



## Molecular Docking and Rescoring Studies of EGFR Inhibitor Ligands Using Prime MMGB/SA Approach

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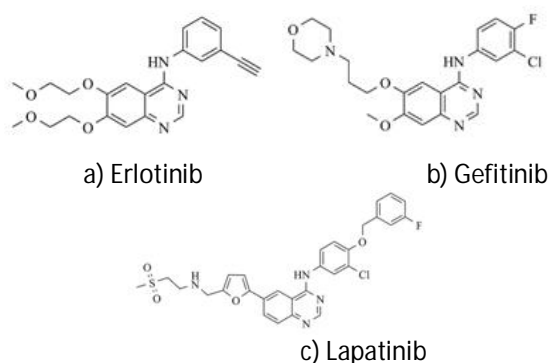
### ABSTRACT

Epidermal growth factor receptor (EGFR) being a priority target in anticancer drug research, it was used for evaluating the efficacy of 29 molecules chosen from Binding database ([www.bindingdb.org](http://www.bindingdb.org)). The known inhibitors are originated from a diverse chemical space but without exception all of them act at the Adenosine triphosphate (ATP) binding site of the enzyme. The efficacy of Erlotinib was evaluated as a reference molecule for the entire study. We performed Insilco molecular docking using Schrodinger LLC., software (GLIDE SP and GLIDE XP) and Molegro Virtual Docker (MVD.2011.4.3.0). We performed rescoring of the GLIDE scores using PRIME MM-GBSA module of Schrodinger LLC. IC<sub>50</sub> values were computed using the rescored values of PRIME MM/GBSA using [www.sanjeevslab.org](http://www.sanjeevslab.org) for all the molecules. The molecules BDB: 50102417 and BDB: 50162990 showed promise as molecule which required further attention. This study also helped us to understand the inhibitor mechanisms of EGFR structure. The docked values for Glide using SP and XP were -8.25 and -9.01Kcal/mol for molecule BDB: 50102417 whereas for BDB: 50162990 the values were -8.54 and -9.51Kcal/ml. The corresponding rescored values (binding energy) values were -39.72, -34.49 Kcal/mol in SP and XP for BDB: 50102417 and the molecule BDB: 50162990 showed values of -36.71, -31.88 Kcal/mol in SP and XP scores. In MVD, the BDB: 50162990 molecule of Set-A got Mol Dock score of -196.984Kcal/mol and rerank score of -148.371Kcal/mol respectively, the BDB: 50102417 molecule of Set-B got Mol Dock score of -134.816 Kcal/mol and Rerank score of -111.697 Kcal/mol respectively.

**Keywords:** EGFR inhibitors, Binding DB, Molecular Docking, Rescoring, MM-GB/SA, BDB 50102417, BDB 50162990.

### INTRODUCTION

Cancer is continuing to be a major health problem in developing as well as developed countries. Surpassing heart diseases, it has become the number one killer due to various worldwide factors<sup>1,2</sup>. The epidermal growth factor receptor (EGFR) is cellular trans-membrane tyrosine kinases that is over-expressed in a significant number of human tumors (e.g., breast, ovarian, colon, and prostate), their expression levels often correlate with vascularity, and is associated with poor prognosis in patients<sup>3</sup>. A number of small molecule EGFR kinase inhibitors have been evaluated in cancer clinical trials<sup>4</sup>. For example (Fig.1) anilinoquinazoline-containing compounds Gefitinib (Iressa)<sup>5</sup> Erlotinib (Tarceva)<sup>3</sup> and Lapatinib (Tykerb, also known as GW572016) were recently approved for the treatment of HER2-positive metastatic breast cancer<sup>6</sup>.



**Figure 1:** These are the approved drugs improved over lead molecules in market

Many more compounds are still under evaluation in clinical trials for the treatment of cancer. There are presently two main classes of EGFR inhibitors that can be used in cancer therapy and they are quinazoline derivatives<sup>7</sup> and the pyrimidine derivatives<sup>8</sup> consisting of ATP-competitive small molecules. To discover new effective EGFR inhibitors, investigators usually need to synthesize many compounds and test their corresponding activities by cell based biological assay experiments, which is usually time-consuming and manpower expensive<sup>9</sup>. Consequently, it is of practical interest to develop reliable tools to predict biological activities before synthesis. Bioinformatics tools and computer aided drug design process have a great potential in not only reducing the cost but also in the efficiency with which they can be designed<sup>10</sup>. Several novel tools and techniques in the recent past have helped in the speeding up of drug discovery process such as molecular docking, linear interaction energy methods, pharmacophore designing and QSAR etc<sup>11</sup>. QSAR models of EGFR inhibitors have been recently investigated with encouraging results<sup>9,11</sup>. This approach helps the scientist to predict the activities of a series of newly designed drugs before making a decision whether or not to synthesize them and assay them. It also helps in identification of key structural features and help in the assessment of their biological activity.

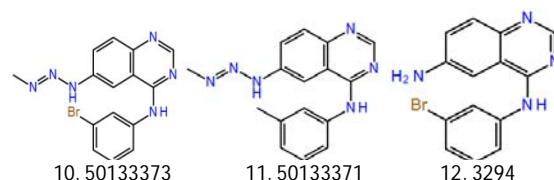
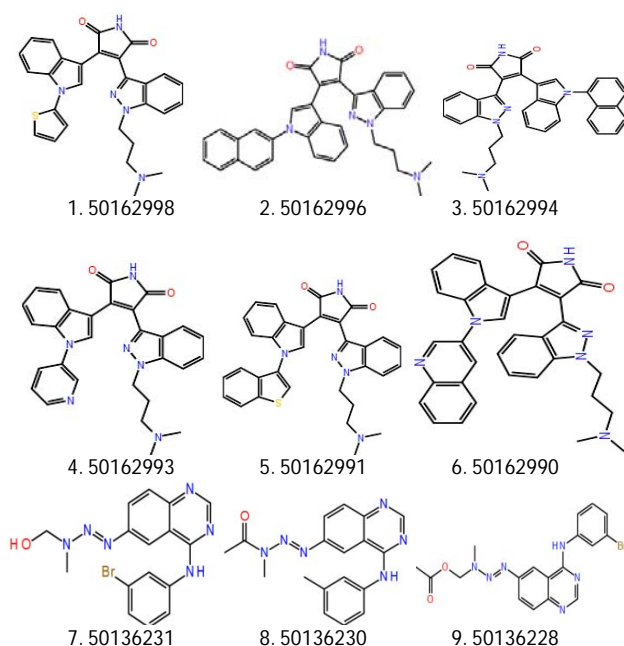
The present study was to search for potential ligands for EGFR from a compound database which has not been previously explored for human EGFR. We chose the best molecular docking programs available currently being

used widely such as GLIDE and MVD and applied the linear interaction energy model (MM-GB/SA) for rescoring of the docked ligands to find out the best free energy of binding for these ligands. The experimental IC<sub>50</sub> were obtained from the pubchem (pubchem.ncbi.nlm.gov) and binding database (www.bindingdb.org) and tried to correlate these values with the rescored values.

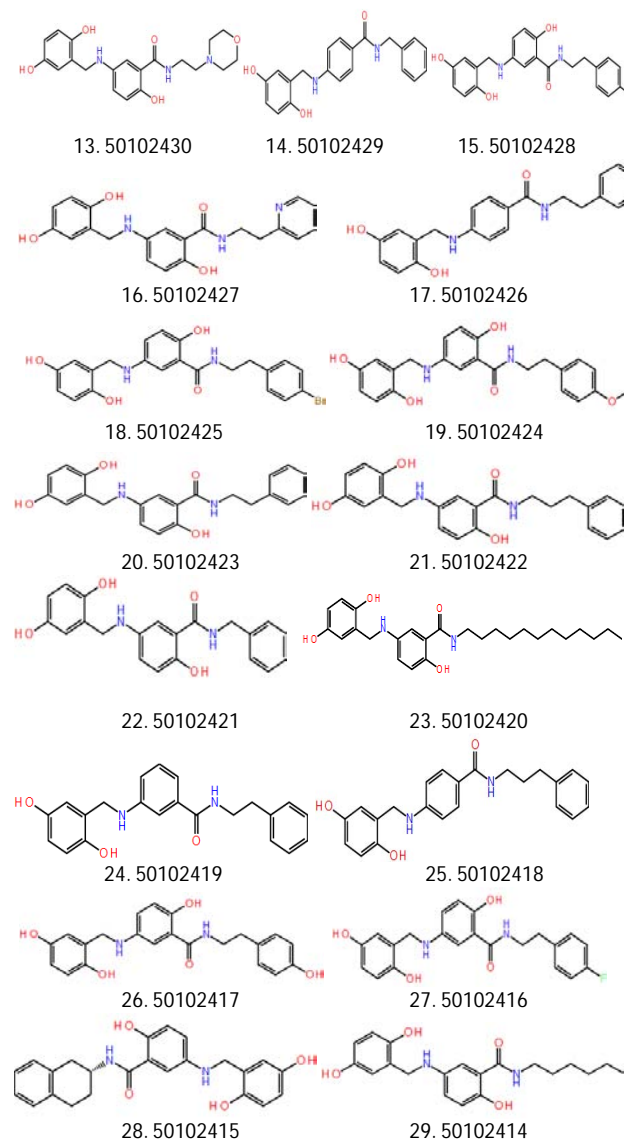
## MATERIALS AND METHODS

Human EGFR protein has more than 50 structures in the PDB database but available in different resolutions. We chose the structure of 1M17\_A based on the sequence similarity (98% similarity with mouse sequence), resolution (2.6Å) and literature<sup>3</sup>. The initial X-ray crystallographic coordinates of Human EGFR (PDB ID: 1M17) were downloaded from Protein Data Bank (<http://www.rcsb.org>). This protein was subjected to the protein preparation wizard of Schrodinger software in the Maestro interface<sup>12</sup>. A small but promising group of compound database consisting of 34 molecules was retrieved in SDF format from binding database. We excluded 5 molecules from this database as they were having molecular weight of more than 700 Daltons that is against the Lipinski's rule of five. The remaining 29 ligands as shown in Fig.2 were divided into two sets A and B with 12 and 17 molecules respectively based on their structural similarities. The Set-A with 12 ligands was further segregated as 6 indazol derivatives and 6 as quinazolin derivatives. The Set-B consists of 17 ligands of [(2, 5-dihydroxyphenyl) methylamine] derivatives. The ligands were subjected to ligand preparation using the ligand preparation wizard (Lprep) of Schrodinger software in the Maestro interface<sup>12</sup>. We are calculating their docking scores, binding free energies and ADMET properties.

### SET-A



### SET-B



**Figure 2:** Depiction of the twenty nine molecular structures downloaded from Binding Databank (www.bindingdb.org) being divided as Set A and Set B based on Molecular structural differences.

The IC<sub>50</sub> values of the 29 ligands are present in the *BindingDB* site which is also present in pubchem database; these values were collected along with their structures in the beginning of our work in nanomolar units. These IC<sub>50</sub> values can't be used for the statistical analysis; hence these values will be converted into pIC<sub>50</sub> values which are otherwise called as activity values from the internet site (www.sanjeevslab.org).

$$\text{Activity} = \text{pIC}_{50} = 1/\log \text{IC}_{50} = -\log \text{IC}_{50}$$

### Glide (Schrodinger, Inc.) methodology

The Glide (Grid-Based Ligand Docking with Energetic, version 5.5) algorithm approximates a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using a series of hierarchical filters. The Glide docking methodology has been described in detail<sup>13</sup>. The best 10 poses and corresponding scores have been evaluated using Glide in Standard precision mode (Glide SP) and in Extra Precision mode (Glide XP) of Glide algorithm for each ligand of the data set.

$$GScore = a * vdW + b * Coul + Lipo + Hbond + Metal + BuryP + RotB + Site$$

### Molegro Virtual Docker Methodology

Software Molegro Virtual Docker (MVD) v 4.3.0 along with graphical user interface, MVD tools was utilized to generate grid, calculate dock score and evaluate conformers. The non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Docking was performed by following the steps in MVD user manual<sup>14</sup>. Two types of dock scores such as Mol Dock score and Re-rank score of ligands were calculated in docking.

### Prime MM-GBSA methodology

This application calculates the free energy binding between the receptor and a ligand in its complex. The protocol of Prime/MM-GBSA module of Schrödinger software<sup>15</sup> is followed to re-rank the docked conformations of each ligand obtained from the Glide (both SP and XP methods) to estimate the binding free energy  $\Delta G_{bind}$ . Energy minimization for the complexes using OPLS\_2005 force field within Macro Model was performed.

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

### ADME/T properties

The ADME/T properties of all the ligands were calculated from the Qikprop 2.3 module of the Schrödinger suite<sup>16</sup>. QikProp settings determine which molecules are flagged as being dissimilar to other 95% of the known drugs. The Qikprop helps us in analyzing the pharmacokinetics and pharmacodynamics of the ligands by accessing the drug like properties Predicted significant ADME/T properties such as permeability through MDCK Cells (QPlogMDCK), Qik Prop predicted log IC50 value for blockage of K<sup>+</sup> channels (QPlogHERG), QikProp predicted gut-blood barrier (OPPCaco) and violations of the Lipinski's rule of five (LROF). The molecular properties are provided in Table 1 and Glide, MVD docking and Prime MM-GBSA Re-scoring values are listed in Table 2.

### RESULTS

In Glide docking, out of 29 ligand molecules docked two molecules has got least glide scores, they are BDB: 50162990 and BDB: 50102417 molecules as the best

from Set-A and Set-B respectively, which have got good dock scores. From these two molecules the BDB: 50162990 molecule is having the least glide score both in SP -8.540660 Kcal/mol and in XP -9.519830 Kcal/mol from Set-A and BDB: 50102417 molecule is having the least glide score both in SP -8.255964 Kcal/mol and in XP -9.019752 Kcal/mol from Set-B. We obtained glide score values of -7.148196 Kcal/mol, -8.431376 Kcal/mol in SP and XP mode for Erlotinib molecule. Rest of the 27 molecules has got least Glide scores either in SP or XP but not in both.

In MVD, the BDB: 50162990 molecule of Set-A got MolDock score of -196.984Kcal/mol and Re-rank score of -148.371 Kcal/mol respectively, the 50102417 molecule of Set-B got MolDock score of -134.816 Kcal/mol and Re-rank score of -111.697 Kcal/mol respectively. The reference molecule Erlotinib got -129.444 Kcal/mol, -97.6263 Kcal/mol MolDock and Re-rank scores respectively. The 50162991 molecule of Set-A and 50102425 molecule of Set-B got even better docking scores in MVD but they got very high scores in Glide. The BDB: 50162990 and BDB: 50102417 are the only molecules that showed the least docking scores both in Glide and MVD ligand docking.

The output of the Prime/MM-GBSA contain two types of binding free energies, binding energy with ligand strain given in DG1 and the binding energy without ligand strain energy is given in DG2. The BDB: 50102417 molecule of Set-B is having the least rescoring values, with DG1 values -39.72 Kcal/mol, -34.49 Kcal/mol in SP and XP respectively and the DG2 values as -45.63 Kcal/mol, -47.31 Kcal/mol in SP and XP respectively, thus it stands as the best molecule where as the molecule BDB: 50162990 molecule of Set-A went back in the race with DG1 values as -36.71 Kcal/mol, -31.88 Kcal/mol in SP and XP respectively and DG2 values -44.36 Kcal/mol, -43.38 Kcal/mol in SP and XP respectively. The binding energy values of erlotinib molecule which is taken as reference drug for our studies are DG1 values -41.41Kcal/mol, -39.88Kcal/mol in SP and XP respectively, DG2 values -44.60 Kcal/mol, -42.74 Kcal/mol in SP and XP respectively.

### DISCUSSION

An effective way to predict binding structure of a substrate in its receptor is docking simulation, which has been successfully used in many applications. Docking procedures basically aims to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, it describes a process by which two molecules fit together in 3-Dimensional space. The binding site of 1M17 was visualized as five main pharmacophoric regions: hydrophilic region formed of adenine binding site, sugar and phosphate regions in addition to two hydrophobic areas I and II. It is also known that most of the kinase inhibitors share common properties like low molecular weight for small molecules and hydrophobic



heterocycles which act by competing with ATP for binding in kinase ATP binding site.

**Table 1:** Binding Database index and Pubchem index of Molecules along with their Molecular properties, Molecular formula, Molecular weight, Activity IC50 values in nano molar units taken from Binding databank site, their PIC50 values calculated from (www.sanjeevslab.org) and their IUPIC nomenclature

BDB ID	CID pubchem	Mol. formula	MW g/mol	IC50 nM	PIC50 nM	IUPAC NAME
Erlotinib	176870	C <sub>22</sub> H <sub>23</sub> N <sub>3</sub> O <sub>4</sub>	393.435	NA*	NA	N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine
50162998	10185160	C <sub>28</sub> H <sub>25</sub> N <sub>5</sub> O <sub>2</sub> S	495.598	7000	5.154	3-[1-(3-dimethylaminopropyl)indazol-3-yl]-4-(1-thiophen-2-ylindol-3-yl)pyrrole-2,5-dione
50162996	10209082	C <sub>34</sub> H <sub>29</sub> N <sub>5</sub> O <sub>2</sub>	539.635	6100	5.214	3-[1-(3-dimethylaminopropyl)indazol-3-yl]-4-(1-naphthalen-2-ylindol-3-yl)pyrrole-2,5-dione
50162994	10143584	C <sub>34</sub> H <sub>29</sub> N <sub>5</sub> O <sub>2</sub>	539.635	10000	5	3-[1-(3-dimethylaminopropyl)indazol-3-yl]-4-(1-naphthalen-1-ylindol-3-yl)pyrrole-2,5-dione
50162993	10163439	C <sub>29</sub> H <sub>26</sub> N <sub>6</sub> O <sub>2</sub>	490.563	3800	5.420	3-[1-(3-dimethylaminopropyl)indazol-3-yl]-4-(1-pyridin-3-ylindol-3-yl)pyrrole-2,5-dione
50162991	10187378	C <sub>32</sub> H <sub>27</sub> N <sub>5</sub> O <sub>2</sub> S	545.658	5600	5.251	3-[1-benzothiophen-3-ylindol-3-yl]-4-[1(3-dimethylaminopropyl)indazol-3-yl]pyrrole-2,5-dione
50162990	10302405	C <sub>33</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>	540.623	10000	5	3-[1-(3-dimethylaminopropyl)indazol-3-yl]-4-(1-quinolin-3-ylindol-3-yl)pyrrole-2,5-dione
50136231	11349700	C <sub>16</sub> H <sub>15</sub> BrN <sub>6</sub> O	387.238	110	6.958	[[4-[(3-bromophenyl)amino]quinazolin-6-yl]diazanyl-methyl-amino]methanol
50136230	11198415	C <sub>18</sub> H <sub>18</sub> N <sub>6</sub> O	334.380	578.0	6.238	N-methyl-N-[4-[(3-methylphenyl)amino]quinazolin-6-yl]diazanyl-acetamide
50136228	10094127	C <sub>18</sub> H <sub>17</sub> BrN <sub>6</sub> O <sub>2</sub>	429.275	130	6.886	[[4-[(3-bromophenyl)amino]quinazolin-6-yl]diazanyl-methyl-amino]methyl Acetate
50133373	9885081	C <sub>15</sub> H <sub>13</sub> BrN <sub>6</sub>	357.212	39	7.408	N-(3-bromophenyl)-N'-methyl-diazanyl-quinazolin-4,6-diamine
50133371	982519	C <sub>16</sub> H <sub>16</sub> N <sub>6</sub>	292.343	200	6.698	N'-methyl-diazanyl-N-(3-methylphenyl)quinazolin-4,6-diamine
3294	5328042	C <sub>14</sub> H <sub>11</sub> BrN <sub>4</sub>	315.172	0.78	9.107	N4-(3-Bromophenyl)quinazolin-4,6-diamine
50102430	10452792	C <sub>20</sub> H <sub>25</sub> N <sub>3</sub> O <sub>5</sub>	387.435	50000	4.301	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-(2-morpholin-4-ylethyl)benzamide
50102429	10593843	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub>	348.401	100000	4	N-benzyl-4-[(2,5-dihydroxyphenyl)methylamino]benzamide
50102428	10740438	C <sub>22</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>4</sub>	412.872	46000	4.337	N-[2-(4-chlorophenyl)ethyl]-5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-benzamide
50102427	10499929	C <sub>21</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub>	379.415	15000	4.823	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-(2-pyridin-2-ylethyl)benzamide
50102426	10761144	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub>	362.427	200000	3.698	4-[(2,5-dihydroxyphenyl)methylamino]-N-phenethyl-benzamide
50102425	10504027	C <sub>22</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>4</sub>	457.323	35000	4.455	N-[2-(4-bromophenyl)ethyl]-5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-benzamide
50102424	10716388	C <sub>23</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub>	408.453	100000	4	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-[2-(4-methoxyphenyl)ethyl]benzamide
50102423	10619651	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	378.427	10000	5	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-phenethyl-benzamide
50102422	10500718	C <sub>23</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	392.454	1000	6	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-(3-phenylpropyl)benzamide
50102421	10428841	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	364.400	8000	5.096	N-benzyl-5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-benzamide
50102420	10343317	C <sub>26</sub> H <sub>38</sub> N <sub>2</sub> O <sub>4</sub>	442.597	35000	4.455	5-[(2,5-dihydroxyphenyl)methylamino]-N-dodecyl-2-hydroxy-benzamide
50102419	10044189	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub>	362.427	500000	3.301	3-[(2,5-dihydroxyphenyl)methylamino]-N-phenethyl-benzamide
50102418	10738302	C <sub>23</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	376.454	33000	4.481	4-[(2,5-dihydroxyphenyl)methylamino]-N-(3-phenylpropyl)benzamide
50102417	10620637	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub>	394.426	300000	3.522	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-[2-(4-hydroxyphenyl)ethyl]benzamide
50102416	10318571	C <sub>22</sub> H <sub>21</sub> FN <sub>2</sub> O <sub>4</sub>	396.417	4000	5.397	5-[(2,5-dihydroxyphenyl)methylamino]-N-[2-(4-fluorophenyl)ethyl]-2-hydroxy-benzamide
50102415	10787405	C <sub>24</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	404.465	4000	5.397	5-[(2,5-dihydroxyphenyl)methylamino]-2-hydroxy-N-(1,2,3,4-tetrahydronaphthalen-2-yl)benza
50102414	10406106	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	358.436	900	6.045	5-[(2,5-dihydroxyphenyl)methylamino]-N-hexyl-2-hydroxy-benzamide

NOTE: In the table molecules of SNO 2–13 represents SET-A and molecules of SNO 14-30 represents SET-B.

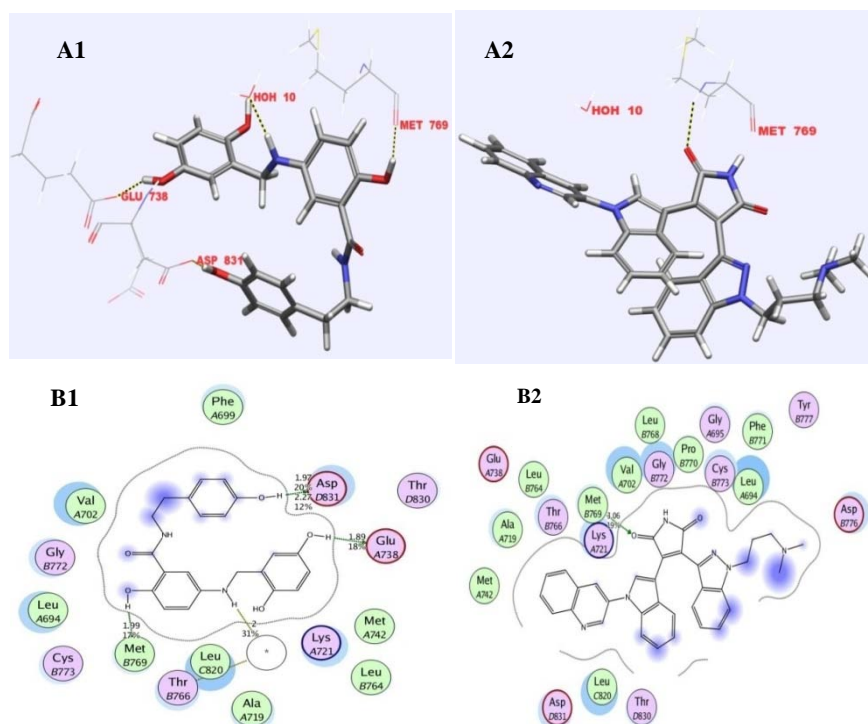
**Table 2:** Binding Database index along with Glide scores in SP and XP, MVD Mol dock values, its re-rank values and Prime MM-GBSA rescoring values in SP and XP. Binding free energy that includes ligand strain as DG1 and Binding free energy of complex alone as DG2.

BID	Glide SP	Glide XP	MVD Mol Dock	MVD Rerank	Glide Energy SP	Glide Energy XP	$\Delta G_{bind}$ SP DG1	$\Delta G_{bind}$ XP DG1	$\Delta G_{bind}$ SP DG2	$\Delta G_{bind}$ XP DG2
Erlotinib	-7.148196	-8.431376	-129.444	-97.6263	-50.490565	-50.166342	-41.41	-39.88	-44.60	-42.74
50162998	-5.934834	-7.864300	-189.499	-139.521	-53.310819	-58.702974	-22.07	-17.73	-37.07	-29.81
50162996	-7.846112	-6.165351	-191.737	-128.049	-65.008028	-60.480589	-15.38	-23.15	-33.36	-36.58
50162994	-7.234867	-7.117469	-185.953	-114.84	-56.438744	-66.280610	-18.68	-20.98	-24.80	-32.40
50162993	-7.299373	-7.428041	-164.756	-120.088	-53.492216	-56.331047	-26.70	-24.99	-33.91	-38.79
50162991	-6.179759	-6.489483	-209.95	-153.377	-52.566148	-63.401441	-16.87	-22.16	-31.69	-36.07
50162990	-8.540660	-9.519830	-196.984	-148.371	-67.105856	-66.458333	-36.71	-31.88	-44.36	-43.38
50136231	-6.407398	-7.818558	-102.329	-91.0394	-47.247333	-47.679379	-28.33	-32.53	-32.03	-34.95
50136230	-7.574168	-5.226777	-109.174	-96.9778	-43.887697	-44.214936	-30.10	-33.45	-32.37	-36.90
50136228	-5.093836	-5.649680	-113.583	-97.8377	-49.515064	-47.253517	-22.30	-29.52	-24.01	-30.99
50133373	-6.740066	-7.106242	-109.972	-93.8549	-43.742680	-42.497615	-29.00	-30.00	-30.47	-31.36
50133371	-6.963125	-7.117464	-110.834	-95.3594	-40.387212	-40.212685	-26.59	-28.62	-28.44	-30.15
3294	-5.539208	-7.069045	-92.0359	-81.1921	-39.558122	-39.445401	-20.45	-26.66	-22.52	-27.93
50102430	-7.799049	-7.142730	-125.175	-107.485	-55.001747	-55.237989	-37.97	-44.42	-36.61	-44.63
50102429	-7.585338	-6.517910	-117.395	-100.567	-52.015931	-49.474755	-27.36	-34.56	-26.02	-30.03
50102428	-7.859082	-8.154711	-127.692	-108.975	-57.333135	-57.018495	-38.54	-46.74	-25.14	-37.22
50102427	-7.878340	-7.186955	-124.719	-106.432	-57.973449	-57.431106	-30.99	-41.76	-24.78	-36.99
50102426	-7.462499	-6.807600	-113.022	-98.4002	-53.631507	-53.074579	-31.38	-38.29	-31.49	-43.62
50102425	-7.342426	-6.827995	-135.053	-111.989	-57.453021	-54.063434	-27.02	-36.37	-30.77	-38.96
50102424	-7.188777	-7.105667	-124.349	-103.023	-56.205434	-56.089033	-31.34	-38.88	-26.00	-36.80
50102423	-7.683234	-7.659811	-125.075	-106.665	-56.217999	-54.522865	-31.80	-39.83	-27.63	-39.71
50102422	-8.226105	-8.251268	-126.258	-106.663	-58.791227	-56.853971	-30.60	-40.08	-30.57	-37.00
50102421	-8.037560	-7.588743	-116.722	-100.4	-56.830894	-53.408634	-35.26	-42.66	-33.75	-41.25
50102420	-7.496508	-8.282052	-111.918	-96.1479	-60.134435	-62.589301	-32.15	-43.63	-36.58	-46.91
50102419	-7.753651	-7.017079	-124.735	-99.4279	-54.792925	-54.556446	-31.19	-38.08	-28.01	-34.02
50102418	-7.828320	-4.990621	-121.541	-98.5143	-55.083139	-51.380929	-22.16	-33.36	-27.67	-41.42
50102417	-8.255964	-9.019752	-134.816	-111.697	-60.140897	-59.173387	-39.72	-45.63	-34.49	-47.31
50102416	-7.847415	-7.937068	-133.165	-112.777	-56.048750	-54.894729	-37.36	-45.51	-26.57	-38.05
50102415	-7.921863	-7.994692	-122.129	-104.976	-57.300426	-57.559089	-30.84	-35.07	-28.98	-38.00
50102414	-7.377034	-7.049184	-131.466	-107.709	-53.597588	-53.457347	-34.02	-42.87	-30.88	-42.83

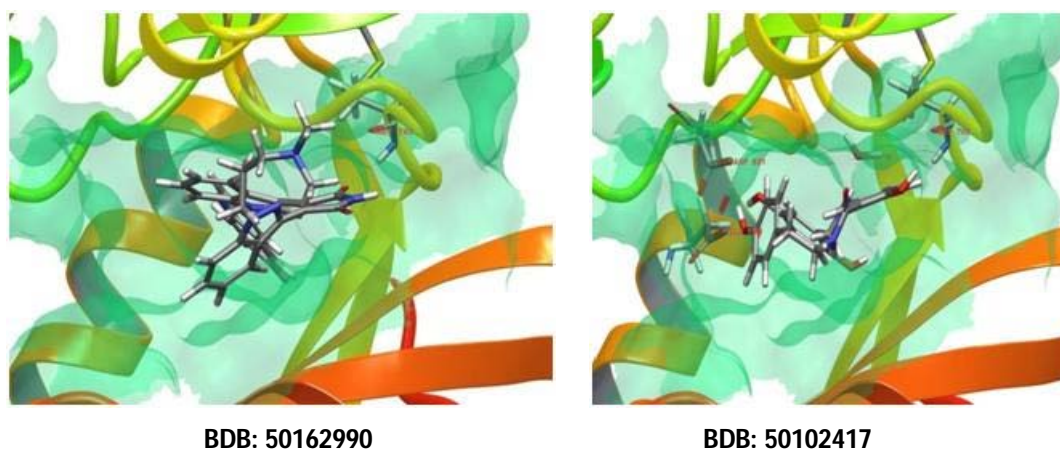
NOTE: In the table molecules of SNO 2–13 represents SET-A and molecules of SNO 14-30 represents SET-B.

The molecule BDB: 50162990 are forming a single hydrogen bond with the protein; the keto oxygen at 5<sup>th</sup> carbon of pyrrole forms H-bond with the amide hydrogen of MET-769 residue. The other oxygen atom of the molecule BDB: 50162990 are not within the reach of any other amide groups to form the H-bond, same is the position of the nitrogen atoms of the molecule- BDB: 50162990. In contrast to the molecule- BDB: 50162990 the molecule- BDB: 50102417 is forming 4 H-bonds. The 3 –OH groups of the molecule- BDB: 50102417 is donating its hydrogen to the amide residues of the protein, Glu-738, Met-769 and Asp-831 respectively to form H-bonds. The other nitrogen atom N1 is not within H-bond range of the Thr-766 amide, hence the water molecule bridges this gap. The N1H donates its hydrogen to the water

molecule to form H-bond, the water molecule forms H-bond with Thr-766, thus a bridging H-bond is formed with water molecule between Thr-766 and molecule- BDB: 50102417. We know that, more the hydrogen bonds between the molecules more they will be stable. Hence BDB: 50102417 can be more stable in the protein than the BDB: 50162990 ligand. In Glide SP interactions most of the molecules are not showing the hydrogen bond with the MET 769 residue, although it is given in hydrogen bond constrain before docking. But we can see the hydrogen bond in XP docking with all the ligands that shows the superiority of the Glide XP docking; this example shows XP docking of Glide is more preferable. Interaction plots are given as shown in Fig .3.



**Figure 3:** A1, A2 are the interaction plots of BDB: 50102417 and BDB: 50162990 respectively taken from Glide. B1, B2 are interaction plots of BDB: 50102417, BDB: 50162990 respectively taken from MOE



**Figure 4:** Docked poses of ligand's BDB: 50162990 and BDB: 50102417 respectively showing their positions in the active site of the protein molecule 1M17\_A.

The Monte Carlo multiple minimum (MCM) method implemented in Macro Model was used to perform the conformational analysis in the unbound state. All low energy conformers within 5.0kcal/mol were retained. To better account for the protein flexibility, the best pose for each inhibitor was energy minimized in the bound state. The conjugate gradient minimization scheme that uses the Polak-Ribiere first derivative method (PRCG), considered the best general method for energy minimization. Applying MD simulations greatly increases computational efficiency and provides a method with a time scale compatible with synthetic chemistry-biological test cycles. In addition, a recent study suggests that a single, relaxed structure for each complex provides superior result when compared to the standard averaging over MD trajectories.

In Prime MM-GB/SA the same two molecules which are best in docking are showing least binding energy, the BDB: 50162990 molecule of Set-A, and the BDB: 50102417 molecule of Set-B. When compared to docking scoring functions, the MM-GB/SA procedure provided more accurate docking poses, improved enrichment in the virtual screening of databases, and superior correlation between calculated binding affinities and experimental data in the lead optimization.

The molecules BDB: 50162990 and BDB: 50102417 are having good ADME/T properties. BDB: 50102417 molecule of Set-B is having much resemblance in properties with Erlotinib molecule, Floctafenine and Carvedilol are the two similar type molecules in common to both erlotinib and molecule BDB: 50102417. By this we can say that the molecule BDB: 50102417 is having good

chances to be a lead molecule, if some structural modifications are done keeping in consideration of developing ADME/T properties which can be done as the extension to this work.

### CONCLUSION

Computational drug designing has come a long way and is definitely the promise of tomorrow to discover many more new drugs in the coming years. The molecules showing promise (BDB: 50102417 and BDB: 50162990) require further studies to be carried by taking more structural derivatives of these molecules by pharmacophoric mapping and screening. Our work further reaffirms that hydrogen bonding plays a very important role in structure and function of biological molecules and especially in inhibition of a complex. We could also identify that rescoring of the docking values gives the best prediction of ligands about their interaction with protein and helps us in sorting them and choosing the best one out of a group or library of ligands. MM GB/SA based method is one of the best technique for prioritization of lead molecules while screening compound databases using molecular docking protocols. Further studies on these molecules using pharmacophore and QSAR methods hold promise for further development of the leads into drugs.

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