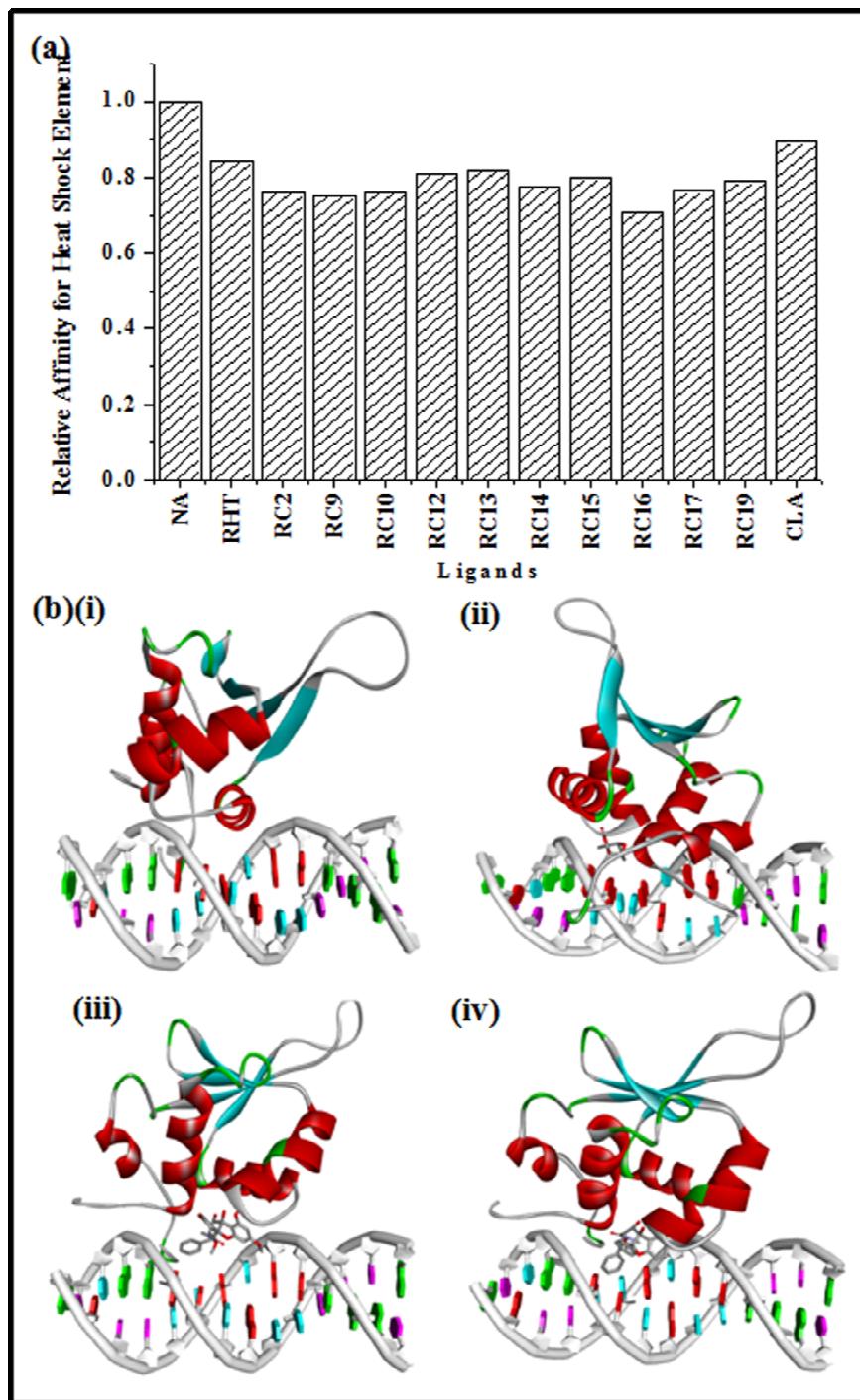


Fig. 6 (a) Molecular docking and interaction pattern analysis of HSF1D protein with the Hsp70 Heat Shock Element (HSE) (a) Relative variation in the binding energy of HSF1D with the HSE with or without ligands. (b) Binding pattern of HSF1D with HSE (i) in the absence of any ligand and in the presence of (ii) CLA, (iii) RHT and (iv) RC17

#### 4. Conclusion

The knowledge gained through this investigation would help in better understanding of the molecular interaction basis between HSF1 and its ligands. It would also enhance the design and discovery of HSF1 targeted anti-cancer drug. The present study demonstrated that Rohinitib and Cantharidin based novel hybrid inhibitors had a better HSF1 binding potential in comparison to their parent molecules. The hybrid ligands interacted with most of the HSF1 active site residues including Ala17, Phe61, His63, Asn65, Ser68, Arg71 and Gln72, crucial for interaction with nucleic acid. The ligands were also found to have good druglikeness, good intestinal absorption, good aqueous solubility and were non carcinogenic, non mutagenic and biodegradable under the aerobic conditions. The simulation analysis suggested formation of stable complex between HSF1 and ligands, restraining the movement of active site residues. Also, the HSF1-ligand complex had a reduced affinity for the Hsp70 promoter HSE in comparison to unbound native protein. Conclusively, RHT-CLA hybrid ligands can be used as a potential inhibitor of HSF1 for non-oncogene target based cancer therapy.

#### Author's Contributions

TA and AK conceived the original idea of the research work. TA, NA implemented the experiments. TA, AK, TKM and HA wrote and reviewed the main body of the manuscript.

#### Conflicts of Interests

Authors declare no conflicts of interest.

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## FIGURE CAPTION LIST

- **Fig. 1** Role of HSF1 protein in the cancer progression. Under normal condition, HSF1 is present in cytoplasm as an inactive monomer bound by Hsp90, Hsp70 and Hsp40. Under proteotoxic stress (usually observed in cancerous cells), Hsp90 is released from the HSF1-chaperone complex. The HSF1 thereby forms an inactive homotrimeric complex and migrates to the nucleus where it gets activated by subsequent hyperphosphorylation. The activated trimeric form of HSF1 binds to heat shock element (HSE) present in stress proteins, inducing the transcription of Hsp40, Hsp70, Hsp90, BAG3 and other chaperones, thus, promoting cancer cell survivability. Thus, herein we hypothesized that RTH-CLA based hybrid inhibitors would block HSF1-HSE interaction, resulting in death of cancerous cells.
- **Fig. 2** Chemical structures of the Rohinitib (RHT) and Cantharidin (CLA) based hybrid ligands targeted towards Heat Shock Factor 1.
- **Fig. 3** Structural Analysis of Heat Shock Factor 1 DNA Binding Domain (HSF1D). (a) Superimposition of the HSF1D before (green) and after (orange) simulation of 10ns and the Ramachandran plot of the protein after simulation; (b) The active site residues of HSF1D after simulation run (nucleic acid binding residues are shown in pink); (c) Root mean standard deviation (RMSD) plot of the HSF1D protein backbone as a function of time; (d) Root mean standard fluctuation (RMSF) plot of HSF1D amino acid residues of during the simulation run; (e) Variation in number of hydrogen bonds formed between HSF1D and surrounding solvent molecules; (f) Superimposition of active site residues before (blue) and after (pink) simulation of 10ns.
- **Fig. 4** Molecular docking and interaction pattern analysis of the ligands with HSF1D protein (a) The best binding posses (corresponding to highest binding affinity) of the ligands with HSF1D; (b) Interaction analysis of the ligands with HSF1D active site residues using LigPlot<sup>+</sup>; (c) Interaction analysis of the ligands with HSF1D active site

residues using Discovery Studio Visualizer 4.1 Client. Here in the ligands corresponds to

(i) CLA, (ii) RHT, (iii) RC15 and (iv) RC17

- **Fig. 5** (a) Root mean standard deviation (RMSD) plot of the protein backbone as a function of time; (b) Root mean standard fluctuation (RMSF) plot of amino acid residues in the complex during the simulation run; (c) Variation in number of hydrogen bonds between protein and ligand; (d) Root mean standard deviation (RMSD) plot of the active site residues as a function of time. All the analysis was carried out as a function of time at 300K. The black and red plot designates the ligand RC15 and RC17 in complex with HSF1D protein respectively.
- **Fig. 6** (a) Molecular docking and interaction pattern analysis of HSF1D protein with the Hsp70 Heat Shock Element (HSE). (a) Relative variation in the binding energy of HSF1D with the HSE with or without ligands. (b) Binding pattern of HSF1D with HSE (i) in the absence of any ligand and in the presence of (ii) CLA, (iii) RHT and (iv) RC17

#### TABLE CAPTION LIST

- **Table 1** Binding energy of the RHT – CLA based hybrid ligands as analyzed from Hex 6.3 and Autodock 4.2 softwares. The inhibition constant values were obtained from Autodock 4.2 software.
- **Table 2** Binding energy and the fold decrease in the affinity of HSF1 for Hsp70 HSE in presence of RHT – CLA based hybrid ligands as analyzed from Hex 6.3. For the analysis, HSF1-HSE interaction in absence of any ligand was considered as control (NA\*).