Chapter - 5

IDENTIFICATION OF NOVEL LIGANDS THROUGH PHARMACOPHORE BASED VIRTUAL SCREENING AND DOCKING ANALYSIS

5.1 Introduction

Computational drug design approaches are used in both lead identification and lead optimization. As discussed in chapter - I, EGFR is a well known anti-cancer target and application of such computational drug design approaches will help towards the development of potent EGFR inhibitors and ultimately lead to the development of new anti-cancer agents. Efforts have been made for the identification of new compounds as EGFR inhibitors through computational virtual screening. It is a very useful tool for molecular modeling and hit identification (McGauhey et al., 2007).

An abundance of structural information, indicated by both the ever-increasing availability of 3-dimensional (3D) protein structures and the readiness of free conformational databases of commercially available compounds, such as NCI (https://cactus.nci.nih.gov/ncidb2.2/; Ihlenfeldt et al., 2002), ZINC (http://zinc.docking.org/; Irwin & Shoichet, 2005), Asinex, (http://www.asinex.com/), etc., supply a broad platform for virtual screening. Recent studies reveal that the screening of compounds against specific target through virtual screening performs better than high throughput screening (Doman et al., 2002; Polgár et al., 2005; Schuster et al., 2010).

At the same time, new technology enables the implementation of more accurate and sophisticated pharmacophore models in the screening of millions of compounds within a manageable period and reduces the amount of experimental work (Doman et al., 2002). Nowadays, virtual screening based on pharmacophore models is widely accepted by many medicinal chemists (Drews, 2000; McInnes, 2007; Horvath, 2011; Gao et al., 2010) and expected to play a more important role in future drug discovery efforts (Sun, 2008).

Different types of interactions such as hydrogen bonding, charge transfer, electrostatic, and hydrophobic interactions have been observed in drug receptor complexes. The 3D
pharmacophore explains the spatial arrangement of chemical features responsible for these interactions of small molecule inhibitors with a macromolecular target protein. Hence, it is limited to representing one single mode of action, i.e., representing the binding mode of ligands that bind to the same target (Wolber, 2005). Screening of chemical libraries and mapping of the molecules using a specified pharmacophore facilitates the creation of virtual hit list. Molecules present in the virtual hit list satisfy the requirements of the model and have high probability to be active in experimental testing. Therefore, to filter promising ligands from large collections of compounds and enrichment of active molecules for experimental investigations in a variety of chemical databases can be done by virtual screening (Bajorath, 2002; Tanrikulu et al., 2013).

5.1.1 Applications of pharmacophore based virtual screening

Pharmacophore-based VS is widely used in various steps of the drug discovery process and facilitates the initial selection of compound classes as well as the optimization of compound properties as discussed below.

5.1.1.1 Identification of Lead compound

During the last few years, a number of studies describing the application of pharmacophore based virtual screening in lead identification for a specific disease related target, which may be developed into drug molecules for the treatment of the specified disease has been reported (Murgueitio et al., 2014; Krautscheid et al., 2014; Joung et al., 2014; Lu et al., 2015; Temml et al., 2014; Singh et al., 2015). For example, novel ligands for histamine H3 receptor and chemokine receptor CXCR2 were identified by (Lepailleur et al., 2014; Ha et al., 2015) respectively. Both the identified ligands were also used to treat Alzheimer’s disease and the authors were the first to report compounds with this dual mechanism of action.

5.1.1.2 Structure Activity Relationship

As mentioned earlier, a pharmacophore model gives an idea of the important features involved in the binding mode of active molecules to their respective target. To establish the structure activity relationship (SAR), the dataset molecules are discriminated as actives and inactives with the trained pharmacophore model. Differences in the experimentally observed biological activities of a set of compounds can be rationalized based on the presence/absence of chemical groups, represented by pharmacophore features, in the respective molecules. SARs can be accepted during model building, thereby illuminating the underlying mechanisms for the (absent) biological activity (Kaserer et al., 2015). Features responsible for the interaction of
compounds with the p – glycoprotein drug binding site were explained with the help of pharmacophore models (Ferreira et al., 2011). Sometimes, the pharmacophore models are developed from the previously established SAR and can be used to identify novel bioactive molecules (Flohr et al., 2002).

5.1.1.3 Target selectivity

Some of the molecules for example steroids having the common core structures and are found in both exogenous and endogenous bioactive compounds. These compounds often lack selectivity. The selectivity of these compounds may be identified by substituting the chemical moieties specifically leading to additional hydrophobic or ionic interactions and hydrogen bonds which permit to distinguish the target of interest (Ayan et al., 2012; Delvoux et al., 2014; Marchais-Oberwinkler et al., 2011; Koch et al., 2004 & 2005).

5.1.1.4 Previously reported studies for pharmacophore based virtual screening

Pharmacophore replacement of a pyrimidine ring in 5 –hydroxy – 4-keto motif of isoflavone genistein and superposition of this on EGFR tyrosine kinase inhibitor 4 – 3( -chlorophenylamino) – 6, 7dimthoxyquinazoline offered a novel 3′-chloro-5,7-dihydroxyisoflavone which binds more potently with the Cys 773 of sugar pocket of EGFR tyrosine kinase (Traxler et al., 1999). Six compounds were selected as good inhibitors of Spleen tyrosine kinase (Syk) through pharmacophore based virtual screening against Specs, NCI, MayBridge, and Chinese Nature Product Database (CNPD) (Xie, et al., 2009).

A pharmacophore of one hydrogen-bond acceptor (HBA), one hydrophobic point (HY), and two ring aromatics (RA), was identified for a set of known p38 MAP kinase inhibitors. Using this pharmacophore as a query, in a search against Medchem database, 30 new ligands were identified. Docking of these ligands into the active site of p38 MAP kinase led to the identification of one potential lead (Suresh, 2012).

A pharmacophore model consisting of ARPPP was generated for diverse classes of EGFR inhibitors. This obtained model was used as query to search ZINC database and 10 novel potential EGFR inhibitors were identified (Gupta et al., 2011). The effect of steric and electrostatic field interactions on EGFR inhibitors of quinazoline analogs was studied using CoMFA analysis which enabled the identification of novel ligands by virtual screening (Noolvi & Patel, 2013). A pharmacophore model consisting of three acceptors (A), one donor (D) and two areomatic rings (R) AAADRR for 40 pyrrolo[3, 2-d]pyrimidine derivatives was developed
and using this pharmacophore as a query, five different chemical databases were searched which resulted in the identification of 12 compounds as inhibitors of EGFR (Sudha et al., 2015). Four novel ligands of EGFR inhibitors were identified using AADHR pharmacophore (identified for N-benzyl-N-(X-2-hydroxybenzyl)-N0-phenylureas and thioureas derivatives) as a query and search against Zinc database (Thulasibabu, et al., 2014)

5.1.1.5 Combination with other techniques

The consensus approach such as docking, shape based modeling and molecular dynamics (MD) simulations etc., are combined with pharmacophore based virtual screening, which will enhance the number of active molecules in the virtual hit list. In addition a number filters for the oral bioavailability of compounds and rule of three have been framed (Veber et al., 2002; Congreve et al., 2003).

Lipinski’s rule of five is used to select the compounds with desired properties and to eliminate undesirable molecules (Lipinski et al., 1997). Similarly, for the detection and optimization of pan – assay – interference compounds (PAINS) (i.e. group of molecules that are prone to unspecifically interfere with some experimental test systems) the substructure filter can be used (Baell, 2010; 2014).

Multiples of these methods and filters can be included as well. For example, 17 inhibitors of microsomal prostaglandin E2 synthase were selected for testing purpose by applying multiple filters such as Lipinski filter, a pharmacophore-based virtual screening procedure and molecular docking. Among these 17 compounds, two compounds showed good activity in the experimental assay (Noha et al., 2015). Similarly, Temml et al., (2014) used a combination of pharmacophore and shape based virtual screening to identify novel liver X receptor agonists.

In the present work, we have employed pharmacophore based virtual screening, docking in combination with Lipinski’s rule of five filter to process for the identification of new compounds as EGFR inhibitors.

In the previous chapter, we identified two pharmacophores (AADRR & AAPRR) – one for pyrrolopyrimidine and another for pyrrolotriazine analogs of known inhibitors of EGFR. These pharmacophores were used as query to perform virtual screening against Zinc and NCI database compounds. The hits obtained from the virtual screening process were used for docking to identify novel EGFR inhibitors.
5.2 Materials and Methods

5.2.1 Virtual screening

Virtual screening was performed using the validated pharmacophore for pyrrolopyrimidines and pyrrolotriazines of AADRR and AAPRR discussed in detail in chapter IV. These were used as query against Zinc (www.zincdb.org; Irwin, Shoichet, 2004) and NCI chemical databases (www.cactus.nci.nih.gov/ncidb2.2/). Novel and potential compounds suitable for further drug development process were retrieved by using the virtual screening workflow module from Schrodinger software, in which all the generated conformers were used (William, David & Julian, 1996). A Fitness value of 3 was set as criteria for filtering of the retrieved compounds. Then conformer database searching process was performed allowing reorientation of the conformers to identify the matching of the database compounds with the query pharmacophore, matching of minimum of four out of five sites of the common pharmacophore and avoiding of partial matches involving more sites. The compounds which fitted well with the pharmacophoric features of the best hypothesis were retrieved as hits.

5.2.2 Ligand preparation

As discussed in earlier chapter, all the compounds obtained from the pharmacophore based virtual screening, were prepared by using Ligprep module in the Schrodinger. The low-energy 3D structures of all compounds were obtained.

5.2.3 Protein preparation and grid generation

We have used the same target protein structure (PDBID: 3POZ) used in chapter – III. The procedure for protein preparation and grid generation has been discussed in section 3.2.1. In PDB, the protein structure is in complex with the inhibitor molecule viz., N–{2-[4((3–chloro-4[-3-trifluoromethyl) phenoxy] phenyl} amino)-5H-pyrrolo [3, 2-d] pyrimidin-5-yl] ethyl}-3-hydroxy-3-methylbutanamide] (TAK -285). During protein preparation, ligand molecule from the complex structure was removed. After the completion of protein preparation process, the grid was generated around the active site of the target protein. The isolated ligand molecule was optimized by using Ligprep module in Schordinger and the re-docked with the active site of the target protein

5.2.4 Molecular docking
Re-docking of the PDB ligand: N–{2-[4({3–chboro-4-[3-trifluoromethyl] phenoxyl phenyl} amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl] ethyl}-3- hydroxy-3- methyl butanamide] retrieved from EGFR complex structure (PDB ID: 3POZ) was carried out with the active site of the target protein.

Similarly, the highly active inhibitor of pyrrolopyrimidine analogs, 4-[2-chloro-4-[[5-(2-hydroxyethyl)] pyrrolo[3,2-d] pyrimidin-4-yl] amino]phenoxy]-1,3-dihydropyridol-2-one (Pubchem CID: 25026082, PIC50 = 8.40), and pyrrolotriazines analog, 5-[(4-aminopiperidin-1-yl)methyl]-N-(3-chlorophenyl)pyrrolo[2,1-f][1,2,4]triazin-4-amine (Pubchem CID: 21874200, PIC50 = 8.40) were docked with the above target protein to find out their docking scores as well as to understand their mode of binding with EGFR.

The virtual screening workflow in Maestro was used in three phases, by means of three different docking protocols of varying precisions and computational intensities. Using high-throughput virtual screening (HTVS) protocol, docking analysis was carried out for the hits obtained from the pharmacophore based virtual screening. Based on the docking score, about ~30% ligands were selected and subjected for standard precision (SP) docking. Then, ~ 30% of hits from SP docking were continued for more accurate extra precision (XP) docking. All these steps were carried out by using the standard protocols of the program Glide with default parameters (Schrodinger, LLC, New York, NY) (Halgren et al., 2004; Friesner et al., 2006) and the Glide score was used to select the best conformation for each ligand, during the docking process. Absorption, Distribution, Metabolism and Excretion (ADME) properties were calculated for the retrieved hits by using QikProp program.

5.3 Results and discussion

5.3.1 AADRR - Pharmacophore model of pyrrolopyrimidine analogs based virtual screening and docking analysis

AADRR was used as a query pharmacophore to prioritize the potent compounds from Zinc and NCI chemical databases consisting of a total of 203568 molecules with diverged chemical scaffolds. A hit list of 9000 compounds was obtained based on pharmacophore model with fitness value in the range of 1.571 – 2.193. These compounds were further considered for docking studies.

5.3.1.1 Binding mode analysis of experimentally proved tak - 285 inhibitor
Crystallographic analysis shows that N–{2-[4({3–chloro-4[-3-trifluoromethyl) phenoxy]phenyl}) amino]-5H-pyrrolo [3, 2-d] pyrimidine-5-yl] ethyl}-3- hydroxy-3-methylbutanamide (TAK - 285) binds to the EGFR with a DFG-in and α helix C-out conformation. One of the pyrrolo [3, 2-d] pyrimidine ring nitrogen atoms makes a direct hydrogen bond with the main-chain nitrogen of Met793 of the hinge region between the N- and C-lobes. The other ring nitrogen atom makes a water mediated hydrogen bond to Thr854. The bulky 3-trifluoromethylphenyl group occupies a pocket formed by Met766, Cys775, Leu777, Leu788, Thr790, Thr854, and Phe856 (Aertgeerts et al., 2011). From the binding mode analysis of known inhibitors we found that they do not closely match the earlier derived pharmacophores although there are some superficial similarities in the pharmacophoric features such as the number of hydrogen bond donors (D), hydrogen bond acceptors (A) etc.,

The docking results of tak - 285 bind with the receptor protein EGFR with docking score of -12.261 kcal/mol and shows similar hydrogen bond interaction with that of crystallographic data viz., N1 –nitrogen of pyrrolopyrimidine forms hydrogen bond with hinge region NH of Met 793. In addition, ethyl hydroxyl group of pyrrole and hydorxy group of methyl butanamide side chain at N – 5 position of pyrrole ring forms hydrogen bond interaction with Asp 800. Another H – bond was also observed between methyl butanamide hydroxyl group and Cys (797) (Fig 5.1). Through this analysis, it was observed that the inhibition activity may be increased by the presence of electron donating groups at position – 5 of pyrrole ring.

Fig 5.1 – Interactions of PDB ligand tak - 285 with the receptor EGFR
5.3.1.2 Binding mode analysis of highly active compound 4-[2-chloro-4-[[5-(2-hydroxyethyl)pyrrolo[3, 2-d] pyrimidin-4-yl] amino] phenoxy]-1, 3-dihydroindol-2-one

Docking analysis reveals that this compound binds with EGFR with a docking score of -12.281 kcal/mol and the N1 –nitrogen of pyrrolopyrimidine forms hydrogen bond with the hinge region NH of Met 793 and ethyl hydroxyl group of pyrrole forms hydrogen bond with Arg 841. The pi – pi interaction is favored between aromatic ring of indole group to DFG motif residue Phe 856 (Figure 5.2).

Fig 5.2 – Interactions of highly potent pyrrolopyrimidine inhibitor with the receptor EGFR

5.3.1.3 Results of molecular docking studies
The hits obtained from the pharmacophore based screening process were docked using the HTVS protocol. Based on the docking score range (-10.942 to – 7.000 kcal/mol) 2742 molecules were selected. These molecules were used for SP docking which resulted in 843 compounds with a docking score range of -11.953 to -8.00 kcal/mol. Then, these hits were subjected for more accurate XP docking. Based on the binding mode, docking score (range of -11.86 to – 10.86 kcal/mol and ADMET properties including Lipinski’s rule of five, eight molecules were identified, in which all molecules having good docking score and best binding mode and were selected as potential EGFR inhibitors.

5.3.1.4 Binding mode analysis of AADRR pharmacophore based virtually screened inhibitors

The crystallography data and docking result of PDB ligand, highly active compounds of both the pyrrolopyrimidines and pyrrolotriazines reveals that the hinge region residue Met 793 is actively involved in the hydrogen bond formation through the ring nitrogen. Based on these insights, the binding mode analysis was carried out for the virtually screened hits. All the obtained eight hits were well docked into the binding pocket of EGFR target protein with good docking scores. Except two compounds viz., ZINC - 72644877 ZINC – 72288739 remaining six compounds interact through hydrogen bond with Met 793. In addition, ZINC – 53800236 & ZINC – 79318504 compounds form hydrogen bonds with Asp 855. Further, ZINC – 55565095 & NCI – 21264 compounds also form pi –pi interactions with Phe 856. The binding mode analysis revealed that the hits obtained shared a common intermolecular interaction with the target protein residue Met 793 as well as also forms the pi – pi interactions similar to that of highly potent inhibitor with good docking scores (Fig 5.3 a, b, c, d, e, f, g & h). The presence of amino functional group in the structural scaffolds of the hits was found to be responsible for the hydrogen bond formation with the EGFR tyrosine kinase target protein. The varied range of obtained hits confirmed that the pharmacophore model could retrieve novel compounds with similar features to that of existing EGFR tyrosine kinase inhibitors. The structural features of the lead molecules contain amino group, mainly responsible for the formation of hydrogen bond with the EGFR target protein.
Fig 5.3 (a) – Interactions of NCI database compound - N-hydroxy-5-(4-hydroxy-3,5-ditert-butyl-phenyl)-N-methyl-1H-triazole-4-carboxamide (ZINC ID: 212464)

Fig 5.3 (b) – Interactions of Zinc database compound -- N-[3-(2-oxo-1-piperidyl)phenyl]-1H-indazole-7-carboxamide (ZINC ID: 25238067)
Fig 5.3 (c) – Interactions of Zinc database compound - N-[[3-(isopropoxymethyl)phenyl]methyl]-1H-indazole-7-carboxamide (ZINC ID:53800236)

Fig 5.3 (d) – Interactions of Zinc database compound N-(6-benzoyloxy-3-pyridyl)-1H-pyrazole-5-carboxamide (ZINC ID: 55565095)
Fig 5.3 (e) – interactions of Zinc database compound - 3,4-dimethyl-6-oxo-N-[4-[[2S]-tetrahydrofuran-2-yl]methoxy][phenyl]-1H-pyridazine-5-carboxamide  (ZINC ID: 79318504)

Fig 5.3 (f) – Interactions of Zinc database compound 3,4-dimethyl-6-oxo-N-[4-[[2R]-tetrahydrofuran-2-yl]methoxy][phenyl]-1H-pyridazine-5-carboxamide ZINC ID: 79318508
Fig 5.3 (g) – Interactions of Zinc database compound – [4-(thiophene-2-carbonylamino)phenyl] (ZINC ID: 72644877)

Fig 5.3 (h) – Interactions of Zinc database compound – N-[4-[(1R,2S)-2-hydroxycyclohexoxy]phenyl]-4-methyl thiazole-5-carboxamide (ZINC ID: 7288739)
The compound ID and IUPAC names, their docking score, type and the residues involved in the interactions for each compound as listed in Table 5.1.

**Table 5.1 - Compound IDs, Docking score & Interaction patterns and residues involved in interactions of obtained hits.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound ID and IUPAC names</th>
<th>Docking Score</th>
<th>Interacting residues &amp; type of interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZINC- 21264 - N-hydroxy-5-(4-hydroxy-3,5-ditert-butylphenyl)-N-methyl-1H-triazole-4-carboxamide</td>
<td>-11.37</td>
<td>Met 793, Gln 791, Phe 856</td>
</tr>
<tr>
<td>2</td>
<td>ZINC – 25238067 - N-[3-(2-oxo-1-piperidyl)phenyl]-1H-indazole-7-carboxamide</td>
<td>-10.99</td>
<td>Met 793, Lys 745</td>
</tr>
<tr>
<td>3</td>
<td>ZINC – 53800236 - N-[3-(isopropoxymethyl)phenyl]methyl]-1H-indazole-7-carboxamide</td>
<td>-10.86</td>
<td>Met 793, Asp 855</td>
</tr>
<tr>
<td>4</td>
<td>ZINC – 55565095 - N-(6-benzylxoy-3-pyridyl)-1H-pyrazole-5-carboxamide</td>
<td>-11.08</td>
<td>Met 793, Phe 856</td>
</tr>
<tr>
<td>5</td>
<td>ZINC – 79318504 - 3,4-dimethyl-6-oxo-N-[4-[[2S]-tetrahydrofuran-2-yl]methoxy]phenyl]-1H-pyridazine-5-carboxamide</td>
<td>-11.60</td>
<td>Met 793, Asp 855</td>
</tr>
<tr>
<td>6</td>
<td>ZINC – 79318508 - 3,4-dimethyl-6-oxo-N-[4-[[2R]-tetrahydrofuran-2-yl]methoxy]phenyl]-1H-pyridazine-5-carboxamide</td>
<td>-11.86</td>
<td>Met 793</td>
</tr>
<tr>
<td>7</td>
<td>ZINC – 72644877 - [4-(thiophene-2-carbonylamino)phenyl]</td>
<td>-10.86</td>
<td>Asp 855</td>
</tr>
</tbody>
</table>
5.3.2 AAPRR - Pharmacophore model of pyrrolothiazine analogs based virtual screening and docking analysis

Using AAPRR as a query pharmacophore, searching of compounds from Zinc and NCI chemical databases, a hit list of 17522 compounds was obtained with fitness value range of 1.571 – 2.193. These compounds were further considered for HTVS, SP and XP docking studies similar to that of previous section 5.3.1.

5.3.2.1 Binding mode analysis of highly active compound 5-{[(4-aminopiperidin-1-yl)methyl]-N-(3-chlorophenyl)pyrrolo[2,1-f][1,2,4]triazin-4-amine

Docking analysis reveals that the compound binds with the target protein with the docking score of -12.281 kcal/mol and the N1 –nitrogen of pyrrolothiazine forms hydrogen bond with hinge region NH of Met 793 and amino group attached with piperidine nucleus forms hydrogen bond with Asp 855 & Asn 842 (Figure 5.4).

Fig 5.4– Interactions of highly potent pyrrolothiazine inhibitor with the receptor EGFR.

As discussed in the section 5.3.1.3, 5362 molecules out of 17522 molecules were obtained from HTVS with a docking score range of -10.670 to -6.000 kcal/mol; continuation of these hits to SP docking resulted 1620 compounds with a docking score in the range of -10.772 –
8,000 kcal/mol were subjected to XP docking. Finally, six molecules were selected with a docking score range of -11.862 to -10.966 kcal/mol with highest binding scores and good binding mode.

5.3.2.2 Binding mode analysis of AAPRR pharmacophore based virtually screened inhibitors

Comparing the binding mode of AAPRR pharmacophore based screening hits with the crystallography data and highly potent active analog of pyrrolotriazine revealed that the interactions with EGFR of all the hits shares a common pattern of hydrogen bond formation with Met 793.

Further, the Zinc database compound 79856172 also forms hydrogen bond interaction with the DFG motif residue Asp 855, similar to that of interaction of highly potent compound. Interestingly, 59186833, 79090657 & 79683299 forms Pi – Pi interaction with Phe 856 of DFG motif residue (Fig 5.5 a, b, c, d, e & f). The presence of amino functional group in the different structural scaffolds of obtained hits was found to be responsible for the hydrogen bond formation with the EGFR target protein and may be responsible for inhibitory activity. The varied range of obtained hits confirmed that the pharmacophore model could retrieve novel scaffolds and compounds with similar features to that of existing EGFR inhibitors.

Fig 5.5 (a) – Interactions of Zinc database compound – (5E)-5-[(4-benzyloxy-3-methoxyphenyl)methylene]-2-imino-1-methyl-imidazolidin-4-one (ZINC ID: 59186833)
Fig 5.5 (b) – Interactions of Zinc database compound – 4-[(3R)-3-(1H-benzimidazol-2-ylmethyl)-1-piperidyl]-5,6,7,8- tetrahydropyrido[3,4-d]pyrimidine (ZINC ID: 79856172)

Fig 5.5 (c) – Interaction of (1R)-N-[2-(2-methoxyphenyl)ethyl]-N-methyl-1-[3-((1H-benzimidazol-2-ylmethyl)amino)propyl]-4-phenyl-1H-indole-3-carboxylic acid (ZINC ID: 67867548)
Fig 5.5 (d) – Interactions of Zinc database compound – N-[[2-(pyrrolidin-1-ylmethyl)phenyl]methyl]-1,3-benzodioxole-4-carboxamide (ZINC ID: 79090657)

Fig 5.5 (e) – Interactions of Zinc database compound – N-(2-imidazo[1,2-a]pyridin-2-ylethyl)-5-propyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (ZINC ID: 84582093)

Fig 5.5 (f) – Interactions of Zinc database compound – 2-hydroxy-N-(3-isoindolin-2-ylpropyl)benzamide (ZINC ID: 79683299)
The compound ID and IUPAC name, their docking score, type and the residues involved in the interactions for each compound as listed in Table 5.2

**Table 5.2 - Compound ID and IUPAC name, docking score & interaction patterns and residues involved in interactions of the hit molecules.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound ID and IUPAC name</th>
<th>Docking Score</th>
<th>Interacting residues &amp; type of interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>59186833 - (5E)-5-[(4-benzylxy-3-methoxyphenyl)methylene]-2-imino-1-methylimidazolidin-4-one</td>
<td>-11.862</td>
<td>Met793, Phe856</td>
</tr>
<tr>
<td>2.</td>
<td>67867548 - 4-[(3R)-3-(1H-benzimidazol-2-ylmethyl)-1-piperidyl]-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine</td>
<td>-11.414</td>
<td>Met793</td>
</tr>
<tr>
<td>3.</td>
<td>79090657 - (1R)-N-[2-(2-methoxyphenyl)ethyl]-N-methyl-1-[3-(3-pyridyl)-1,2,4-oxadiazol-5-yl]ethanamine</td>
<td>-11.288</td>
<td>Met793, Phe856</td>
</tr>
<tr>
<td>4.</td>
<td>79683299 - N-[2-(pyrrolidin-1-ylmethyl)phenyl]methyl]-1,3-benzodioxole-4-carboxamide</td>
<td>-10.966</td>
<td>Met793, Thr858, Phe856</td>
</tr>
<tr>
<td>5.</td>
<td>79856172 - N-(2-imidazo[1,2-a]pyridin-2-ylethyl)-5-propyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine</td>
<td>-10.898</td>
<td>Met793, Asp 855</td>
</tr>
</tbody>
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5.3.3 Pharmacokinetic prediction of lead molecules obtained from AADRR and AAPRR pharmacophore based screening and docking analysis

The analysis of passing of this Lipinski’s rule of five is significant during drug development process through which the lead molecules can be generated and are eliciting most satisfactory ADME properties. Pharmacophores based virtually screened and docked compounds were thoroughly analyzed for their Lipinski’s properties and all the compounds are obeying the rule of five. The normal range and the compounds properties are given in Table 5.3 (a) & (b)

Further, the percentage for human oral absorption is also identified and all the eight compounds are in the range of 82% – 100%. These results show that all the pharmacokinetic properties are in the acceptable range. Hence, these compounds can be used as potential drug like molecules of EGFR inhibitors.

Table 5.3 (a) – AADRR based screened and docked hits of ID and their Obtained Drug Likeness Profile

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>mol_MW (&lt;500)</th>
<th>Donor HB (&lt; 5)</th>
<th>Accept HB (&lt; 10)</th>
<th>QPlogP o/w (&lt; 5)</th>
<th>Rule of five Compliance</th>
<th>Percent Human Oral Absorption (&gt; 80 high, &lt; 25 poor)</th>
<th>QPlogS (−6.5 to 0.5)</th>
<th>QPlog HERG (below −5)</th>
<th>QPlog BB (−3 to 1.2)</th>
</tr>
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<tr>
<td>ZINC79318508</td>
<td>343.382</td>
<td>0</td>
<td>5</td>
<td>3.308</td>
<td>Yes</td>
<td>94.349 (-4.883 -5.304)</td>
<td>-1.139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZINC79318504</td>
<td>343.382</td>
<td>0</td>
<td>5</td>
<td>3.22</td>
<td>0</td>
<td>93.853 (-4.645 -5.109)</td>
<td>-1.105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>212464</td>
<td>414.261</td>
<td>5</td>
<td>6</td>
<td>2.664</td>
<td>0</td>
<td>82.827 (-5.017 -6.882)</td>
<td>-1.695</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZINC55565095</td>
<td>294.312</td>
<td>1</td>
<td>4</td>
<td>3.287</td>
<td>0</td>
<td>95.939 (-4.572 -6.428)</td>
<td>-1.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZINC25238067</td>
<td>334.377</td>
<td>1</td>
<td>5</td>
<td>3.175</td>
<td>0</td>
<td>94.098 (-5.357 -6.027)</td>
<td>-0.922</td>
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<tr>
<td>ZINC72288739</td>
<td>332.417</td>
<td>2</td>
<td>6</td>
<td>2.722</td>
<td>0</td>
<td>100 (-3.929 -4.771)</td>
<td>-0.388</td>
<td></td>
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<tr>
<td>ZINC72644877</td>
<td>343.397</td>
<td>1</td>
<td>7</td>
<td>2.881</td>
<td>0</td>
<td>100 (-5.388 -4.129)</td>
<td>-0.367</td>
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<td></td>
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</tbody>
</table>
Table 5.3 (b) – AAPRR based screened and docked hits of ID and their Obtained Drug Likeness Profile

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>mol_MW (&lt;500)</th>
<th>Donor HB (&gt;5)</th>
<th>Accdpt HB (&gt;10)</th>
<th>QPlogP o/w (&lt;5)</th>
<th>Rule of five Complianc</th>
<th>Percent Human Oral Absorption (&gt; 80 high, &lt; 25 poor)</th>
<th>QPlogS (−6.5 to 0.5)</th>
<th>QPlog HERG (below −5)</th>
<th>QPlog BB (−3 to 1.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZINC53800236</td>
<td>323.394</td>
<td>1</td>
<td>4</td>
<td>4.088</td>
<td>0</td>
<td>100</td>
<td>−5.256</td>
<td>−5.97</td>
<td>0.711</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Factors of Lipinski’s rule of five</th>
<th>Pharmacokinetic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>59186833</td>
<td>318.334 1 7 2.366 0 90.803 −4.526 −6.332 −0.847</td>
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<tr>
<td>67867548</td>
<td>345.441 1 8 1.154 0 77.442 −2.024 −3.32 −0.01</td>
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<tr>
<td>79090657</td>
<td>302.307 1 7 1.547 0 84.652 −3.205 −4.577 0.746</td>
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<td>79683299</td>
<td>296.427 1 6 2.289 0 89.145 −2.638 −5.948 0.082</td>
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<td>79856172</td>
<td>315.389 0 5 3.41 0 100 −4.038 −6.081 −0.41</td>
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<tr>
<td>84582093</td>
<td>339.415 1 4 4.181 0 100 −4.452 −6.68 0.463</td>
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</tbody>
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5.3.4 Conclusion

In this study, based on the pharmacophores AADDR and AAPRR of pyrrolopyrimidines and pyrrolotriazines inhibitors of EGFR, virtual screening was performed against NCI & ZINC database compounds. The obtained hits were subjected to filtering process to check the selected pharmacophore features. The interaction patterns of these compounds were identified by docking analysis. Based on the docking scores, binding modes, residues involved in the interactions and ADME properties, a total of 14 novel compounds were identified. These 14 compounds may be considered for in vitro studies as inhibitors of EGFR Tyrosine kinase.