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Insights in Binding Mechanism Of Benzofuran Salicylic Acid Inhibitors against Mycobacterium Protein Tyrosine Phosphatases (PTP1B) using Molecular Modelling and Simulation Approaches

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ABSTRACT : The protein tyrosine phosphatase 1B (PTP1B) being virulent phosphatase secreted by *Mycobacterium tuberculosis*, which is essential for the survival and persistence of the bacterium in the host and is proposed as a promising drug target against tuberculosis infection. Here *in silico* docking studies of series of benzofuran salicylic acid derivatives as inhibitors for PTP1B were performed. Based on interactions with proximal domain, three compounds were selected as the best. Molecular Dynamics Simulation study was carried out for these PTP1B docked complexes for better understanding of binding mode, binding free energy and stability. MGBSA calculation showed that binding free energy in terms of enthalpy of top three PTP1B complexes were well correlated with experimental IC_{50} and molecular determinants in binding were also identified. Based on binding free energy (ΔG), compound 4g (6-hydroxy - 2-phenyl -3-{2-[3- (trifluoromethyl) phenyl]ethynyl}-1- benzofuran -5-carboxylic acid) which has more favorable binding free energy compared to other compounds and hence can be chosen as a potent inhibitor against tuberculosis.

KEYWORDS: Protein Tyrosine phospahatse B; Benzofuran Salicylic acid inhibitor; Docking and Simulations, Tuberculosis; MMGBSA

I. INTRODUCTION

Tuberculosis (TB) is a contagious and air-borne complication caused by Myobacterium Tuberculosis (Mtb) . As per World Health Organization (WHO) reports, 1.5 million people died due to TB, and new 9 million TB cases have been registered in 2013 [**1**]. The capacity to sustain and reproduce in macrophage phagosome paves the way for survival of Mtb. The acidification of lysosome contents and bacterial destruction is prevented by obstructing the normal mechanism of phagosome progression into phagolysosome of the host defence system. Inactivation of macrophages allows for dissemination of Mtb to other parts of body, it is destruction of oxidative radicals which allows the survival of bacteria, Hence oxidative free radicals (Reactive Oxygen Speices) is an important mechanism to cure from tuberculosis. Therefore, there is an urgent need to identify novel therapeutic targets for TB treatment as well as new drugs. In the last decade, exo-enzymes protein tyrosine phosphatase A (PTPA) and B (PTP1B) have emerged as promising therapeutic targets to discover new anti-TB agents [2,3,4,5].These enzymes are secreted into the host macrophage cell by *Mycobacterium tuberculosis* (Mtb) and attenuate host immune defenses by interfering with the host signaling pathways [6,7], Thereby, PTPA and PTP1B inhibition by small molecules could impact Mtb survival in the host and open the way for the development of innovative therapeutic strategies [8].

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II. RELATED WORK

The localization outside of the mycobacterial cell wall, which is difficult to penetrate, renders these enzymes as attractive drug targets. Protein tyrosine phosphatases (PTPs) catalyze the hydrolysis of the phosphorylated tyrosine residues of protein substrates, which is a hallmark of cellular signal transduction. These dephosphorylation activities of PTPs have been implicated in a variety of cellular processes and abnormal PTPs activities may cause various diseases including cancer, diabetes, and immune deficiencies [8]. As per recent studies are concerned, 38 members of PTP family (21 receptor-type PTPs and 17 non receptor-type PTPs) have been characterized and are known to have specificity for the phosphorylated tyrosine substrates [9]. Most PTPs share a highly conserved catalytic module that plays a crucial role in the enzymatic dephosphorylation. PTPs conventionally share core structural features viz. a catalytic domain containing four - stranded parallel β sheets that connects to the helix dubbed as P-loop and it harbors the catalytic cysteine nucleophile within the invariant sequence HCX_5R. To regulate the activity and confer higher substrate specificity, other regions such as FPD loop containing the general acid and pTyr recognition loop (Q loop) coordinate the substrate pTyr and catalytic water molecule for catalysis (Fig:1a) [10]. Reported sequence alignment of P-loop of PTPs between Mtb and human suggests that residue F 161 S, K 164 I, D 165 G, are not conserved, thus these residues can be exploited to design selective Mtb PTP1B inhibitor [11]. In addition to conserved PTP1B active site, the enzyme contains two helix lid domain (α7 and α8) that serve to protect catalytic cysteine from oxidative inactivation.

Recent studies revealed that rapid conformational gating of PTP1B lid constitute a reversible physical blockade that masks active site and retards oxidative inactivation. Since mobile lid element has evolved as novel means of protecting the active site against host chemical defence through conformational gating, it is this lid (in close conformation) which protects the PTP1B to subvert dephosphorylation.

PTP1B – OMTS [(oxalylamino-methylene)-thiophene sulfonamide] complex represents a partially open lid conformation, since enzyme active site remains buried and there is no clear route for inhibitor to access the active site without further lid movement. There is a large excursion of helix α8 with little change in position of α7. The closed form of lid protects the active site from oxidation [12]. Recently developed high resolution time dependent single molecule spectroscopy, has shown that PTP1B lid spontaneously transits from previously observed close conformation to much more open conformation that has not been captured crystallographically. Local folding and unfolding of α-3A helix has influence on the conformational dynamics of lid helix α8. Lid residues (197-235) represent an insertion unique to mycobacterial PTPs . The lid begins in helix α7, a long alpha helix that includes two full turns of 3_{10} helix (190-196). Helix α7 ends at Asp212 and is connected by five residue linker to α8 (218-225), which is followed by 10 residue loop. Two adjacent lids (α7,α8) are capable of independent motion. Thus, oxidative resistance of PTP1B mediated conformational dynamics represents a novel adaptation by Mtb species to oxidative changes [13].Hence the ability to inhibit specific protein phosphatases could have clinical importance and may provide alternative treatments of diseases. Furthermore, specific phosphatase inhibitors are invaluable tools for elucidating the function of individual PTPs within cells and for studying phosphatase catalytic mechanisms [14]. In the current study we performed molecular docking studies of reported benzofuran salicylic analogs which possess excellent potency and selectivity against PTP1B [15]. Further, 10 ns conventional molecular dynamics simulations of PTP1B in complex with top benzofuran analogs have been carried out and compared for their dynamic behaviour with known synthesized inhibitor (OMTS) as well as free PTP1B.

III. MATERIALS AND METHODS

3.1 Preparation of the Ligands and Protein

The reported benzofuran analogs were prepared using Ligprep module of Schrodinger 09 [16,17] for probable tautomeric and ionization states at pH = 7 ± 1 followed by minimization with OPLS 2005 force field. The protein preparation of PTP1B (PDB ID:2OZ5) was performed using Protein preparation wizard of Schrodinger 09 where missing hydrogen atoms , bond orders were assigned followed by energy minimization.[16,17]

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3.2 Molecular Docking

The receptor grid was prepared keeping the cocrystal (OMTS) ligand on PTP1B (PDB ID:2OZ5) at the centre of grid with 20Å edges bearing catalytic site. Initially, docking study of the cocrystal (OMTS) was performed on prepared receptor grid for cross-validating the binding mode with respect to X-ray crystal structure. Further, molecular docking was performed for benzofuran analogs against PTP1B using Glide XP 5.8 Program [18,19]**.** The top analogs based on docking score as well as binding interaction with catalytic residues were selected for induced fit docking [20], and results were compared with the cocrystal after Glide XP. The docked conformations corresponding to the lowest free energy (or highest score) provided by Glide program were selected as the most probable binding pose of top compounds.

3.3 Molecular Dynamic Simulation

To understand the dynamics and binding affinity of top ranked of benzofuran analogs in depth, molecular simulation studies were performed and results were compared with known OMTS inhibitor as well as apo protein. Due to inherit flexibility of OMTS and available space in active site, the two molecule of OMTS can bind with PTP1B in proximal and distal pocket as observed in crystallographic structure (PDB ID:2OZ5). Since top benzofuran analogs bound in proximal site of PTP1B, we had taken OMTS bound in proximal binding site for simulation and free energy calculation for comparison. For protein simulation, protein was parameterized using AMBER FF99SB force [21,22], and inhibitors were parameterized using General Amber Force Field (GAFF) embedded with antechamber module [23]. Further, protein ligand complex structure was solvated in the TIP3P water box (with buffering distance of 10 Å) and charge neutralization was carried out using counter ions of Na+ and Cl- counter ions. The first phase of energy minimization was carried with 1000 cycle of steepest descent and 1500 cycle of conjugate gradient with solute atom restrained by a harmonic potential with force constant by 10 kcal/mol$Å^2$ followed by the second phase of energy minimization which was carried out using conjugate gradient method for 2500 cycle for full system minimization without any restraint. Further, the two phase of equilibration, first with positional restraint on solute atom (protein and inhibitor) and other one without restraint, were run for bringing the temperature and pressure of each system with 300 K and 1 atm, respectively, for making isothermal-isobaric ensemble system. The temperature and pressure of each system were stabilized around 300K using Barendsen temperature coupling (Thermostat) and langevin barostat, respectively [24]. The equilibration was done for 1ns with 2 fs integration time step, all atoms belonging solute atoms were restrained by harmonic potential with force constant 10 kcal/mol$Å^2$. For constraining hydrogen linked bonds, SHAKE algorithm [25] was used . The equilibrated system with periodic boundary condition was used to perform constant temperature and pressure simulation for 10 ns with 1 fs integration time step using leapfrog integrator [26]. Simulation trajectory was saved on 1ps of simulation time and further used for structural analysis such as root mean square deviation of overall protein (RMSD), root mean square fluctuation in residue wise (RMSF) and radius of gyration (RGYR). Principal Component Analysis (PCA) of trajectories was calculated using Prody program [27]. The hydrogen bonding and hydrophobic occupancies were calculated between inhibitors and PTP1B using LIGPLOT [28]. Equilibration and simulation processes were validated as a function of potential energy of the system. The MD calculations were performed on 8- GPU cluster built in CAS in Crystallography & Biophysics University of Madras, Chennai.

The binding free energy was calculated for best benzofuran salicylic analogs and OMTS inhibitor with help of MM-GBSA method which are implemented in MMPBSA.py script of AMBER12 [29]. The first step in MM-GBSA is to generate multiple snapshots from the stable MD production trajectory of the complexes, Here 500 snapshots were collected , at 100 ps intervals of 10 ns simulated trajectory. For each snapshot, free energy is calculated for each molecular species and the ligand binding free energy is estimated as follows.

$\Delta G_{binding} = G_{complex} - G_{receptor} - G_{ligand}$

$\Delta G_{Tot} = \Delta G_{gas} + \Delta G_{solv}$

$\Delta G_{gas} = \Delta E_{ele} + \Delta E_{vdw}$

$\Delta G_{solv} = \Delta E_{polar\text{-}solvation} + \Delta E_{non\text{-}polar}$

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IV. RESULTS AND DISCUSSION

4.1 Docking studies of Benzofuran salicylic analogs with PTP1B

In this study, docking of benzofuran salicylic acid analogs have been carried out against PTP1B using Induced fit docking module of Schrodinger 09. Rational binding mode has been discussed and the binding free energies in terms of docking score are determined.Chemical diagram of top ranked benzofuran salicylic inhibitors and cocrystal – OMTS are shown in Fig 1b. Well predicted binding mode of compounds at the proximal pocket and the binding orientations were compared with OMTS inhibitor bound in crystal structure of PTP1B (Table :1). The cocrystallized structure of PTP1B with OMTS contains two site for binding such as proximal and distal pocket. The OMTS bound in proximal pocket is more favorable due to involvement of interaction with P-loop as well as FPD loop which are important for catalytic reaction. OMTS bound in proximal site with one π-π stacking interaction between Phe98 and one of the phenyl ring of OMTS, and a π-cation interaction involving Arg166 and pyrozole ring. 3-chlorophenyl moiety of OMTS is bound in hydrophobic pocket containing residues such as Ile203, Leu232,Leu199, Val131,Leu101 whereas charged carboxylic moiety of OMTS makes hydrogen bond with charged residues such as Arg166, Asp165, Lys164, Ala162 of P-loop (Fig :2). Similarly, in compound 4f, fluoro substituted benzyl ring interacts electrostatically with charged residue pocket involving Glu32, Arg56, Glu60, Arg 63, Arg64, and Arg210 whereas phenolic oxygen of compound 4f interacts with Asp165 via hydrogen bonding. The carboxylic acid moiety of compound 4f forms hydrogen bonds with Phe161,Ala162 and, salt bridges with Lys164 and Arg166. Similar salt bridge interaction was also found with Lys164 and Arg166 with carboxylic moiety of compound 4i. Replacement of diflurophenyl group of 4f with phenoxy phenyl in compound 4i lead to the formation of polar contacts with residues like Ser57, Ser91, His94 and π- cationic interactions were also involved with Arg59. In compound 4g, trifluoro methyl benzene moiety forms π- π stacking with the residue Phe161 of P loop, and carboxylic moiety interacts via hydrogen bond as well as salt bridge with Arg166. The increased activity reported in the literature could be due to the formation of hydrogen bonding with Ser57, π-cation interaction with Arg59 compared to 4f, and is due to the substitution of phenoxy phenyl moiety in 4i. Benzofuran carboxylic acid moiety of compound 4i and 4f showed similar salt bridge interactions (Lys164, Arg166), and hydrophobic interaction (Val231, Leu232, Leu227, Ile203, Met206, Ile206, Phe211 and Phe98). It is due to the substitution of tri-fluromethyl benzene moiety in compound 4g that has paved the way for polar contact formation of benzofuran carboxylic acid moiety as compared to compound 4f and compound 4i where these moieties form hydrophobic contacts.

V. MOLECULAR DYNAMICS SIMULATION

In order to understand the conformational stability of top ranked benzofuran analogs complex with PTP1B, molecular dynamics simulation has been carried out and compared with cocrystal (OMTS) and apo-protein. Based on thermodynamic properties and stability in terms of potential energy, all complexes are found to be stable during the simulation. Chimera [30] and VMD [31] were used for visualization of molecular dynamics trajectories. Structural deviation of these complex as well as apoprotein were analyzed using root mean square deviation of overall protein (RMSD), residue wise root mean square fluctuation (RMSF) and radius of gyration (RGYR) from 10 ns simulated trajectories. RMSD suggests that after 7 ns, all complexes (cocrystal OMTS, compound 4f,4g,4i) and free protein got stablized and structural deviations had converged (Fig: 3). Initially complexes were found to have more deviation compared to free PTP1B, it seems ligand induced conformational changes occurred with top benzofuran analogs as well as cocrystal inhibitor. Radius of Gyration (RGYR) also suggests that due to inhibitor binding, there is negligible structural change in complexes during simulations (Fig: 4). RMSF reveals that conformational restriction in active site loops such as P-loop and FPD loop compared to apo protein. Out of top three benzofuran analogs, compound 4g showed more comformational restriction in these catalytic loops. The studies also reveal that due to inhibitors binding at lid, there is more structural deviation in lid domain (α7, α8) compared to apo protein (Fig: 5)

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5.1 Inter molecular interaction of top benzofuran salicylic scaffold analogs with PTP1B

Hydrogen bond interaction between PTP1B and top benzofuran salicylic analogs calculated from last 5 ns simulated conformations revealed that though compound 4f, 4i and 4g showed hydrogen bonding interaction patterns with Ala162 and Asp165 of P-loop similar to OMTS inhibitor (Fig: 6), Compound 4g also exhibit additional hydrophobic interactions with Cys160, Ala162, Leu164 of P-loop.And, among the top benzofuran analogs, compound 4g makes more hydrophobic interactions with Met206 of α7 lid helix compared to cocrystal. All benzofuran derivatives mostly interact with α3 helix hydrophobically (Fig: 7). Hydrophobic interaction of FPD loop was more prominent with compound 4f and 4i, whereas 4g interacts mostly with P-loop residues.

5.2 Principal component analysis (PCA) of simulated trajectory of best compound 4g and cocrystal OMTS and free PTP1B

In order to characterize conformational dynamics of lid domain as well as catalytic loops in protein, PCA was performed. The conformational changes among the benzofuran salicylic analogs compared to apo and cocrystal suggest that movement of lid domain (comprising the helices α7 and α8) varied among the complexes. It is observed that lid domain moves away from the active site in OMTS and apo form while with best compound 4g, lid moves towards active site (Fig: 8). Similarly, in catalytic P-loop, significant movement was found in cocrystal as well as in apo form compared with compound 4g where its movement was restricted. In addition to this, FPD loop movement was observed towards active site in compound 4g similar to apo protein, where as in cocrystal, its movement is away from active site. PCA also suggested that displacement of α3 helix is found away from active site in case of cocrystal and apo compared to compound 4g where displacement is towards active site. The α3A helix moves away from active site in apo where as in compound 4g, α3A moves towards active site compared to cocrystal where its movement is restricted.

5.3 Binding free energy calculation:

The top benzofuran salicylic acid inhibitors bound to PTP1B have been used for free energy calculations from simulated trajectories. On the basis of molecular mechanics generalized born/surface area (MMGB-SA) computation and normal mode analysis (NMA), the obtained results indicate the rank of calculated free energy (ΔG_{Tot}) of these inhibitors are in excellent agreement with experimental results (IC_{50}) (Table :2). Out of three benzofuran derivatives, compound 4g showed best binding free energy (ΔG_{Tot}= -26.62 kcal/mol) compared to compound 4f (ΔG_{Tot} = -19.29 kcal/mol) and 4i (ΔG_{Tot} = -18.95 kcal/mol). Electrostatic energies and polar solvation contribution are the largest, whereas van der waals and non-polar contribution are lesser in terms of change in enthalpy (ΔH). Similarly, entropy changes also played an important role in free energy change (ΔG_{Tot}). We also calculated binding free energy (ΔG_{Tot}) for OMTS, but ΔG_{Tot} for top benzofuran salicylic acid inhibitors found to be high which conflicted to experimental IC_{50} .It may be due to inherit flexibility of OMTS, enormous available space in active site and due to computational time constraints, mere simulated conformations from 10 ns is insufficient to calculate proper binding free energy. It can be the reason for more standard variations in calculated binding free energy and higher entropy for cocrystal OMTS molecule. Further free energy decomposition of catalytic residues has also been carried out to identify molecular determinants for top benzofuran salicylic acid inhibitors (Fig 9A-E). Results suggest that in the complex with compound 4g, residues such as, Leu101, P-loop containing residues viz: Cys160, Phe161, Ala162, Asp165, Leu203 and Met206 of α7, Leu227, Val232 from α7-α8 bridge loop dominates the most binding free energies compared to compound 4i and 4f, along with van der waals contribution to Phe161 and Met206. Non-polar contribution from the residues Glu60,Arg 63 of α3 also showed more favorable energy in compound 4g. Electrostatic energy and polar contribution were also found dominating in 4g with residues such as Arg56, Arg59, Arg63, Arg64 of α3 helix, Cys161-Lys164 of P-loop and Arg210 of α7.

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VI. CONCLUSION

The binding modes of PTP1B inhibitors were explored using molecular docking and dynamic simulation. The docking studies revealed that benzofuran salicylic acid inhibitors bind in proximal pocket and have favorable hydrogen bonding, salt bridges and hydrophobic interaction with catalytic P-loop. Dynamic simulation studies of PTP1B complexes suggests existence of ligand induced conformational changes in alpha lid domain which paves opening of entrance gate of active site for substrate recognition. Binding free energy calculation showed good validation with respect to experimental IC_{50} of top benzofuran salicylic acid inhibitors of PTP1B. Further decomposed free energy of catalytic residues identified molecular determinants for binding.Among screened inhibitors, compound 4g is found to be a tight binder at the active site
As well as with the p loop, and thereby this compound can be projected with potential to discriminate the human PTP1B and Myobacterium PTP1B.

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Table 1: Induced Fit Docking (IFD) results of top benzofuran salicylic analog and cocrytsallised OMTS inhibitor

Compounds	Hydrogen Bonding Interactions	Hydrophobic Interactions	Docking Score	Glide energy (kcal/mol)	IC50 (nM)
OMTS	Ala162,Lys164, Asp165,Arg166	Ser57,Arg59,Glu60, Phe80,Pro81, Leu83,Ser91,Hie94, Glu95,Phe98, Leu101, Tyr125, Met126, Glu129, Phe133,Arg136,Cys160,Phe161, Leu199,Ile203,Leu227, Val231, Leu232	-9.66	-77.18	440(+/-100)
Compund 4g	Arg166,Asp 165	Arg59,Glu60,Arg63,Arg64,Pro81,Leu83,Ser91,Hie94,Glu95,Tyr125,Met126,Phe133Arg136,Cys160, Phe161,Arg166,Met 206	-6.07	-46.53	38(+/-2)
Compound 4f	Phe161,Ala162, Lys164,Asp165 ,Arg166	Glu32,Arg56,Glu60,Arg63,Arg64,Phe98,Met126,Cys160,Ile203, Met206,Arg210,Phe211,Thr223, Leu227,Val231	-8.90	-51.98	54(+/-4)
Compound 4i	Ser57	Glu60,Arg63,Pro81,Leu83,Ser91, Hie94,Phe98,Glu95,Arg136, Phe161,Ala162,Lys164,Ala165, Arg166,Ile203,Ile207,Leu227, Leu232,Val231	-9.06	-55.57	43(+/-3)

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Table 2: Binding Free Energy of top benzofuran salicylic analog and cocrystallised OMTS inhibitor

Energy Component	OMTS	Compound 4f	Compound 4g	Compound 4i
IC50(nM)	440(+/-100)	54(+/-4)	38(+/-2)	43(+/-3)
VDW	-61.67 (+/-3.94)	-52.06 (+/-3.31)	-44.76 (+/-3.33)	-60.46 (+/-3.05)
EEL	-124.10 (+/-11.14)	-22.46 (+/-4.78)	-97.61 (+/-10.07)	-15.64 (+/5.47)
EGB	78.58 (+/-8.18)	38.59 (+/-3.73)	97.32 (+/-9.11)	37.50 (+/-3.86)
ESURF	-8.34 (+/-0.32)	-6.43 (+/-0.28)	-6.30 (+/-0.34)	-7.15 (+/-0.25)
DELTA G_{gas}	-185.77 (+/-11.24)	-74.52 (+/4.62)	-142.38 (+/-11.19)	-76.11 (+/-5.18)
DELTA G_{solv}	70.23 (+/-8.22)	32.15 (+/-3.76)	91.02 (+/-8.98)	30.34 (+/-3.90)
Enthalphy(H)	-115.54 (+/-8.50)	-42.37 (+/-3.31)	-51.36 (+/-4.73)	-45.76 (+/3.19)
Entropy(S)	-35.57 (+/-9.26)	-23.08 (+/-4.11)	-24.74 (+/-4.58)	-26.81 (+/8.79)
Delta Total	-79.97	-19.29	-26.62	-18.95

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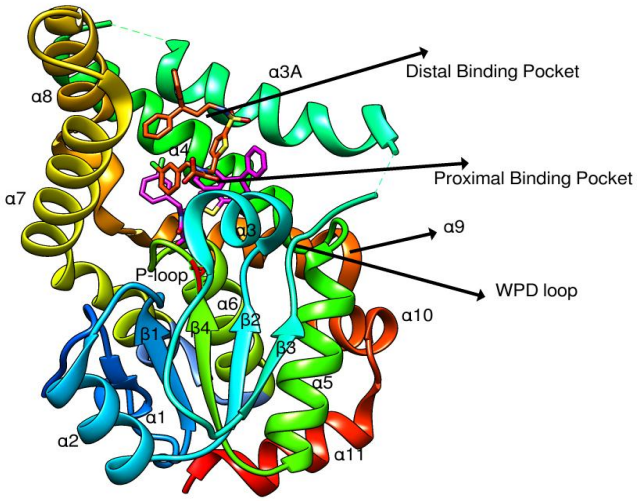
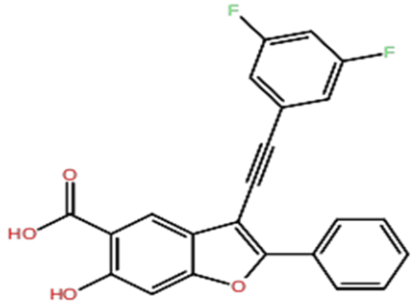
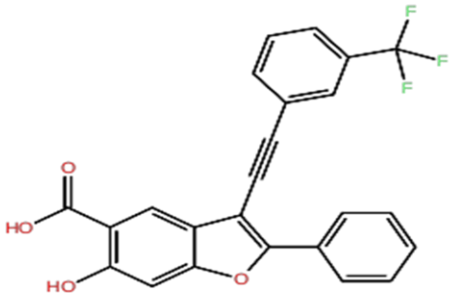


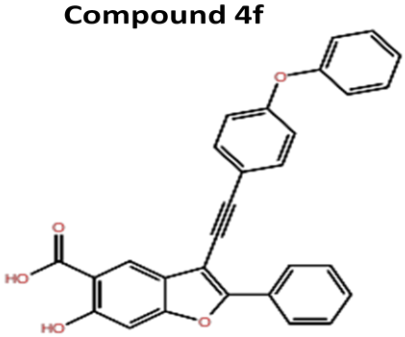
Fig1a: Crystal structure of Mtb Ptp1B bound with OMTS (PDB ID:2OZ5)



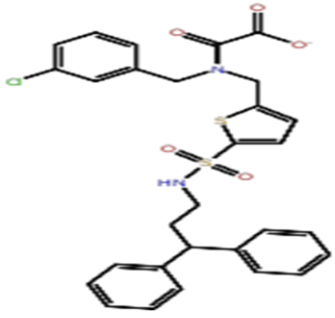
Compound 4f



Compound 4g



Compound 4i



Cocrystal (OMTS)

Fig 1b: Chemical diagram of top ranked benzofuran salicylic inhibitors and Cocrystal OMTS

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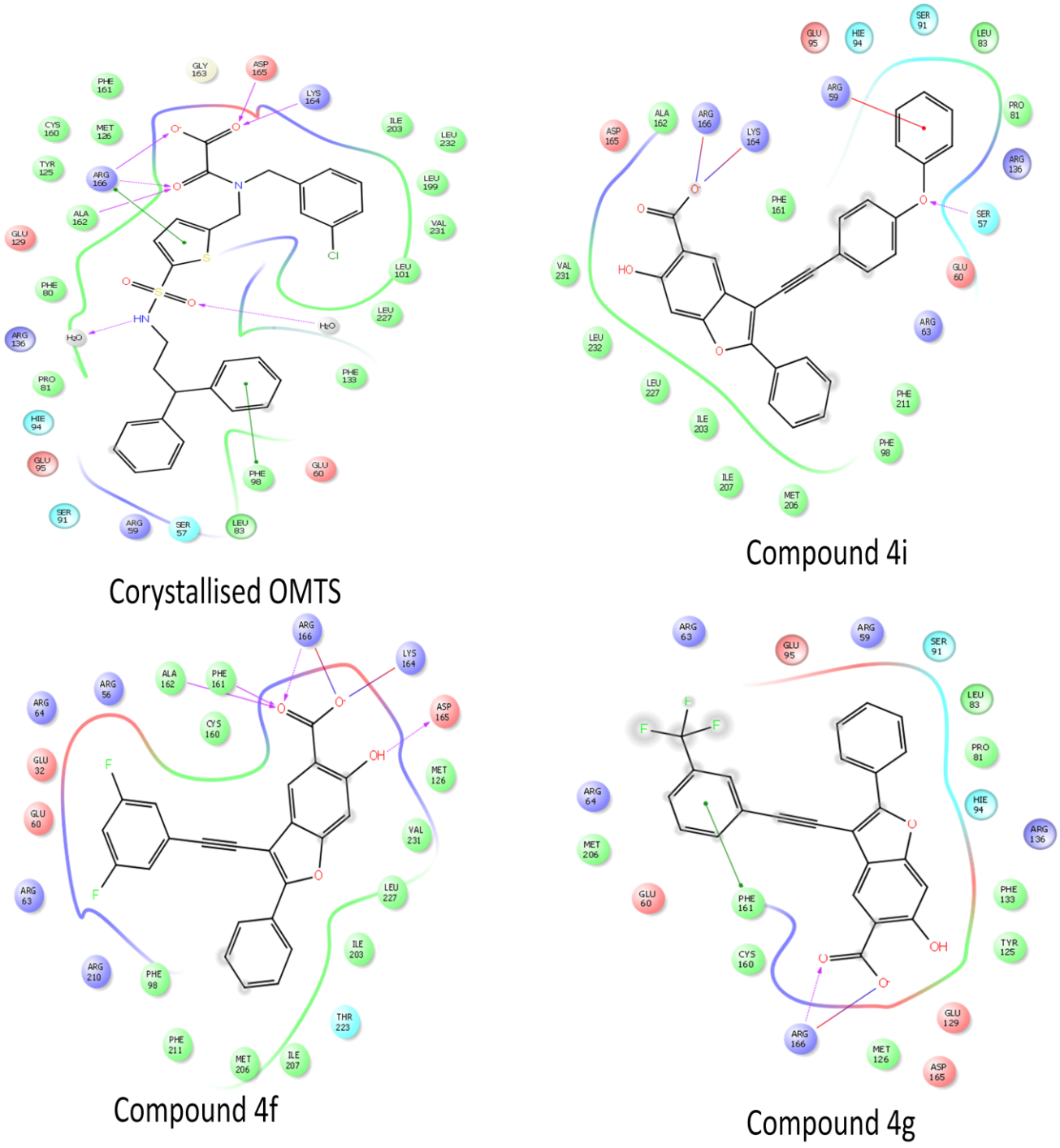

Fig 2: Docking poses of top benzofuran salicylic analog and cocrystallized OMTS inhibitor

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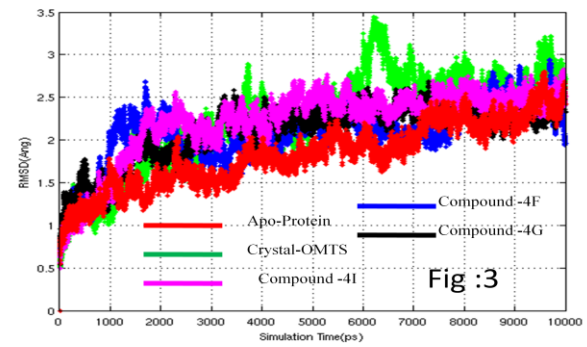

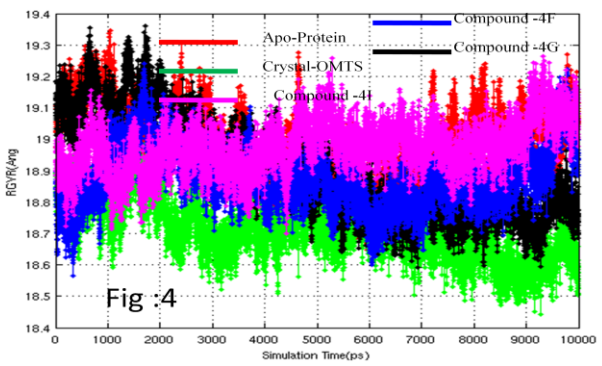

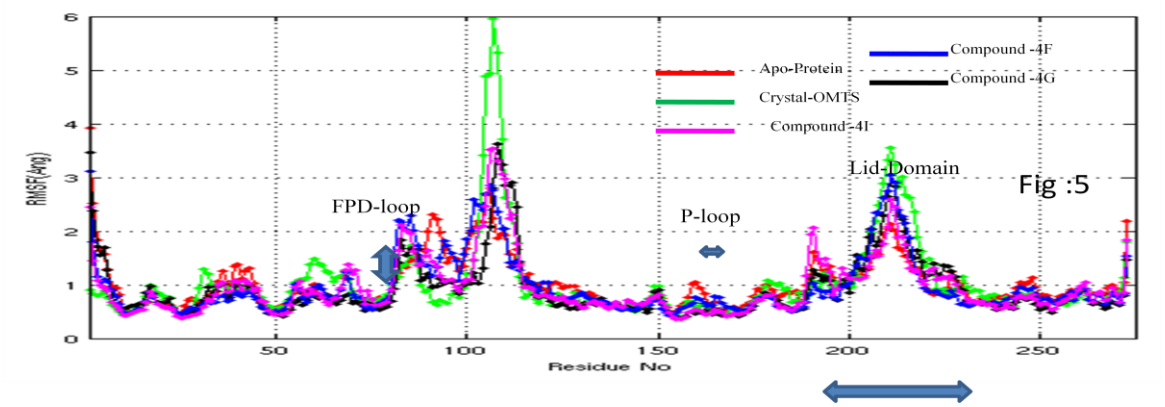

Fig : 3 - RMSD, Fig: 4 - RGYR, Fig: 5 - RMSF of top benzofuran salicylic analog and cocrytsallised OMTS inhibitor and free Ptp1B

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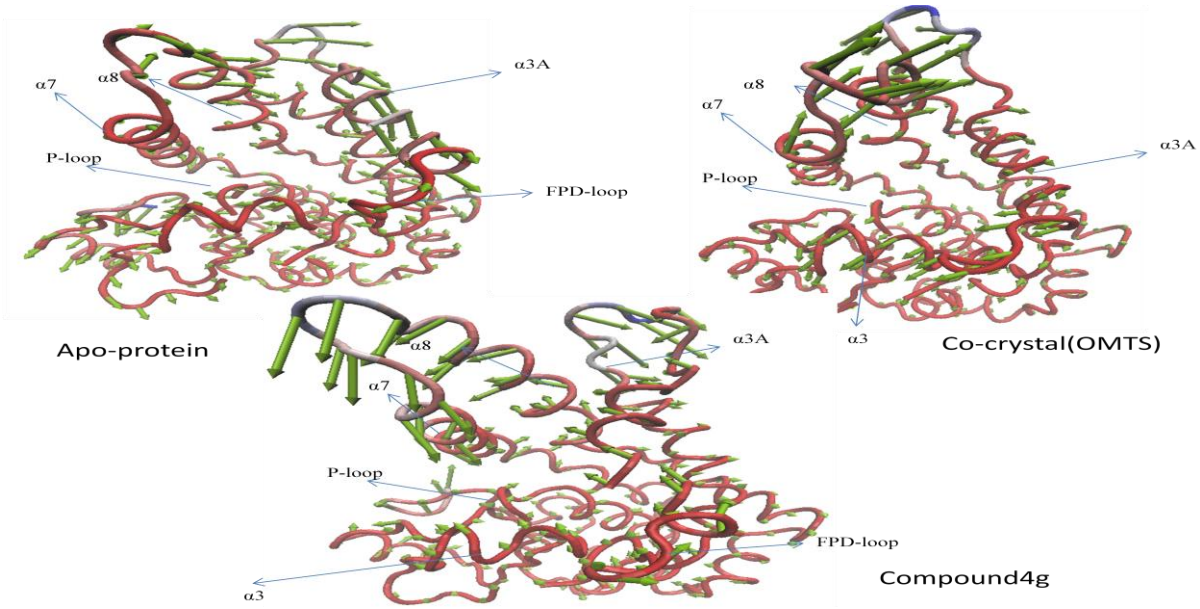

Figure 6 : PCA projection of most variant principal mode (PC1) in initial conformation of apo PTP1B, cocrystal OMTS and best compound 4g

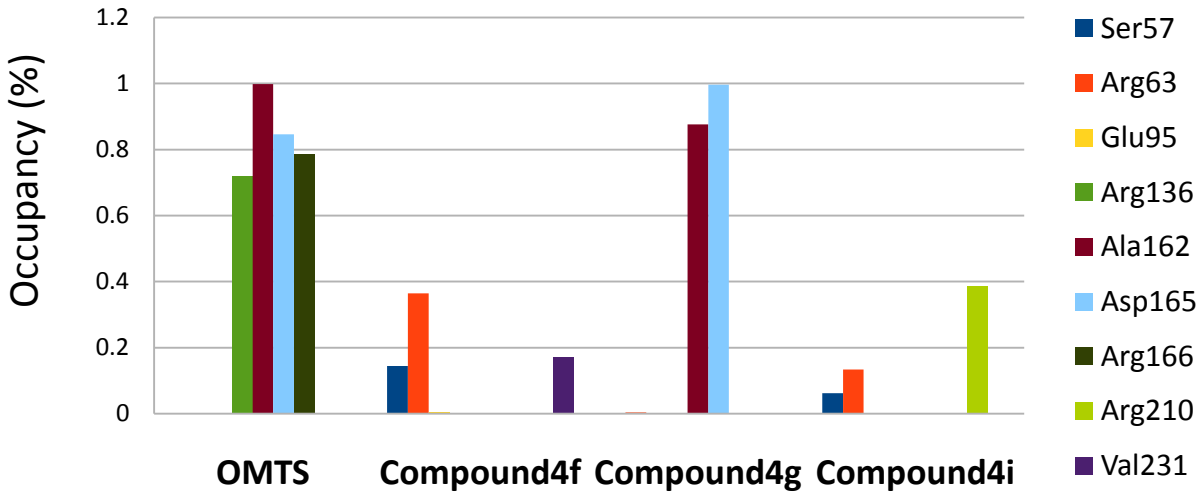

Fig: 7

Occupancy of Hydrogen bond formation among cocrystal and top benzofuran salicylic complexes

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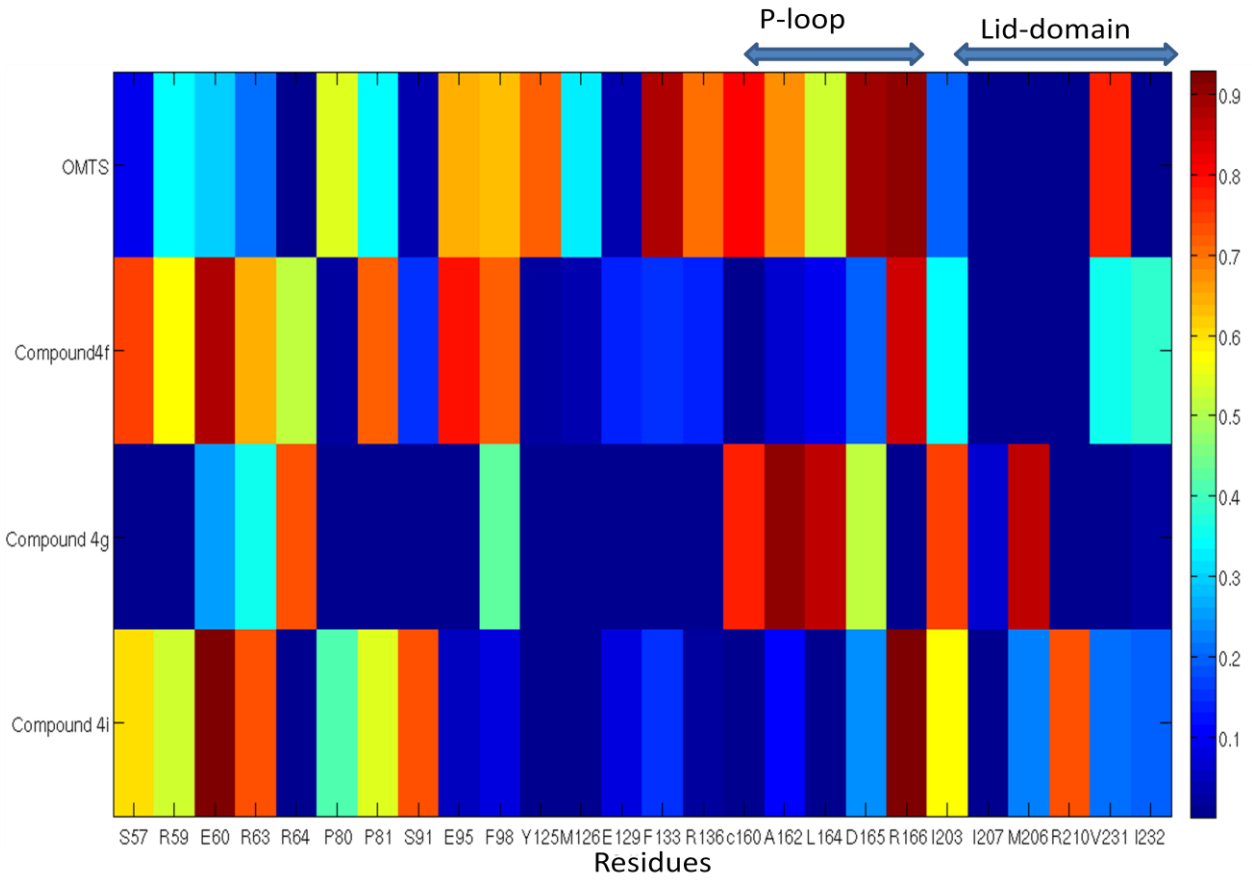

Fig: 8 Occupancy of hydrophobic interaction among cocrystal and top benzofuran salicylic complexes

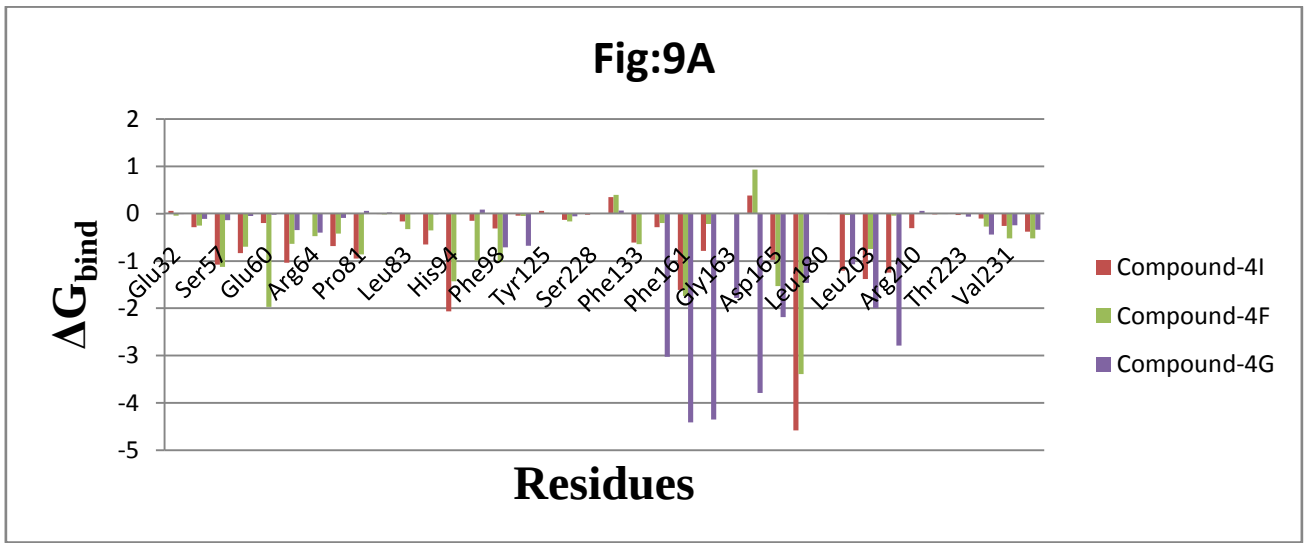

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Fig:9B

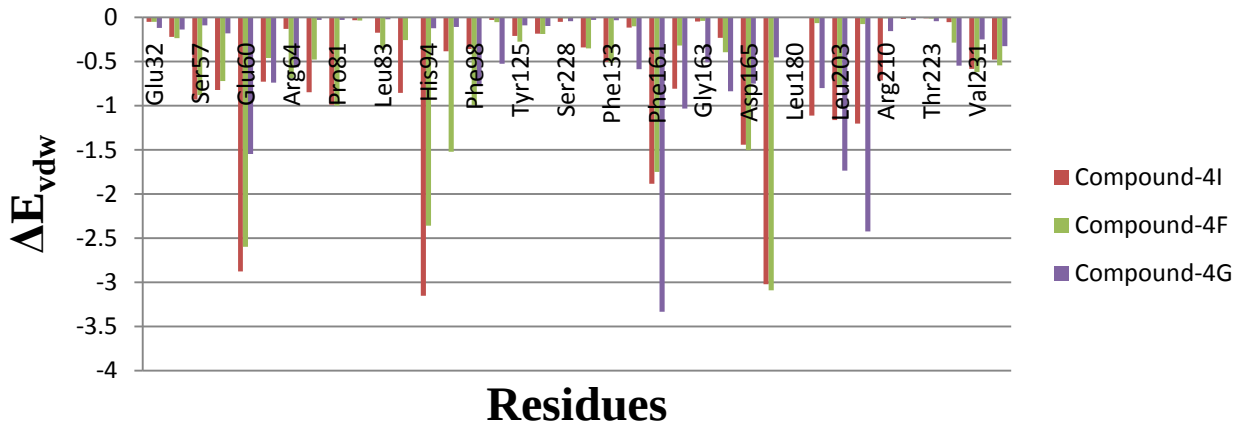
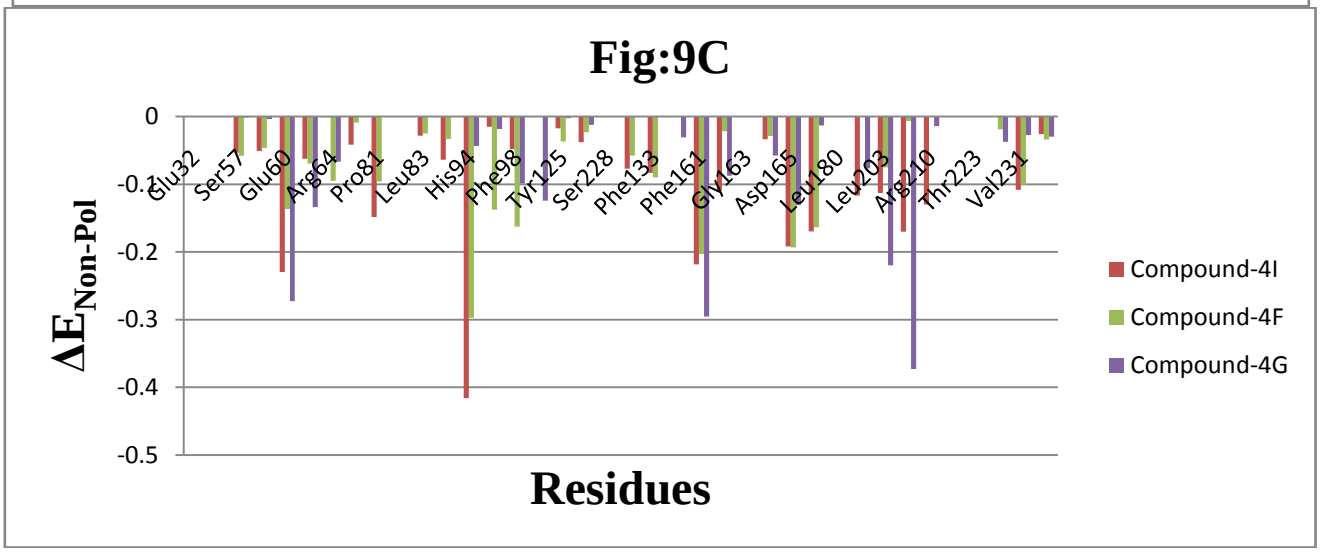


Fig:9C



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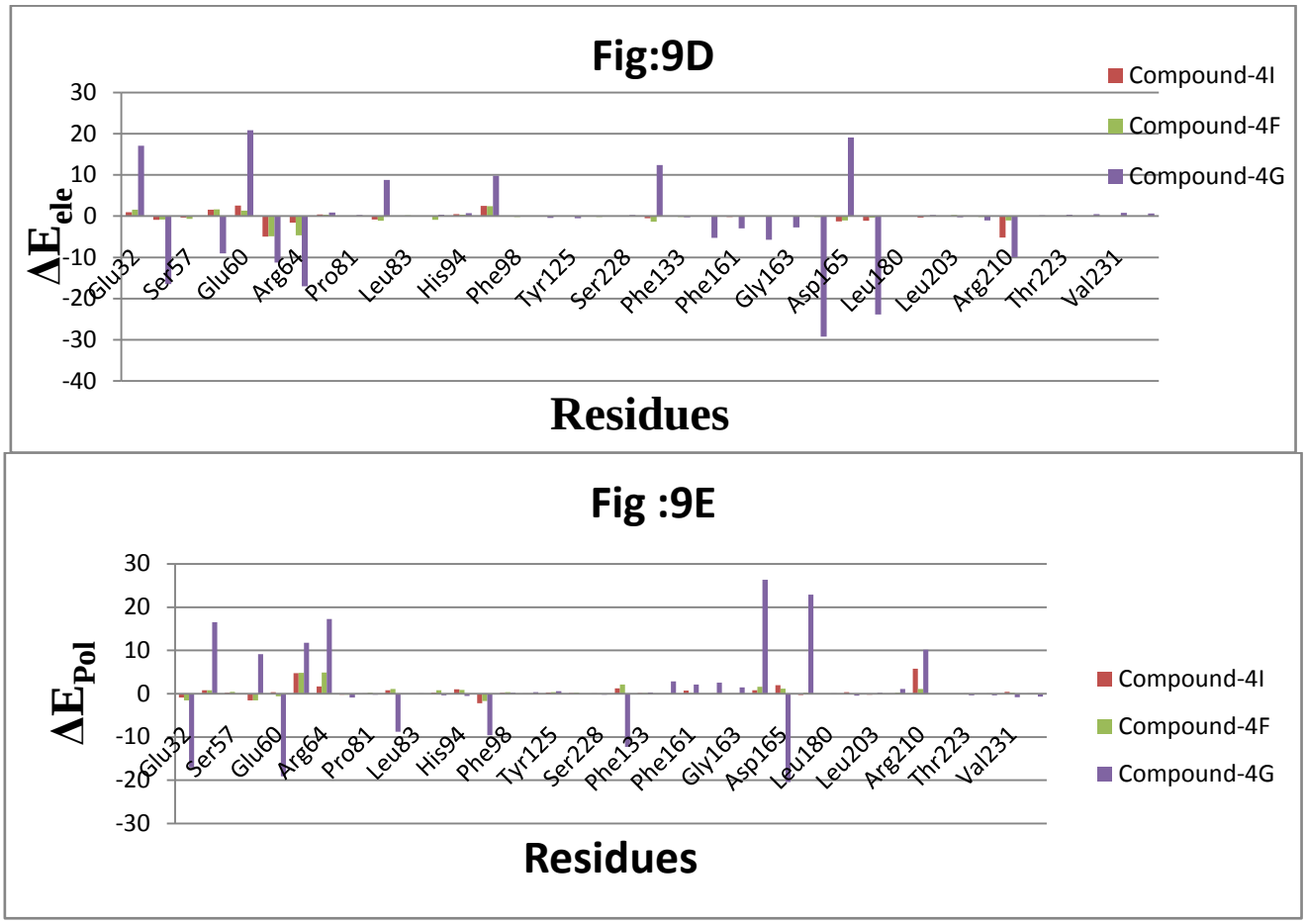

Figure 9 A-E: Decomposition of residual binding free energy into individual contribution (A) Total binding free energy (B) van der Waals (C) Non-Polar (D) Electrostatic (E) Polar Contributions of top benzofuran salicylic analog complexes