ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Virtual screening and molecular dynamics simulation study of hECE-1 protease inhibitors

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ABSTRACT

Human Endothelin Converting Enzyme-1 (ECE-1) is a type II integral membrane protein expressed by endothelial cells of the aorta, lungs, ovary and testis. The role of hECE-1 is to convert big endothelin (ET) into small ET-1. This functional ET-1 acts as a vasoconstrictor. It has been implicated in pathophysiology of cardiac diseases including hypertension, vascular hypertrophy and arthrosclerosis. Regulation of endothelin could be useful in number of cardiovascular diseases. In this study we screened ZINC database using Dockblatster to find out novel inhibitors of hECE-1. Screened compounds were filtered on the basis Lipinski's rule. The selected compounds were then docked using PyRx tool. Finally, ligands ZINC31078067 and ZINC31075404 having lower energy re-docked using Autodock 4.2 by considering Asn 466, Glu 567 and His 632 as flexible residues of hECE-1. A fully solvated molecular dynamics (MD) simulation was performed on docked complexes. The hECE-1-inhibitor complex was found stable throughout the simulation period. The conserved residues ^{565}VNA^{567} of hECE-1 and Zn^{2+} forms strong hydrogen bonding interaction with inhibitor. The selected ligand ZINC-31078067 and ZINC31075404 would act as new potent and selective inhibitors of hECE-1 which could be used as lead drug candidates in the cardiovascular diseases.

Keywords: hECE1 (human Endothelin Converting Enzyme1), Virtual screening, ADME/Tox, Molecular Docking, Molecular Dynamics Simulation.

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ISSN: 0975-8585

INTRODUCTION

Human Endothelin Converting Enzyme-1 (hECE-1) is a type II integral membrane protein expressed by endothelial cells of the aorta, lungs, ovary and testis [1]. The role of hECE-1 is to convert big endothelin (ET) into small ET-1 by cleaving specifically at Trp21-Ile/Val22 sites [2]. An endothelium derived 21 residues vasoconstrictor peptide, endothelin, has been used as one of the most potent vasoconstrictor [3]. ET-1 has been implicated as a mediator of increased vascular tone and vascular remodeling in pulmonary arterial hypertension (PAH) [4]. Circulating ET-1 levels have been correlated with the severity of hemodynamic and with symptoms in patients with congestive heart failure [5]. ET-1 is known to be a potent mitogen that stimulates proto-oncogene expression in vascular and non-vascular cells [6]. Elevated expression of ET-1 has been reported in many tumors and it is believed to be a vital 'hormone' in the growth and progression of prostate, ovarian, colorectal, bladder, breast, and lung carcinomas [6,7]. Because several studies have shown that Endothelin plays a pathogenic role in several disease conditions so regulation of endothelin production could be useful in cardiovascular diseases and human breast cancer [5-8].

The enzyme hECE-1 represents plausible target for pharmacological intervention to the endothelin system because hECE-1 appears to be a novel metalloprotease that has strict substrate specificity [8, 9]. A selective inhibitor for the enzyme can presumably inhibit the production of active endothelin in a highly specific manner. Unfortunately, this avenue of research has been severely hampered in the past because many other metalloprotease inhibitors do not inhibit hECE-1 [9]. The hECE-1 inhibitors should provide renewed promise for progress toward this novel target for therapeutic intervention endothelin system. There has been an attempt made to screen the potential inhibitors of ECE-1 in earlier study [10].

Thus, we used ligand based approach to identify molecules with physical and chemical similarities to known ligands that are likely to interact with the crystal structure of hECE-1 [11-13]. The programme Dockblaster was used to screen 3D ligand containing large libraries of ZINC database [14,15]. Autodock 4.2 used for docking of ligand with hECE-1 and Molecular dynamics was performed over docked complexes [16]. Our work suggests that conserved residues ^{565}VNA^{567} of hECE-1 stabilize inhibitor complex. The interacting oxygen atom of inhibitors with zinc ion replaces the catalytic water molecule thus this non-peptidic inhibitor could serve as a valuable tool to reduce the activity of hECE-1. Here, we report ligands ZINC31078067 and ZINC31075404 specifically inhibit hECE-1 with the performance and pharmacological activity of the compound used as a drug for inhibition of hECE-1 to control various cardiovascular diseases and cancer.

MATERIAL AND METHOD

MODEL PREPARATION AND VIRTUAL SCREENING

The crystal structure of human Endothelin Converting Enzyme (hECE-1) complexed with inhibitor phosphoramidon was used as a starting structure (PDB ID: 3DWB) which is a monomeric mutant C428S with 10 missing residues (^{422}YGTKKTCLPR^{431}) [13]. The hECE-1 crystal structure was then patched with missing region as per previous modelling criteria in

[17]. Henceforth; hECE-1 crystal structure with patched model of 10 missing residues would be called as a whole hECE-1 structure for further discussions in this manuscript. Whole hECE-1 structure with inhibitor phosphoramidon submitted to Dockblaster for prediction of novel inhibitor [13, 14]. Partial charges from the AMBER force field were used for receptor atoms except metal ion zinc parameterized as previously study [18, 19]. The flexible-ligand sampling algorithm in Dockblaster superimposes atoms of the docked molecule as per supplied binding site which represent positions for binding ligand atoms [14]. The ligand selection was performed by bin size; distance tolerance up to 2.0 Å, each ligand configuration was scored for electrostatic, van der Waals interaction complementarity and corrected for ligand desolvation. The high-scoring ligand conformation is minimized with 100 steps of simple rigid-body minimization [14]. We have screened ZINC database of commercially available molecules, containing 3 million compounds were available ready to dock [15].

MOLECULAR DOCKING USING AUTODOCK

The selected ligands were obtained from Dockblaster and filtered on basis of Lipinski's rule [20-22]. The ligands with high docking score were optimized by semi-empirical RM1 method [23] using Spartan pro 06 [24]. PyRx (http://pyrx.sourceforge.net/) virtual screening software for computational drug discovery was used to screen libraries of compounds against potential drug targets. The kollman united atom charges were assigned to receptor atom and charges of zinc interacting residues was manually checked. The zinc parameter such as zinc radius, well depth and charge were assigned as per previous literature [18, 19]. However, the active site was defined using AutoGrid [16]. The grid size was set to 60 X 60 X 60 points with grid spacing of 0.375Å. The Lamarckian genetic algorithms (LGA) consisted 100 run with 27000 generation. The generated docked confirmation within 2 Å RMSD was clustered together and analysed.

The ligands ZINC31078067 and ZINC31075404 having lowest binding energy and RMSD was re-docked with whole hECE-1 structure. The Flexible residues Asn566, Glu667 and His732 of whole hECE-1 were selected as per our unpublished data. Receptor structure was minimized with steepest descent method using Gromacs 4.0.4 to remove internal strain [25]. The parameters, selected flexible residues and metal ion zinc given as per previous section. The active site was defined using AutoGrid [16]. Step size of 1Å for translation and 50^0 for rotation were chosen and the maximum number of energy evaluation was set to 2,500,000. Twenty runs were performed. For each of the 30 independent runs, a maximum number of 27, 0000 LGA operations were generated on a single population of 50 individuals. Operator weights for crossover, mutation, and elitism were default parameters (0.80, 0.02, and 1, respectively). The resulting docked conformations were clustered using threshold RMSD less than 1Å with lowest binding energy.

MOLECULAR DYNAMICS SIMULATION

Whole hECE-1 complexed with ZINC31078067 and ZINC31075404 was used as a starting model for the MD simulation. The topologies of ligands were generated from PRODRG server [26]. The GROMOS force field has been used for molecular dynamics simulation [27, 28]. The complex was fully surrounded by SPC water molecules (water

density was 1.0). Total 15 Na^+ ions were used to neutralize the system. The solvated structure was minimized by steepest descent method for 1000 steps at 300K temperature and constant pressure. The LINCS algorithm [29] was used to constrain bond length. The electrostatics interactions were calculated using PME algorithm [30]. All simulations were performed on HP Workstation with the help of Gromacs 4.0.4 program [25]. The dynamic run was then visualized using VMD (Visual Molecular Dynamics) package [31]. Images after simulation were generated using chimera [32]

RESULT AND DISCUSSION

MODEL PREPARATION AND VIRTUAL SCREENING

The receptor structure of whole hECE-1 was prepared before virtual screening (Fig.1). Partial charges from the united-atom AMBER force field were used for all receptor atoms except for metal ion which is defined as per previous literature [18, 19]. Virtual screening against whole hECE-1 as a receptor was carried using Dockblaster to screen ZINC database of commercially available molecules [14, 15]. Each ligand passes through rapid steric fit filter is scored for electrostatic and van der Waals complementarity and adjusted for partial ligand desolvation due to solvent occlusion. The top 100 property matches decoy were selected which shows pose fidelity below 2.0 Å with 60% enrichment as given [14]. The selected hits were transferred through lipinski filter using FAF2/ADME/tox server [21]. The ligand which satisfy lipinski rule but some are excluded due to high internal energy, conformational flexibility, Novelty and Diversity [20, 22]. The selected ligands were optimized using RM1 method [23]. Finally, we have selected top 12 ligands having Dockblaster score observed in Fig. 2A were used for further study.

DOCKING USING PyRx AND AUTODOCK

The receptor structure of Whole hECE-1 was prepared in Autodock wizard. The kollman united atom charges were assigned to receptor atom. The active site residues and zinc ion interacting residues charges and protonation states were properly designed using previous result of metalloprotease docking study [18, 19]. The 12 optimized ligand hits were redocked using Autodock 4.0 wizard of PyRx software with whole hECE-1 receptor. The generated docked conformations were analyzed for binding energy, intermolecular energy and internal energy of each conformation was observed and clustered within 2Å (Fig 2B). The top ranking two ligands (ZINC31078067 and ZINC31075404) and receptor complexes were analysed for hydrogen bonding, hydrophobic and hydrophilic interactions.

The ligands ZINC31078067 and ZINC31075404 were redocked with whole hECE-1 with flexible residues Asn566, Glu667 and His732 as reported in our unpublished data using Autodock 4.2. [16]. Grid size was redefined which centered on selected flexible residues. The grid size was set to 40 X 52 X 45 points with a grid spacing of 0.375Å centered on the selected flexible residue present in active site of enzyme. The grid box includes the entire binding site of the enzyme and provides enough space for the ligand translational and rotational walk. After 270000 LGA operations the generated conformations were selected on the basis of lowest binding energy and clustered RMSD less than 1Å. The complex of

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whole hECE-1 with ligand ZINC31078067 and ZINC31075404 was selected for molecular dynamics simulation.

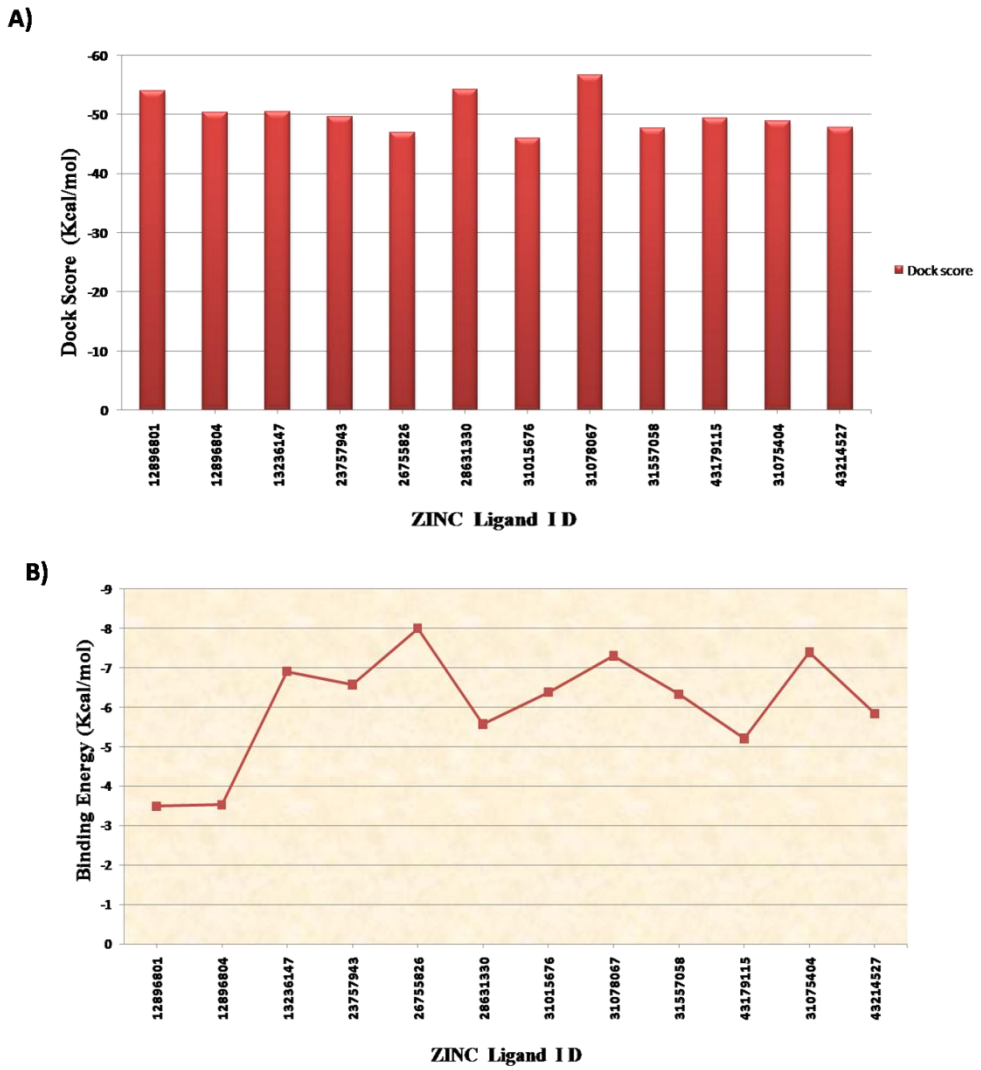

Fig 2: A) Showing Dock score of zinc ligand with whole hECE-1 given by Dockblaster.
B) Showing Binding energy of zinc ligand with whole hECE-1 given by AutoDock.

MOLECULAR DYNAMICS OF WHOLE hECE-1 WITH INHIBITOR

The molecular dynamics simulations have been performed over the complex of whole hECE-1, ligand (ZINC31078067 and ZINC31075404) and zinc ion to understand the coordination and their dynamic behavior with each other. We were interested to find out hydrophilic, hydrophobic and hydrogen bonding interactions of ligands (ZINC31078067 and ZINC31075404) with whole hECE-1 and zinc atom. We monitored set of parameter to verify protein structure over entire simulation run. The RMSD result shows stable behavior of whole hECE-1 as can be seen from Fig. 3A. The average RMSD value found relatively low (0.28nm) throughout 10 ns duration of MD simulation. The results of Rg (Radius of gyration) also indicate stable behavior of structure over the total simulation time (Fig. 3B). RMSF calculation has also been made to describe the basic dynamics of the protein for the C-alpha atoms. Small values for the rigid structural elements and larger values for the ends and

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loops can be seen from the RMSF values (Fig. 3C). In order to identify the specific interactions between ligands and whole hECE-1, we measured direct hydrogen bonding interactions between whole hECE-1 and ligands (ZINC31078067 and ZINC31075404), water-mediated H-bonds (so-called water bridges) and hydrophobic (Lennard-Jones) interactions. H-bonds were recognized on the basis of commonly used geometrical criteria [33]. Overall these results indicate that the structure was stable over the entire simulation period.

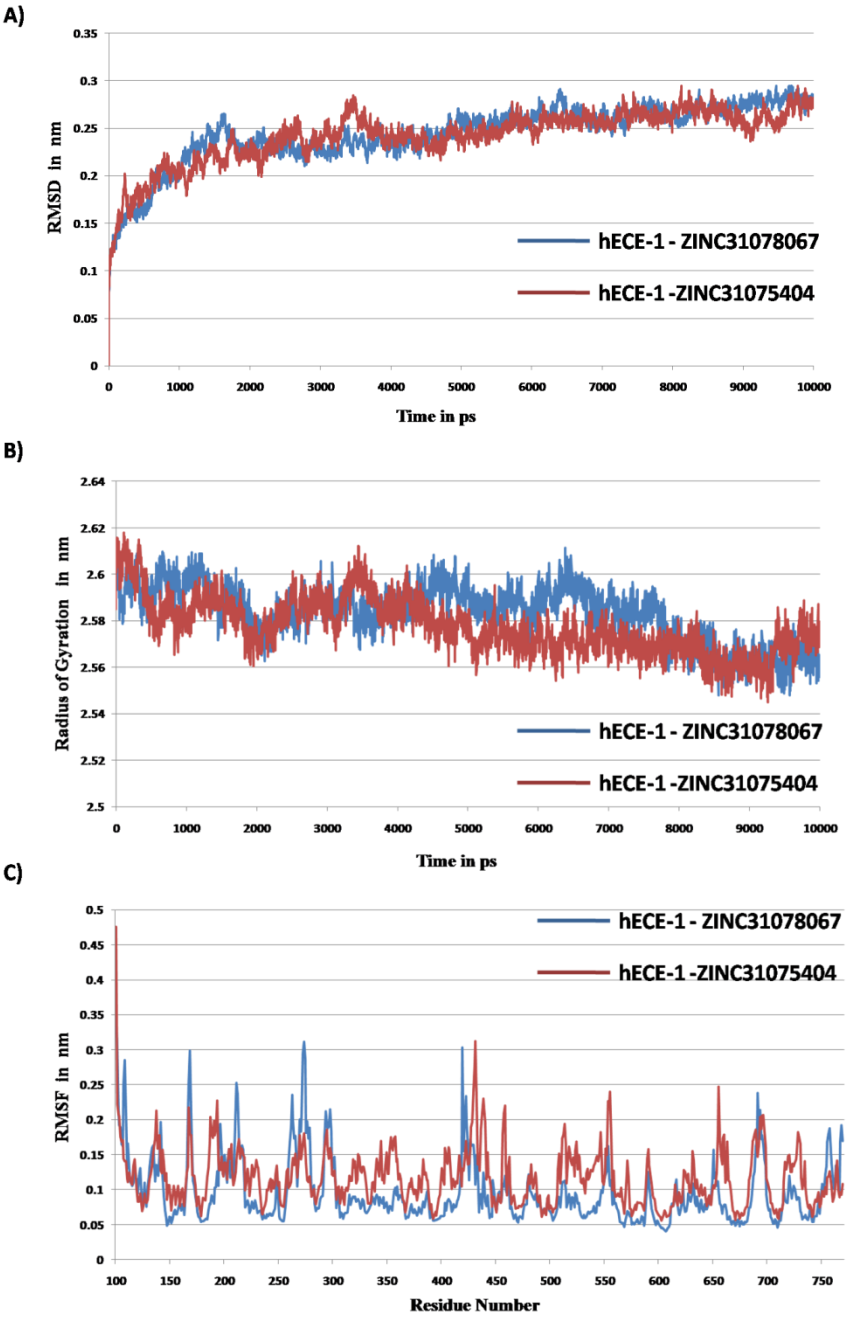

Fig 3: A) Showing RMSD of whole hECE-1-ZINC31078067 complex (blue) and whole hECE-1-ZINC 31075404(red) for 10 ns MD simulation.

B) Radius of gyration (Rg) of whole hECE-1-ZINC31078067 complex (blue) and hECE- 1- ZINC 31075404(red) for 10 ns MD simulation.

C) Root Mean Square Fluctuation (RMSF) of hECE-1 residues in whole hECE-1- ZINC31078067 complex (blue) and hECE-1 - ZINC 31075404 (red) during 10 ns MD simulation.

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STRUCTURE OF WHOLE hECE-1 AFTER SIMULATION

The overall structure of whole hECE-1 was stable during entire simulation. The complexes were analyzed after simulation to check interaction of ligands (ZINC31078067 and ZINC31075404) with whole hECE-1. The whole hECE-1 ectodomain associated with an N-terminal domain and C-terminal domain. C-terminal domain contains catalytic site and metal ion Zinc (Fig.1). The hydrophobic surface calculations were performed to understand nature of pockets and ligand interaction with whole hECE-1.

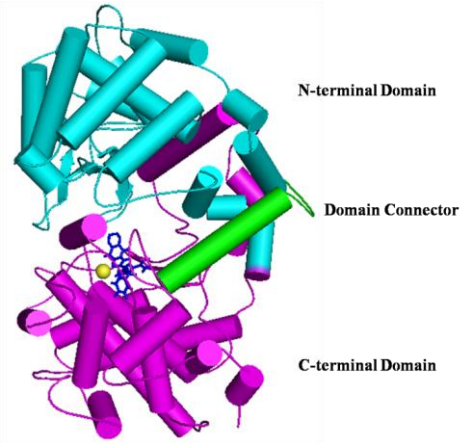

Fig 1: MD simulated structure of whole hECE-1 showing N-terminal domain (Cyan) and C-terminal domain of ECE-1 (magenta) with green colored connecting Helix and loop with Inhibitor (Blue) and Zinc ion (yellow).

LIGAND INTERACTION WITH WHOLE hECE-1

The whole hECE-1 active site was split in the two regions S1 and S2 pockets. The S1 pocket of hECE-1 has S1' and S2' subsite which is non selective and hydrophobic in nature [34]. The hydrophobic surface calculation shows S1 pocket is colored white to orange red indicate hydrophobic nature of this pocket (Fig. 4). The hydrophobic moiety of ligand ZINC31078067 was fixed in S1 pocket while hydrophobic region of ligand ZINC31075404 exposed to S1 pocket and hydrophilic ring turn towards S2' subsite of hECE-1 (Fig. 4A and 4B). The S1' subsite of S1 pocket make strong interaction with both the ligands. The conserved tripeptide ^{565}VNA^{567} present in all members of M13 subfamily [13]. The conserved tripeptide makes hydrogen bonding with both ligands as depicted in Fig. 5 (Table 1 and 2). The S2' subsite and ligand interaction was also observed in complex. The residue Ser 735 of hECE-1 makes hydrogen bonding with both ligand (Fig. 5, Table 1 and 2). Ligand ZINC31078067 interact with Arg 145 (NH11) of hECE-1 takes part in ligand stabilization also observed in previous study of hNEP (Fig.5A) [35]. The hECE-1 residue His 732 interacts with ligand ZINC31075404. Furthermore, these residues form complex hydrogen bonding network which stabilizes hECE-1 and ligand (ZINC31078067 and ZINC31075404) complexes. The hydrophobic environment of S1 pocket generated by residues Phe 149, Trp 714 and Trp 153 which forms strong interaction with hydrophobic region of ligands (ZINC31078067 and ZINC31075404).

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Table 1.Hydrogen bonding interactions between whole hECE-1 and ligand ZINC31078067 along with zinc ion.

Sr. No.	Residues	Distance in Å
1	Glu 667 OE2…Zn 671	2.291
2	Glu 667 OE1…Zn 671	2.104
3	His 611 NE2…Zn 671	2.186
4	Zin 672 OAW…Zn 671	2.059
5	Zin 672 OAT….Zn 671	2.873
6	Arg 145 NH11…OAQZin 772	1.904
7	Val 565 O…HBK Zin 772	2.579
8	Asn 566 OD1…H111 Zin 772	2.418
9	Ala 567 O…H112 Zin 772	2.405
10	Ser 735 O…HAK Zin 772	2.662
11	Trp 714 O…HAL Zin 772	2.884

Table 2. Hydrogen bonding interactions between whole hECE-1 and ligand ZINC 31075404 along with zinc ion.

Sr. No.	Residues	Distance in Å
1	Glu 667 OE2…Zn 771	2.377
2	Glu 667 OE1…Zn 771	2.302
3	His 611 NE2…Zn 771	2.282
4	Glu 508 OE2…Zn 771	2.099
5	Glu 508 OE1…Zn 771	2.091
6	Zin 772 O12….Zn 771	1.948
7	Asn 566 HD22…N31 Zin 772	2.435
8	His 732 ND1…H4 Zin 772	2.900
9	Asn 566 OD1…H117 Zin 772	2.670
10	Ala 567 H…O19Zin 772	1.870
11	Ala 567 O…H118 Zin 772	3.076
12	Phe149 HE2…O33 Zin 772	2.124
13	Ser 735 OG…H120 Zin 772	2.682
14	Val 604 HG11…O16 Zin 772	2.373
15	Val 565 O…H20 Zin 772	2.614

INTERACTIONS WITH METAL ION DURING MD SIMULATION

In the hECE-1 crystal structure Zn^{2+} ion interacts with His 607, His 611 and Glu 667 with inter-atomic distances of 2.2 Å, 2.1 Å, and 1.8 Å respectively [13]. MD simulated structure of whole hECE-1 with ZINC31078067 shows that Zn^{2+} interacts with Glu 667 (OE1, OE2) and His 611 (NE2) and OAW and OAT of ZINC31078067 as shown in Fig. 6A and Table 3. Zinc ion has been found hepta-coordinated with bidentate carboxyl group of Glu 667, His 611 (NE2), OAW and OAT of ZINC31078067 and oxygen atoms of two water molecules (Fig. 6A). In the earlier study zinc has been shown hexa-coordinated [36]. Our simulated structure shows that zinc is hepta coordinated due to fluctuations in the atoms OAW and OAT of ZINC31078067 in mono and bidentate manner (Fig. 6A). The atoms OAW and OAT of ZINC31078067 interacts with zinc in bidentate manner from 4000 ps to 6000 ps, whereas they also show monodentate interaction for rest of the period during simulation (Fig. 6A). The monodentate interaction of ligand ZINC31078067 via atom OAW with zinc through

entire simulation, second atom OAT replaces the catalytic water molecule involved in catalytic process. Such type of coordination between Zn^{2+} and Phosphoramidon has been observed in previous study and it could be important for the inhibition of catalytic activity of enzyme [13].

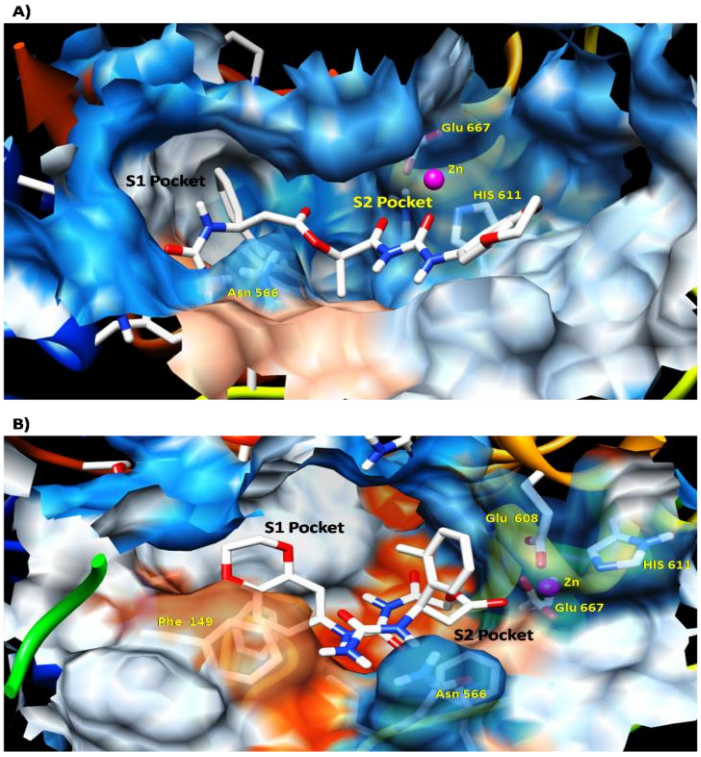

Fig 4: Depicts hydrophobic surface calculations of hECE-1 with inhibitors, A) ZINC31078067, B) ZINC 31075404 found in S1 and S2 Pockets of hECE-1.

The another MD simulated complex of whole hECE-1 with ZINC31075404 shows the zinc coordination with Glu 667(OE1, OE2), His 611 (NE2), Glu 608(OE1, OE2), water (OW) and O12 atom of ZINC31075404 with interatomic distance as depicted in Fig. 6B and Table 4. In this complex we found zinc hepta coordinated due to fluctuation of water and Glu 608 (OE1 and OE2). The Glu 608 OE1 and OE2 show monodentate coordination up to 6000 ps with Zinc and water molecule is in close contact with zinc. After 6000ps water molecule was moved apart from zinc and Glu 608 (OE1 and OE2) atom shows bidentate coordination of with zinc till end of the simulation (Fig. 6B).

Table 3. Hydrogen bonding interactions of Zinc ion in complex whole hECE-1 and ligand ZINC 31078067.

Sr.No	Residues	Distance in Å
1	Glu 567 OE2…Zn 671	2.292
2	Glu 567 OE1…Zn 671	2.105
3	Zin 672 OAW…Zn 671	2.060
4	Zin 672 OAW…Zn 671	2.873
5	His 511 NE2…Zn 671	2.184
6	Water 3640 OW…Zn 671	1.922
7	Water 3652 OW…Zn 671	2.056

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Table 4. Hydrogen bonding interactions with Zinc ion in complex whole hECE-1 and ligand ZINC 31075404.

Sr. No.	Residues	Distance in Å
1	Glu 567 OE2…Zn 671	2.378
2	Glu 567 OE2…Zn 671	2.301
3	His 511 NE2…Zn 671	2.280
4	Glu 508 OE2…Zn 671	2.099
5	Glu 508 OE1…Zn 671	2.092
6	Water 3688 OW…Zn 671	2.015
7	Zin 672 O12…Zn 671	1.947

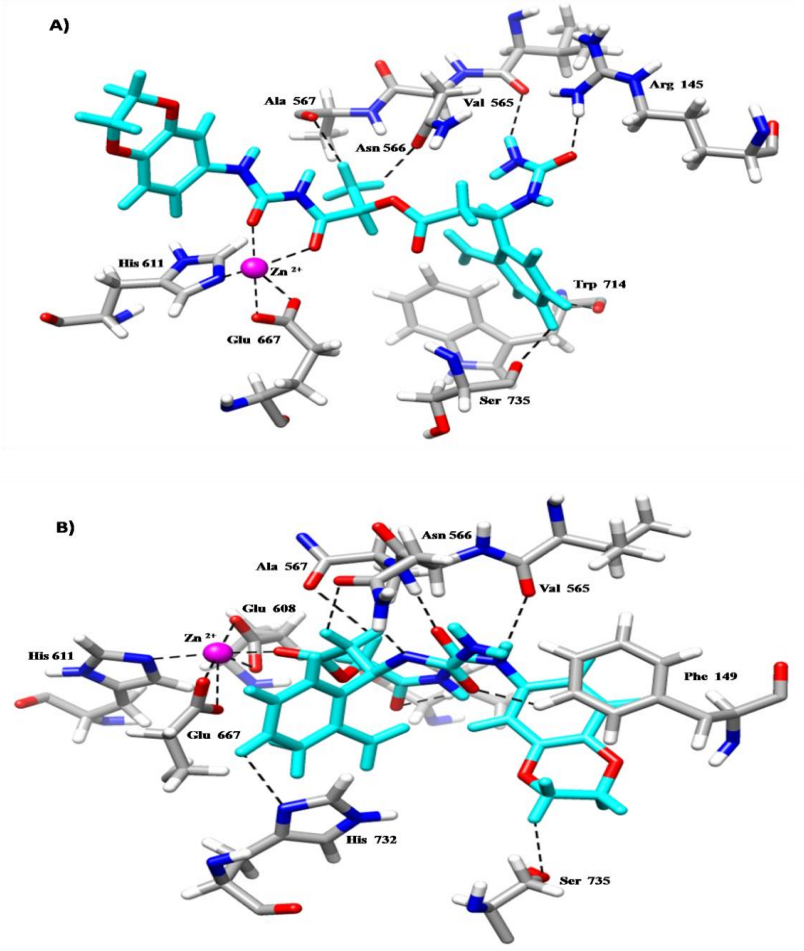

Fig 5: Hydrogen bonding(Black dotted line) interaction of hECE-1 residues (Grey color) with inhibitors (Cyan), A) ZINC31078067 B) ZINC31075404.

CONCLUSION

The complexes of whole hECE-1 with ligands (ZINC31078067 and ZINC31075404) have been found stable throughout the MD simulation period (Fig. 3). Both the ligands form strong hydrophobic interactions with S1 pocket of whole hECE-1 while they are stabilized by hydrogen bonding with conserved tripeptide ^{565}VNA^{567} present in all members of M13 subfamily. The ligands stability was due to hydrophobic and hydrophilic interaction with whole hECE-1. In this study we have utilized combination of various scoring function and docking program to find selective inhibitor for hECE-1. Finally, we state that these ligands

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ZINC31078067 and ZINC31075404 obtained by insilico study could be used as potential inhibitors of hECE-1 and might be used as lead structures in cardiovascular diseases.

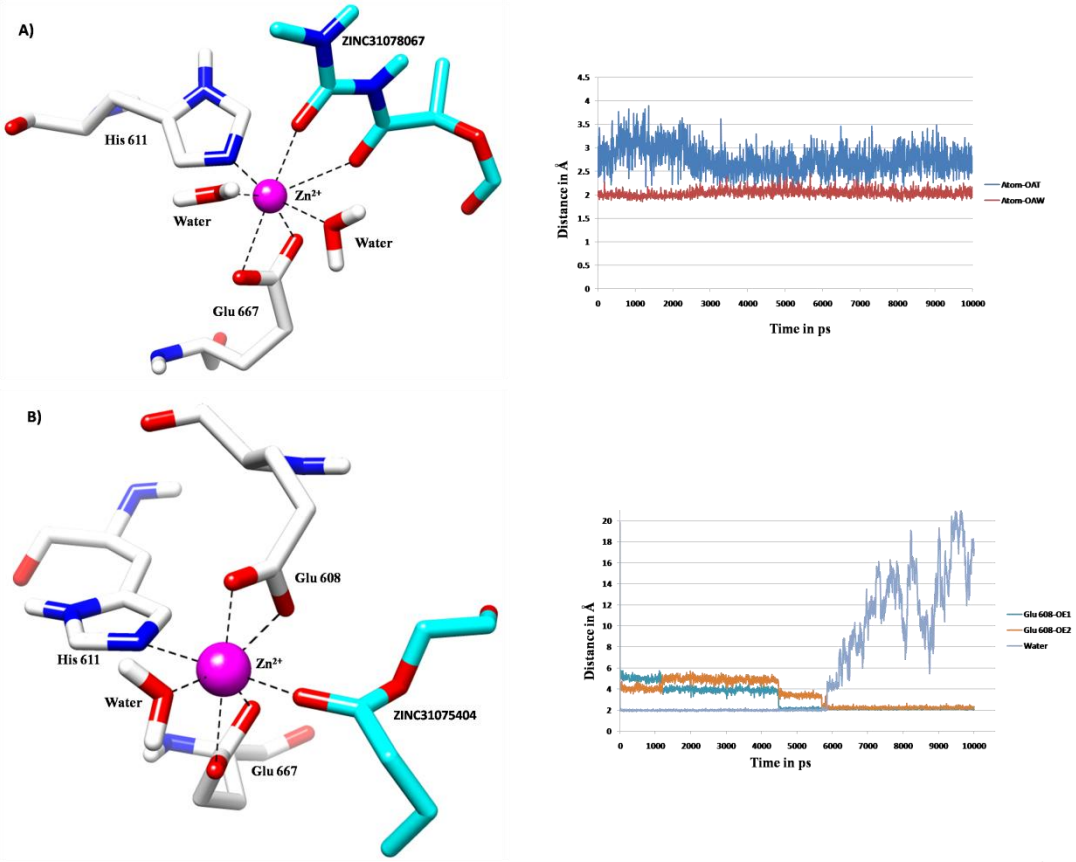

Fig 6: A) Left side shows ZINC31078067 (cyan) interaction with hECE-1 residues (CPK) and Zn^{2+} (magenta), black dotted lines are hydrogen bonds and right side shows hydrogen bond distance of atom OAW and OAT of ligand ZINC31078067 during entire simulation
B) Left side Interaction of ZINC 31075404 (Cyan) with hECE-1 residues (CPK), Zn^{2+} ion (magenta) and black dotted line shows hydrogen bonding. Right side shows the hydrogen bond distance of Glu 608(OE1 and OE2) and water molecule throughout simulation time

CONFLICT OF INTEREST

All Authors have no conflict of interest

ACKNOWLEDGEMENT

CBJ is thankful to Shivaji University, Kolhapur for providing fellowship in the form of Departmental Research Fellow (DRF). MJD is gratefully acknowledged to Department of Science and Technology, New Delhi for providing fellowship as research assistance under the scheme DST-PURSE. KDS is thankful to Department of Biotechnology, New Delhi for financial support under the scheme DBT-IPLS to Department of Microbiology, Shivaji University, Kolhapur. KDS is also thankful to UGC, New Delhi for financial support.

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