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IDENTIFICATION OF POTENTIAL INHIBITORS OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE BY VIRTUAL SCREENING AND DOCKING STUDIES

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Keywords:

EGFR inhibitors, Virtual screening and docking, Tyrosine kinase inhibitors, Pyridopyrimidine Analogs, Anticancer Agents

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ABSTRACT: Understanding the mode of inhibition through the crystal structures of EGFR kinase domain complex with small molecule inhibitors provides us to search for potential ligands of EGFR. The binding mode analysis of pyridopyrimidine analog reveals that the presence of hydrogen bonds between the target protein amino acid Met 793 (i.e. the hinge region which is responsible for catalytic activity of the domain) and the ring nitrogen of the small molecule inhibitor. In addition, amino acid Asp 855 and Lys 745 were also involved in the interaction. The present work focuses on the identification of novel ligands through computational approach. By screening of pubchem database compounds, based on the bioactive and structural similarity of a pyridopyrimidine derivative (highly potent inhibitor of EGFR), 117 compounds were identified and all are having kinase inhibition activity but were not analyzed in EGFR. Using GLIDE software, the docking was done and complete analysis of the binding mode results in eleven compounds having same pattern of interactions and these compounds may be further analyzed by *in vitro* for their EGFR kinase inhibition activity.

INTRODUCTION: The three dimensional structure of EGFR consists of three domains namely, extra cellular ligand binding domain, transmembrane domain and cytoplasmic domain[1, 2]. Upon binding of the specific ligand EGF to its ligand binding domain, the monomeric receptor becomes a dimer and activates receptor autophosphorylation through the cytoplasmic tyrosine kinase catalytic domain [3, 4, 5]. This catalytic activity initiates downstream regulation of many signaling pathways receptors, which are responsible for cancer cell proliferation, arresting of apoptosis process and stimulation of metastasis [6].

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Hence, developing small molecular compounds to inhibit EGFR is considered as an important therapeutic approach for treating a variety of cancers [7, 8, 9]. To achieve this goal small molecule inhibitors which compete with either the ligand binding domain or ATP binding pocket of the cytoplasmic tyrosine kinase domain have been approved as anticancer drugs [10].

Specifically, a number of quinazolin based small molecules such as Gefitinib & Erlotinib were approved for treating Non Small Cell Lung Cancers (NSCLC) [11, 12] and they competitively inhibit with the ATP binding pocket leads to downstream signaling of EGFR [13, 14]. With similar mechanism of action other quinazolin derivatives namely, Vandetanib and Lapatinib (dual inhibitor of EGFR and HER2 tyrosine kinases) were developed for treating medullary thyroid & metastatic breast cancers [15, 16, 17].

Understanding the mechanism of inhibition of this receptor by these molecules was made easy by the availability of crystal structures of EGFR inhibitor complexes. For example, the crystal structure of EGFR in complex with lapatinib (PDB ID: 1XKK), shows two hydrogen bonds. One of the hydrogen bond is between the quinazoline ring nitrogen (N1) and main chain NH of Met 769.The other water mediated hydrogen bond is between N3 and side chain of Thr 830 [18].

Similarly, analyzing the structure of EGFR – Gefitinib complex (PDB ID: 2ITO), it is observed that the ring nitrogen N1 forms hydrogen bond with Met 793, the residue of hinge region which connects the N and C lobes. The ligand molecule is surrounded by the side chains of Lys 745, Leu 788 and Thr 790 [19].

Though the therapeutic effect of quinazoline based molecules on different cancers have been well established, many side effects like diarrhea, skin rashes, nausea, vomiting and haemorrhage were also reported [20]. Search for alternative therapeutics with less toxicity is essential to overcome these side effects of quinazoline analogs. Analogs of pyridopyrimidine, pyrollopyrimidine and pyrollotriazine are experimentally studied and proved as anti cancer agents and these competitively bind to the ATP binding pocket of EGFR [21, 22, 23, 24]. For example, analysis of the binding mode of dual inhibitor 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) with EGFR in the crystal structure (PDBID: 3POZ) enlightens the important residues for interactions.

All kinases possess a bilobed folding i.e. N-terminal and C – terminal in which the N-terminal lobe consists of mostly strands and one helix and the later consists of majority of helix. These two lobes are interconnected by a hinge region and are divided by an inwards cleft which comprises ATP binding site. The size of the ATP binding pocket is based on the spatial accommodation of the two lobes which are in turn represented by the activation state of the domain. The residues associated with catalytic activity of EGFR kinase domain includes the nucleotide phosphate binding loop consisting of Leu718–Val726, alpha helical region of N- terminal lobe (Asn 756 – Val 768), DFG motif (Asp 855–Gly 857), catalytic motif (Arg 836–Asn 842), and activation loop in the C-terminal lobe (Asp 855–Val 876). The dual inhibitor tak - 285 binds with the ATP binding pocket of EGFR in competition with ATP. The inhibition is through the direct hydrogen bond formation between the pyrimidine ring nitrogen and Met 793 found in the hinge region of N and C lobes; a water mediated hydrogen bond is also formed with the other ring nitrogen to Thr 854.

Further, a pocket comprising of Cys 775, Met 766, Leu 777, Leu 788, Thr 790, Thr 854 and Phe 856 provides space for accommodation of bulky moiety of the ligand [21]. All these structural information and use of virtual screening and docking studies provide a novel as well as target specific active ligand molecules. Adopting computational or insilico approach of virtual screening process provides list of suitable chemical candidates as EGFR inhibitors and docking studies are used to understand the interaction pattern of the receptor with its inhibitors [25]. For example Cavasotto, et al., (2006) identified 39 novel ligands as anti EGFR through virtual screening and docking studies and they experimentally discovered that the compound C (4)-N (1)-substituted pyrazolo [3, 4-d] pyrimidine [MSK-039] inhibits EGFR tyrosine kinase activity with low-micro molar concentration [26]. Similarly, Yang, et al., (2011) employed virtual screening against Traditional Chinese Medicine Database (TCM Database Taiwan). Four compounds namely 2-O-caffeoyl tartaric acid, Emitine, Rosmaricine, and 2-Oferuloyl tartaric acid, were identified as potential inhibitors of EGFR with better binding affinity.

Further, through docking analysis, they explained that the interactions are mediated through the formation of hydrogen bond between ligand molecule with either Asp 855 or Lys 716 / 728 of the target protein. The stability of these interactions is further analyzed by molecular dynamic simulation studies [27]. Another structure based virtual screening and docking study carried out by Li, et al., (2012) identified 13 novel compounds from 1, 97, 116 in SPECS database.

Among these 13 compounds 8 compounds were experimentally proved as anti EGFR [28]. In continuation of all these success processes, in this work, we tried and identified novel ligands by applying both structure and ligand based virtual screening and docking analysis to have a look in to search for potential inhibitors of EGFR. Finally, eleven compounds were identified and which form hydrogen bonds, pi –pi, pi – cation & hydrophobic interactions with catalytically important active site residues such as Met 793, Asp 855 & Phe 856 of EGFR. Results from the analysis of binding models and drug like properties point to the likelihood of these compounds as effective EGFR inhibitor candidates.

MATERIALS AND METHODS:

Target Protein structure Retrieval and Preparation: The X-ray crystallographic structure of EGFR kinase domain in complex with 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) inhibitor with a resolution of 1.5 Å (PDB ID: 3POZ) was selected. Using protein preparation wizard from maestro, the selected protein structure was optimized and minimization was done with OPLS (Optimized Potentials for Liquid Simulations) 2005 molecular mechanics force field 27 with a cutoff Root Mean Square Deviation of 0.3 Å [29].

Receptor Grid Generation: The tak-285 inhibitor from the target structure was removed. By using the Receptor Grid generation module, (OPLS2005 force field), grid box was generated in the center of active site region which enables the ligand to rotate freely within the binding pocket and produce several conformational changes [30]. The generated grid box covers the active site residues of Leu 718, Val 726, Ala 743, Lys 745, Met 766, Cys 775, Arg 776, Leu 777, Leu 788, Thr 790, Gln 791, Leu 792, Met 793, Gly 796, Arg 841, Asn 842, Leu 844, Thr 854, Asp 855, Phe 856 & Leu 858.

Selection, Screening and Preparation of ligands: Highly potent pyridopydimidine analog (Binding DB ID: BDBM 3081, Pubchem CID: 5327895) was identified from Binding DB [31]. Based on the structure and bioactive similarity, 117 compounds were retrieved from Pubchem database (http: //www. pubchem.ncbi.nlm.nih.gov/). The dataset consists of pyridopyrimidine, pteridine, naphthyridine, and benzotriazine derivatives. Experimental results of all these compounds show inhibitory activity against various protein kinases but not with definite quantity on EGFR tyrosine kinase.

Hence, these compounds were selected for analyzing potential EGFR kinase inhibition activity. All these compounds were prepared by means of optimizing bond angles, bond lengths and their protonation state, etc., using Ligprep module from maestro [32]. The Pubchem CIDs and their inhibition activity details are given in **Table 1**.

TABLE 1: PUBCHEM CID AND INHIBITION ACTIVITY DETAILS OF SCREENED LIGANDS

S. No	Experimentally observed Kinase inhibition activity	Pubchem CID
1	Abl, c-Src, PDGFr, VEGFr	10345191, 10095463, 10071040, 10070245
2	Bcr-Abl	447077
3	c-Abl	49865422, 46934425
4	Caspase -7	3233059, 3233522, 3233962, 3233469, 3234474, 3235426, 3235500, 3233797
5	c-Src	11530772, 11581459, 11603131, 11675061, 5328723, 5330525, 11749233, 44390508, 44390861, 44390865, 44390870, 44390878, 44390902
6	c-Src, FGFr, PDGF beta receptor	5327891, 5327898, 5327904, 5328703, 5328705, 5328712, 5328713,5328637, 5328638, 5328639, 5328640, 5328645, 5328647, 5328648, 5328704, 5328715, 5328725, 5328717, 5328729, 5328730, 5328732,
7	c-Src, EGFR, FGFr, PDGFr	5327870, 5327871, 5327872, 5327874, 5327882, 5327883, 5327905, 5327906, 5327907, 5327908, 5327909, 5327910, 5327912, 5327913
8	c- Src , KDR, Lck	11785748
9	c –Src, Lck	5330526
10	c-Src, WEE1	44390494, 44390841, 44390876, 44390890, 44390942, 44390910, 44390912, 44390917, 44390921, 44390923, 44390924, 44390925, 44390926, 44390929, 44390937, 44390940, 44390941, 44390944, 44390947, 44390951, 44390953, 44390956, 44390957, 44390958, 44390966, 44390967, 44390968, 44390969,

		44390972, 44390982, 44390985
11	EGFR, HER2	5327865
12	FGFr, PDGFr,	5328649, 10029735
13	HDAC2	3233837
14	Lck	11974313, 11974314, 44414636
15	MAP	23551786
16	P450 – cyp1a2	3233056, 3233170, 3235196, 3233217, 3233262, 3233424, 3233689
17	Src	44425303, 44419466, 11646082, 11595922, 11581460
18	WEE1	5311382

Docking analysis: Highly potent pyridopyrimidine analog 6 – (2, 6 – dichlorophenyl) – 2- (3 – methoxyanilino) – 8 –methyl pyrido [2, 3 –d] pyrimidine – 7- one (Pubchem CID: 5327895) was prepared and docked with the active site of the target protein using Glide [33] with the extra precision (XP) mode, which provides information regarding binding energy and their mode. Similarly, all the 117 prepared ligands were docked with the active site of the target protein. Then properties such as drug like properties, Lipinski's rule of five, permeability for Blood Brain Barrier (QPlogBB), oral absorption percentage, etc., were analyzed by using the Qikprop module [34] for all the ligands.

RESULT AND DISCUSSION: The best docking pose of the highly potent compound 6 – (2, 6 – dichlorophenyl) – 2- (3 – methoxyanilino) – 8 – methyl pyrido [2, 3 –d] pyrimidine – 7- one (Pubchem CID: 5327895) is shown in **Fig 1**. It is noted that the bulky side chain of the ligand molecule is surrounded with many hydrophobic residues and thus produces hydrophobic interactions between the ligand and target protein. This compound binds to the target protein with a docking score of -12.42 Kcal/mol. The interaction mode of this compound reveals that, inhibition activity against EGFR is through a combination of hydrogen bonds as well as hydrophobic interactions with the residues located around the catalytic active site. In this, three hydrogen bonds were formed such as oxygen attached with pyridopyrimidine moiety and the ATP binding pocket hinge region hydrophobic residue Met (793), NH group of methoxy aniline with DFG motif residue Asp (855) and ring nitrogen with Lys (745).

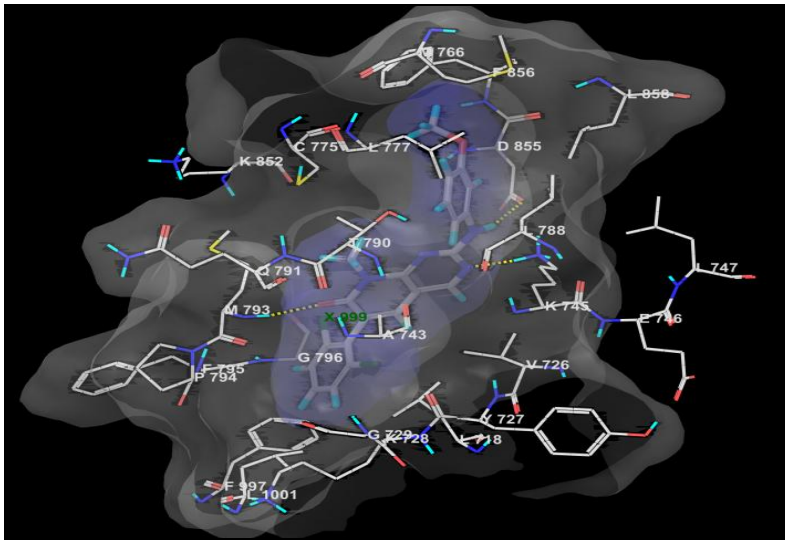
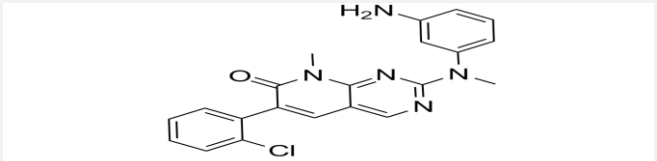
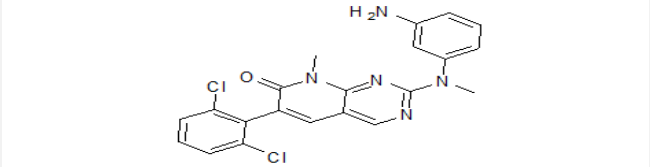
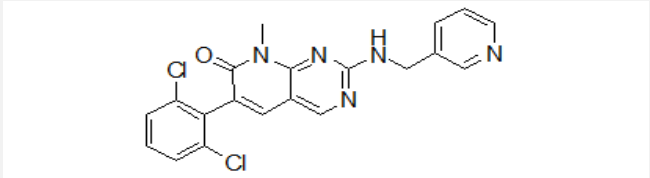
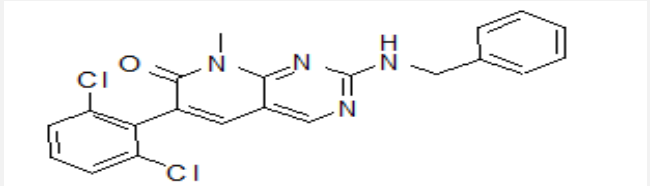
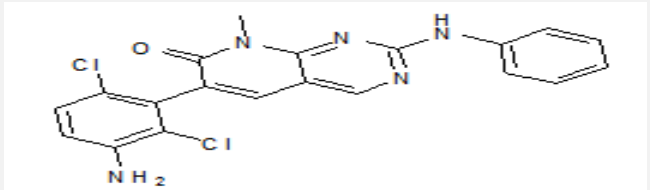
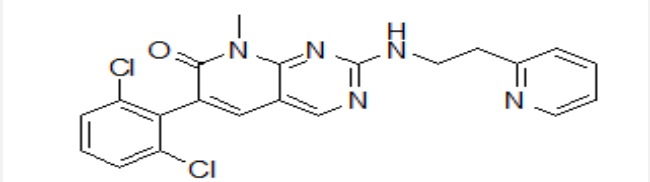


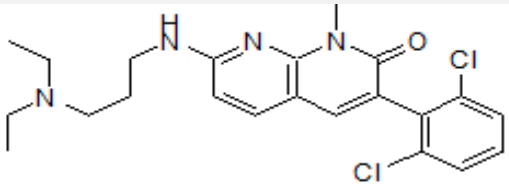
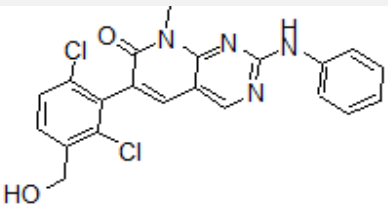
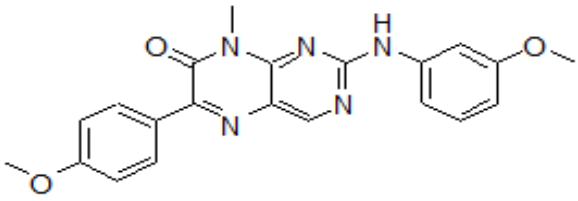
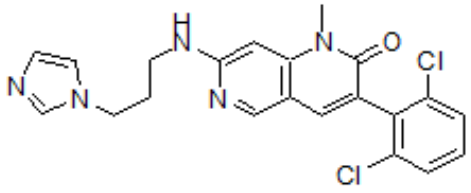
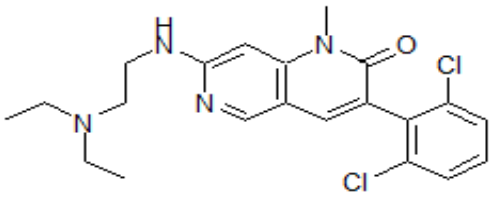
FIG. 1: BINDING OF 6 – (2, 6 – DICHLOROPHENYL) – 2- (3 – METHOXYANILINO) – 8 –METHYL PYRIDO [2, 3 –D] PYRIMIDINE – 7- ONE WITH EGFR KINASE DOMAIN. HYDROGEN BONDS WITH AMINO ACID RESIDUE MET 793, ASP 855 AND LYS 745 ARE REPRESENTED AS DOTTED LINE.

Thorough analysis of the interaction mode, docking scores and the drug like properties of all the 117 compounds were carried out. Results from this analysis reveal that, some compounds did not produce reasonable docking poses as well as not interacting with important active site residues such as Met 793, Asp 855 and Lys 745 and were eliminated.

Finally, eleven compounds consist of seven pyridopyrimidine, three napthyridine and one pteridine derivatives were predicted as potential inhibitors of EGFR. The Pubchem CID, structure, IUPAC name, and docking scores for all these selected ligands are given in **Table 2**.

TABLE 2: PUBCHEM CID, STRUCTURE AND IUPAC NAME AND DOCKING SCORES OF SELECTED ELEVEN COMPOUNDS.

S.no	Pubchem CID	Structure and IUPAC name	Docking scores (kcal/mol)
1.	49865422	 2-[(3-aminophenyl)methylamino]-6-(2-chlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7-one	-10.346
2.	46934425	 2-[(3-aminophenyl)methylamino]-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7-one	-9.532
3.	5327882	 6-(2,6-dichlorophenyl)-8-methyl-2-(pyridin-3-ylmethylamino) pyrido[2,3-d]pyrimidin-7-one	-9.163
4.	5327874	 2-(benzylamino)-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7-one	-9.029
5.	44390969	 6-(3-amino-2,6-dichlorophenyl)-2-anilino-8-methylpyrido[2,3-d]pyrimidin-7-one	-8.696
6.	5327883	 6-(2,6-dichlorophenyl)-8-methyl-2-(2-pyridin-2-ylethylamino)pyrido[2,3-d]pyrimidin-7-one	-8.637

7.	5328727	 3-(2,6-dichlorophenyl)-7-[3-(diethylamino) propylamino]-1-methyl-1,8-naphthyridin-2-one	-8.538
8.	44390951	 2-anilino-6-[2,6-dichloro-3-(hydroxymethyl)phenyl]-8-methylpyrido[2,3-d]pyrimidin-7-one	-8.472
9.	3233262	 2-(3-methoxyanilino)-6-(4-methoxyphenyl)-8-methylpteridin-7-one	-8.409
10.	5328712	 3-(2,6-dichlorophenyl)-7-(3-imidazol-1-ylpropylamino)-1-methyl-1,6-naphthyridin-2-on	-8.375
11.	5328703	 3-(2,6-dichlorophenyl)-7-[2-(diethylamino)ethylamino]-1-methyl-1,6-naphthyridin-2-one	-8.061

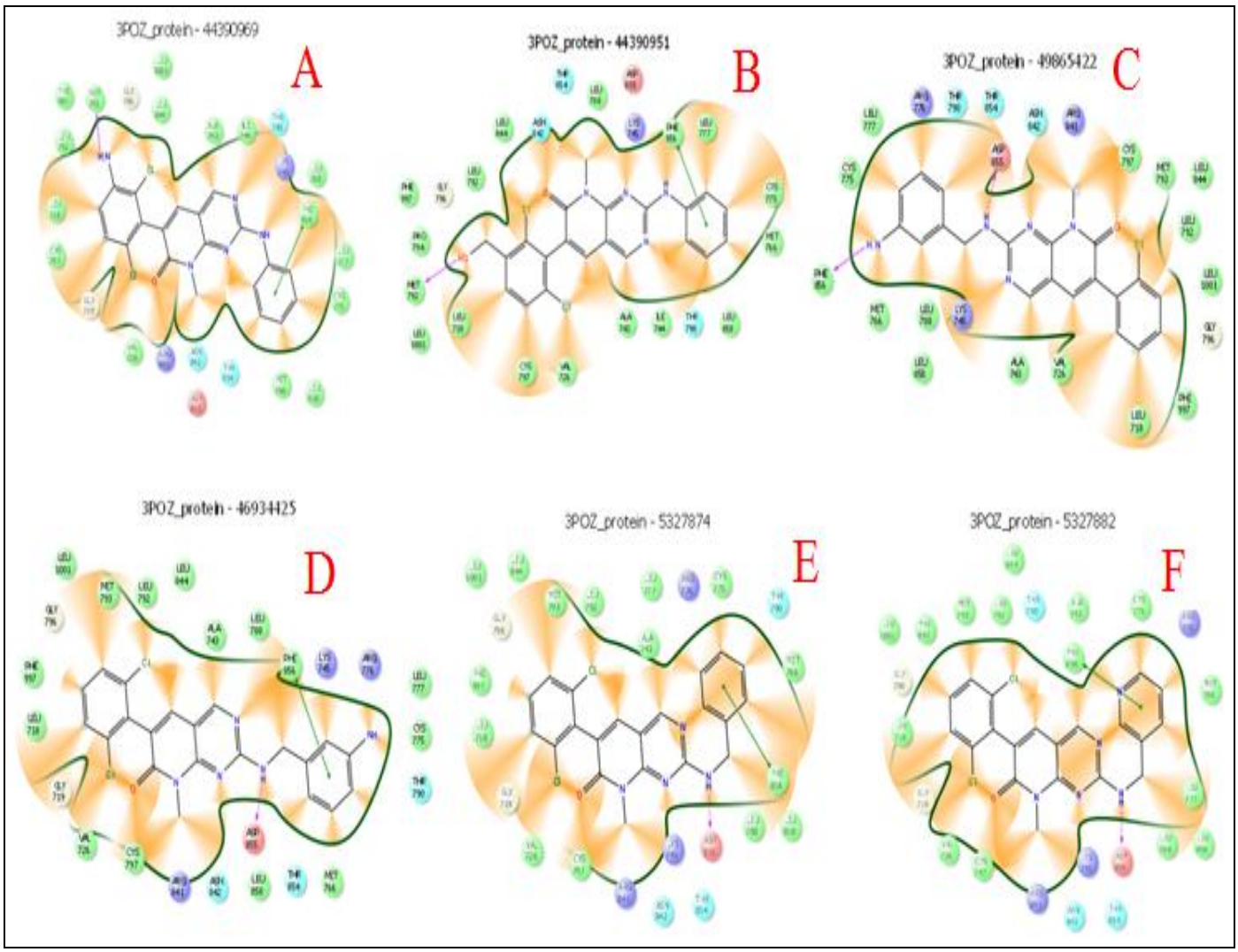
Analyzing the docking models, it is noticed that the pyridopyrimidine derivatives 44390969 and 44390951 forms direct hydrogen bond with hinge region hydrophobic residue of Met 793 through the amino and hydroxyl group respectively. In addition with this interaction, they also forms pi – pi interaction through the aromatic ring with Phe 856 of DFG motif residue (**Fig. 2A, B**).

Similarly, two hydrogen bonds were observed in the binding of another pyridopyrimidine analog 49865422 to EGFR. One direct hydrogen bond is between the phenyl amino group of the ligand and Phe 856 of the target protein. The other side chain hydrogen bond is formed between the methyl amino group of the ligand and Asp 855 of the target residue (**Fig. 2C).**

Binding mode analysis of 46934425, 5327874, 5327882, 5327883 (pyridopyridine analogs), 5328712 & 5328727 (naphtyridin analogs) compounds with the target EGFR reveals that all the compounds form hydrogen bond through methyl amino group of the ligand with DFG motif residue of Asp 855. Another DFG motif Phe 856 also involved in the pi – pi interaction with different aromatic rings present in the ligand molecules.

For example, in 46934425 the amino phenyl ring is involved in the pi – pi interaction (**Fig. 2D**) where as 5327874, 5327882 & 5327883 compounds forms pi – pi interaction through the phenyl ring (**Fig. 2E, F** and **G**). In case of 5328712, the interaction is mediated through the naphthyridin aromatic ring (Fig 2H). In 5328727, instead of pi – pi interaction, pi – cation interaction was observed and it is mediated through diethyl amino group of the ligand (**Fig. 2I).**

Interestingly, it is noticed that in 5328703 (naphthyridin analog), the additional hydrogen bond is between Thr 854 and diethyl amino group of the ligand. The same group also produces cation – pi interaction with DFG motif residue Phe 856. The ethyl amino attached with naphthyridin nucleus forms side chain hydrogen bond with another DFG motif residue Asp 855 (**Fig. 2J**). In pteridine analog, one hydrogen bond is between amino group of methoxy aniline and DFG motif residue Asp 855 and the other hydrogen bond is between the nitrogen of pteridine nucleus and Thr 854 (**Fig. 2K**). Different types of interactions produced by catalytically active site residues of EGFR along with their position are given in **Table 3.**



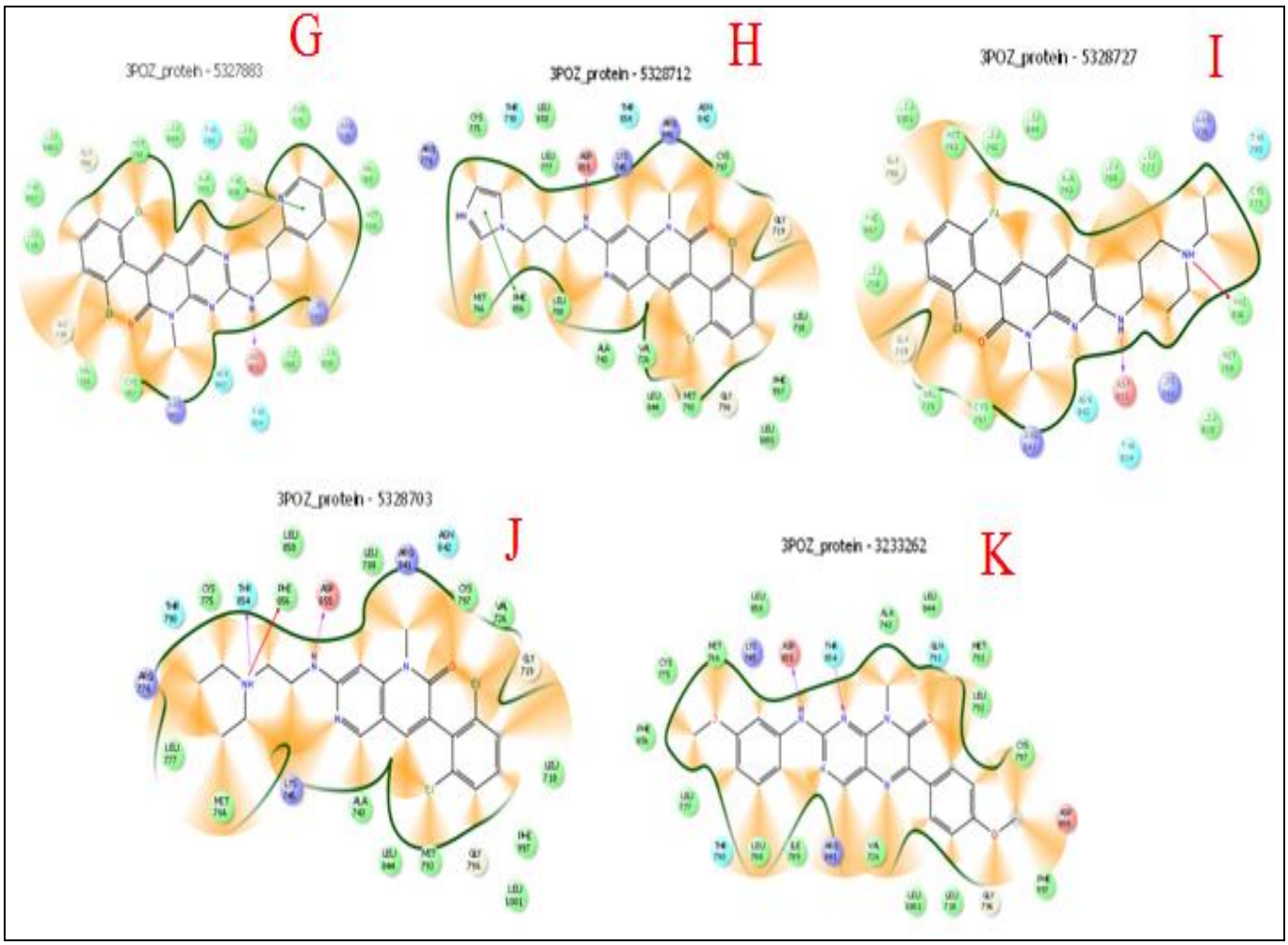


FIG. 2: BINDING POSE OF HIT COMPOUNDS IN EGFR USING LIGINT TOOL FROM MAESTRO. (A) 6 – (3 – amino – 2, 6 – dichlorophenyl) – 2 – anilino – 8 – methyl pyrido [2, 3 –d] pyrimidine -7 – one (Pubchem CID: 44390969), (B) 2 – anilino – 6 – [2, 6 – dichloro – 3 – (hydroxymethyl) phenyl] – 8 – methylpyrido [2, 3 – d] pyrimidin – 7 – one (Pubchem CID: 44390951), (C) 2 – [(3 – aminophenyl) methylamino] -6 – (2 – chlorophenyl) – 8 – methylpyrido [2, 3 – d] pyrimidin – 7 – one (Pubchem CID: 49865422), (D) 2 – [(3 – aminophenyl) methylamino] – 6- (2, 6 – dichlorophenyl) – 8 – methyl pyrido [2, 3 – d] pyrimidin -7 – one (Pubchem CID: 46934425), (E) 2 – (benzylamino) – 6 – (2, 6 – dichlorophenyl) – 8 – methylpyrido [2, 3 – d] pyrimidin – 7- one (Pubchem CID: 5327874), (F) 6 – (2, 6 – dichlorophenyl) – 8 – methyl – 2 – (pyridine – 3-ylmethylamino) pyrido [2, 3 – d] pyrimidin – 7 – one (Pubchem CID: 5327882), (G) 6 – (2, 6 – dichlorophenyl) -8 –methyl – 2- (2 – pyridine – 2-ylethylamino) pyrido [2, 3 – d] pyrimidin – 7 – one (Pubchem CID: 5327883), (H) 3 – (2, 6 – dichlorophenyl) – 7 – (3 – imidazol – 1-ylpropylamino) – 1-methyl – 1, 6 – naphthyridin – 2 –one (Pubchem CID: 5328712), (I) 3 – (2, 6 – dichlorophenyl) – 7- [3 – (diethylamino) propylamino] -1- methyl – 1, 8 – naphthyridin – 2 – one (Pubchem CID: 5328727), (J) 3 – (2, 6 – dichlorophenyl) – 7 – [2 – (diethylamino) ethylamino] -1-methyl – 1, 6 – naphthyridin – 2 – one (Pubchem CID: 5328703) & (K) 2 – (3 – methoxyanilino) – 6 – (4 – methoxyphenyl) – 8 – methylpteridin – 7- one (Pubchem CID: 3233262). Hydrogen bonds are represented by solid pink color, pi –pi bond are in green color solid line and pi – cation bond are in solid line with red color.

TABLE 3: LIGAND IUPAC NAME AND THEIR INTERACTIONS

S. No	Ligand IUPAC name	Residues involved and type of interactions		
		Hydrogen bond	pi – pi interactions	pi - cation
1.	6 – (3 – amino – 2, 6 – dichlorophenyl) – 2 – anilino – 8 – methyl pyrido [2, 3 –d] pyrimidine -7 – one	Met 793	Phe 856	
2.	2 – anilino – 6 – [2, 6 – dichloro – 3 – (hydroxymethyl) phenyl] – 8 – methylpyrido [2, 3 – d] pyrimidin – 7 – one	Met 793	Phe 856	
3.	2 – [(3 – aminophenyl) methylamino] -6 – (2 – chlorophenyl) – 8 – methylpyrido [2, 3 – d] pyrimidin – 7 – one	Phe 856, Asp 855		
4.	2 – [(3 – aminophenyl) methylamino] – 6- (2, 6 – dichlorophenyl) – 8 – methyl pyrido [2, 3 – d] pyrimidin -7 – one	Asp 855	Phe 856	
5.	2 – (benzylamino) – 6 – (2, 6 – dichlorophenyl) – 8 – methylpyrido [2, 3 – d] pyrimidin – 7- one	Asp 855	Phe 856	
6.	6 – (2, 6 – dichlorophenyl) – 8 – methyl – 2 – (pyridine – 3-	Asp 855	Phe 856	

	ylmethylamino) pyrido [2, 3 – d] pyrimidin – 7 – one			
7.	6 – (2, 6 – dichlorophenyl) -8 –methyl – 2- (2 – pyridine – 2-ylethylamino) pyrido [2, 3 – d] pyrimidin – 7 – one	Asp 855	Phe 856	
8.	3 – (2, 6 – dichlorophenyl) – 7 – (3 – imidazol – 1-ylpropylamino) – 1-methyl – 1, 6 – naphthyridin – 2 –one	Asp 855	Phe 856	
9.	3 – (2, 6 – dichlorophenyl) – 7- [3 – (diethylamino) propylamino] -1-methyl – 1, 8 – naphthyridin – 2 – one	Asp 855		Phe 856
10.	3 – (2, 6 – dichlorophenyl) – 7 – [2 – (diethylamino) ethylamino] -1-methyl – 1, 6 – naphthyridin – 2 – one	Asp 855, Thr 854		Phe 856
11.	2 – (3 – methoxyanilino) – 6 – (4 – methoxyphenyl) – 8 – methylpteridin – 7- one	Asp 855, Thr 854		

Analysis of drug like profile of these compounds reveals that the molecular weight, donors, acceptors and octanol / water partition coefficient (solubility) are within the acceptable ranges. Similarly, the absorption properties for all the compounds are 100%. Further, the results show that the compounds have very low Blood Brain Barrier permeability property (**Table 4**).

TABLE 4: COMPOUND CID AND THEIR OBTAINED DRUG LIKENESS PROFILE

Compound Pubchem CID	mol_MW (<500)	Donor HB (< 5)	Accpt HB (< 10)	QPlogP o/w (< 5)	Rule of five Com pliance	Percent Human Oral Absorption (> 80 high, < 25 poor)	QPlog BB (–3 to 1.2)
49865422	391.859	2.56	6.5	3.652	0	100	-0.850
46934425	426.304	2.5	6.5	4.011	0	100	-0.732
5327882	412.277	1	7	3.970	0	100	-0.268
5327874	411.29	1	5.5	4.989	0	100	0.005
44390969	412.277	2.5	6.5	3.677	0	100	-0.702
5327883	426.304	1	6.5	4.733	0	100	-0.159
5328727	433.38	1	6.5	4.696	0	100	0.157
44390951	427.289	2	7.2	3.742	0	100	-0.750
3233262	389.413	1	8.0	3.320	0	100	-0.661
5328712	428.32	1	6.5	4.431	0	100	-0.509
5328703	419.353	1	6.5	4.514	0	100	-0.284

CONCLUSION: Targeting the inhibition of misregulated Epidermal Growth factor Receptor (EGFR) is considered to be an important task in anticancer research. Several drugs targeting both extracellular ad intracellular regions are under clinical development. Especially, developing molecules for oral chemotherapy is a challenging task for clinicians. The structural studies on the mechanism of inhibition of this receptor by small molecule inhibitor provide molecular determinants for the improved and specific inhibition activity of new anticancer drugs. The insights obtained from this study reveal that the hydrophobic residues Met 793, Thr 854, negatively charged residue Asp 855 and Phe 856 play an important role in the inhibition activity of EGFR.

With this knowledge, we identified eleven inhibitors of EGFR by virtual screening and docking analysis. All the selected eleven compounds bind with the important target catalytic active site residues Met 793, Asp 855, Phe 856 and Thr 854. The bioactivity profiles of these compounds are also analyzed which reveal that the compounds are having good drug like profile. Hence these compounds may be used as lead compounds to design novel EGFR inhibitors.

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