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MOLECULAR-INTERACTION STUDIES OF PYRIDINE DERIVATIVES AGAINST EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TARGETING LUNG CANCER

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ABSTRACT: Lung cancer has the highest mortality rate of all cancers, and is the second most diagnosed cancer in both men and women, behind prostate and breast cancer, respectively. It has been reported that more than 1.6 million cases are diagnosed each year along with 1.3 million deaths. Approximately 85%–90% of lung cancer cases are caused by voluntary or involuntary (second hand) cigarette smoking. Lung cancer is mainly divided into two classes, which are non-small cell lung cancer (NSCLC, ~85%) and small cell lung cancer (SCLC, ~15%), according to biology therapy and prognosis. NSCLC could be further divided into squamous cell carcinoma (SCC), adenocarcinoma and large cell lung carcinoma (LCLC). In the present study of protein-Ligand interactions play a key role in structure-based drug design, so by using molecular docking simulation carried out by synthesized 4-(2-fluorophenoxy)-3, 30-bipyridine derivatives investigated their binding affinity against epidermal growth factor receptor. The three dimensional (3D) structure of EGFR was retrieved from Protein Data Bank (PDB ID: 1M17) and docked with synthesized 4-(2-fluorophenoxy)-3, 30-bipyridine derivatives using Glide package (Schrödinger-2014-2). Molecular docking and ADMET properties while Lipinski's rule of five was performed for these synthesized compounds to evaluate their anti-cancer activity. The molecular docking results showed that all compounds having a good binding affinity with active sites amino acids among them 24b exhibited better binding affinity of −9.68 kcal/ mol compared with cocrystal native ligand. The results reveal that these synthesized compounds could be promising candidates for further to treat anti-lung cancer.

KEYWORDS: 4-(2-fluorophenoxy)-3, 30-bipyridine, Molecular docking, ADME, EGFR.

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1. INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the United States. Patients treated with adjuvant chemotherapy have a 5-year survival rate of 25% to 70% depending on the stage, whereas those with advanced disease have a median survival of approximately 8 months when treated with standard platinum-based therapy[1]. While chemotherapy provides useful palliation, advanced lung cancer remains incurable since those tumors that are initially sensitive to therapy rapidly develop acquired resistance. Resistance may arise from impaired drug delivery, extracellular factors, decreased drug uptake into tumor cells, increased drug efflux, drug inactivation by detoxifying factors, decreased drug activation or binding to target, altered target, increased damage repair, tolerance of damage, decreased proapoptotic factors, increased antiapoptotic factors, or altered cell cycling or transcription factors[2]. Epidermal growth factor receptor (EGFR)-mutant non-small-cell lung cancer (NSCLC) was first recognized in 2004 as a distinct, clinically relevant molecular subset of lung cancer[3]. The disease has been the subject of intensive research at both the basic scientific and clinical levels, becoming a paradigm for how to understand and treat oncogene-driven carcinomas. Although patients with EGFR-mutant tumors have increased sensitivity to tyrosine kinase inhibitors (TKIs), primary and acquired resistance to these agents remains a major clinical problem[4]. NSCLC inhibit oncogenic receptor tyrosine kinase pathways, such as the epidermal growth factor receptor (EGFR) pathway. While current EGFR-targeted agents, including erlotinib and gefitinib[5], may result in dramatic responses, a fraction of patients and resistance to these agents frequently develops. In order to select patients most likely to benefit from the blockade of EGFR pathways, investigators have focused on identifying molecular correlates of response to anti-EGFR therapy. New strategies to minimize the risk of resistance to EGFR inhibition have been employed in the development of next-generation EGFR tyrosine kinase inhibitors, such as PF00299804 and BIBW 2992; these include irreversibility of target binding, inhibition of multiple EGFR family receptors, and/or simultaneous inhibition of EGFR and other oncogenic pathways[6]. Over the past decade, a multitude of targeted agents has been explored in the treatment of advanced non-small cell lung cancer (NSCLC)[7]. Thus far, two broad classes of agents have been implemented in clinical practice: (1) vascular endothelial growth factor (VEGF)-directed therapies and (2) antagonists of the epidermal growth factor receptor (EGFR)[8]. The HER2 (ErbB2/neu) protein is a member of the HER (ErbB) receptor family (EGFR, HER2, HER3, and HER4) that expresses tyrosine kinase activity in the intracellular

domain. EGFR and HER2 overexpression are observed in numerous types of cancer, nevertheless, the susceptibility of patients with non-small cell lung cancer (NSCLC) to therapy with EGFR and HER2 tyrosine kinase inhibitors (TKIs) depends on mutations present in the respective coding genes[9] (driver mutations) (Figure:1).

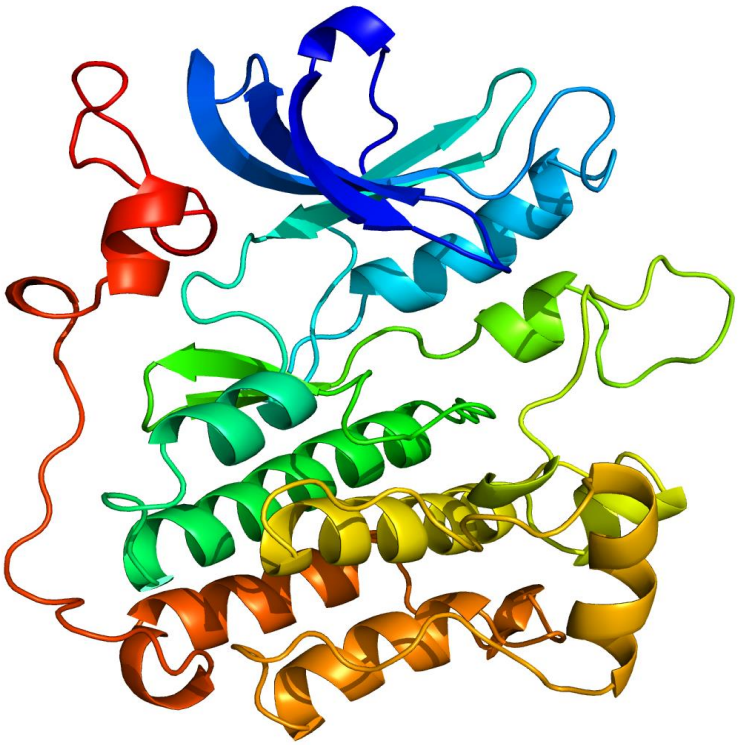

Figure 1: Crystal structure of human Epidermal growth factor receptor (Apo form)

Recent studies suggest the existence of two distinct molecular pathways in the carcinogenesis of lung adenocarcinoma: one associated with smoking and activation of the K-Ras oncogene and the other not associated with smoking and activation of the epidermal growth factor receptor (EGFR)[10]. Drugs targeting the epidermal growth factor receptor, anaplastic lymphoma kinase[11], and vascular endothelial growth factor[12] are now U.S. Food and Drug Administration approved for the treatment of advanced non-small cell lung cancer[13]. Currently, detection of the presence in NSCLC of mutations involving the epidermal growth factor receptor (EGFR) gene and fusion of the N-terminal portion of the protein encoded by EML4 (echinoderm microtubule-associated protein-like 4 gene) with the intracellular signaling portion of the receptor tyrosine kinase encoded by ALK (anaplastic lymphoma kinase gene)—that is, EML4–ALK—and variants has become routine in many centres because patients having tumours harbouring such alterations might benefit from tyrosine kinase inhibitors as part of their treatment regimen[14]. In addition, targeted kinase inhibitors in clinical development for other specific molecular subtypes of NSCLC are covered, including ROS1, BRAF, RET, HER2, KRAS (upstream of the MEK kinase), MET, PIK3CA,

FGFR1, DDR2, VEGFR, and AAK. Expert opinion: In EGFR-mutant NSCLC, there are several kinase inhibitors with promising activity, most notably dacomitinib and CO-1686 in tumors with acquired resistance to EGFR-targeted therapy[15]. In this present study, molecular docking[16] simulation studies were carried out synthesized 4-(2-fluorophenoxy)-3, 30-bipyridine derivatives and cocrystal native ligands[17]. As well as ADME properties also have been carried out.

2. MATERIALS AND METHODS

2.1 Dataset preparation

The synthesized anticancer compounds 4-(2-fluorophenoxy)-3, 30-bipyridine derivatives were retrieved from recent literature. This literature study was showed the synthesis of bipyridine compounds against four cancer cell lines (HT-29, A549, MKN-45, and MDA-MB-231) in vitro/ vivo studies[18].

2.2 Protein preparation

For the molecular docking study were carried out in several anticancer drug target epidermal growth factor receptor (PDB ID: 1M17). Missing hydrogen atoms were added and correct bond orders were assigned, and then formal charges and orientation of various groups were fixed. Following this, optimization of the amino acid orientation of hydroxyl groups, amide groups were carried out. All amino acid flips were assigned and H-bonds were optimized. Nonhydrogen atoms were minimized until the average root mean square deviation reached default value of 0.3 Å. Sitemap 2.3 was used to explore the binding site in the docking studies[19].

2.3 Ligand preparation

Synthesized 4-(2-fluorophenoxy)-3, 30-bipyridine derivatives compound was built using builder panel in Maestro. The compounds were taken for ligand preparation by Ligprep 2.3 module (Schrödinger, USA) which performs addition of hydrogen, 2D to 3D conversion, realistic bond lengths and bond angles, low energy structure with correct chiralities, ionization states, tautomer's, stereochemistries and ring conformations.

2.4 Receptor Grid Generation

In the Receptor Grid Generation, the receptor structure was defined by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids. Ligand docking jobs cannot be performed until the receptor grids have been generated. So a "prepared" structure was used for receptor grid generation.

2.5 Induced fit docking

Induced fit docking (IFD) is one of the main complicating factors in docking studies which predicts accurate ligand-binding modes and concomitant structural movements in the receptor using Glide and Prime modules. In IFD, when a ligand binds to the receptor, it undergoes side chain or backbone conformational changes or both in many proteins. These conformational changes allow the receptor for better binding according to the shape and binding mode of the

ligand[20]. Here, the prepared protein was loaded into the workspace and the sitemap predicted active site was specified for IFD. The grid was calculated about 20 Å to cover all the active site residues defined by the sitemap. The van der Waal's radii of nonpolar receptor and ligand atoms were scaled by a default factor of 0.50. IFD calculations were carried out for synthesis 4-(2-fluorophenoxy)-3,30-bipyridine derivatives with anticancer drug target epidermal growth factor receptor. Following this, 20 conformational poses were calculated where the best conformational pose was selected based on the docking score, glide energy, hydrogen bonding, and hydrophobic bonding interactions.

2.6 Docking method validation

The ligand was docked into the native protein to determine the ability of a Glide docking program to reproduce the orientation and position of the ligand observed in the crystal structure. The docked conformations as calculated Root mean square deviation (RMSD) for with respect to crystal and docked compound using binding superposition studies using chimera 1.11[21].

2.7 ADME properties

Synthesized4-(2-fluorophenoxy)-3, 30-bipyridine series compounds of drug-likeness was determined based on "Lipinski's Rule of Five". ADME and Toxicity studies were considered by taking the parameters as mentioned below. We have analyzed various physiochemical descriptors and pharmaceutically significant properties of 4-(2-fluorophenoxy)-3, 30-bipyridine compounds using QikProp v3.0 tool[22] of Schrodinger software.

2.8 Prime MM-GBSA Free energy calculations

In order to predict the binding mode and free energy for the best-docked complex leads as obtained from Docking, Prime/Molecular Mechanics Generalized Born Surface Area (Prime/MM-GBSA) calculations were applied, which also substantiated to have profound inhibitory synthesized compounds against epidermal growth factor receptor. The Prime/MM-GBSA[23] method based on the docking complex was used to calculate the binding free energy (ΔGbind) of each ligand, using the following equation[24].

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{SOL} + \Delta G_{SA}$$

3. RESULTS AND DISCUSSION

In this molecular docking results of synthesized 4-(2-fluorophenoxy)-3,30- bipyridine derivatives, all compounds were showed better binding affinity with the anticancer drug target protein epidermal growth factor receptor. Two-dimensional scheme of bipyridine derivatives was shown in (Figure: 2).

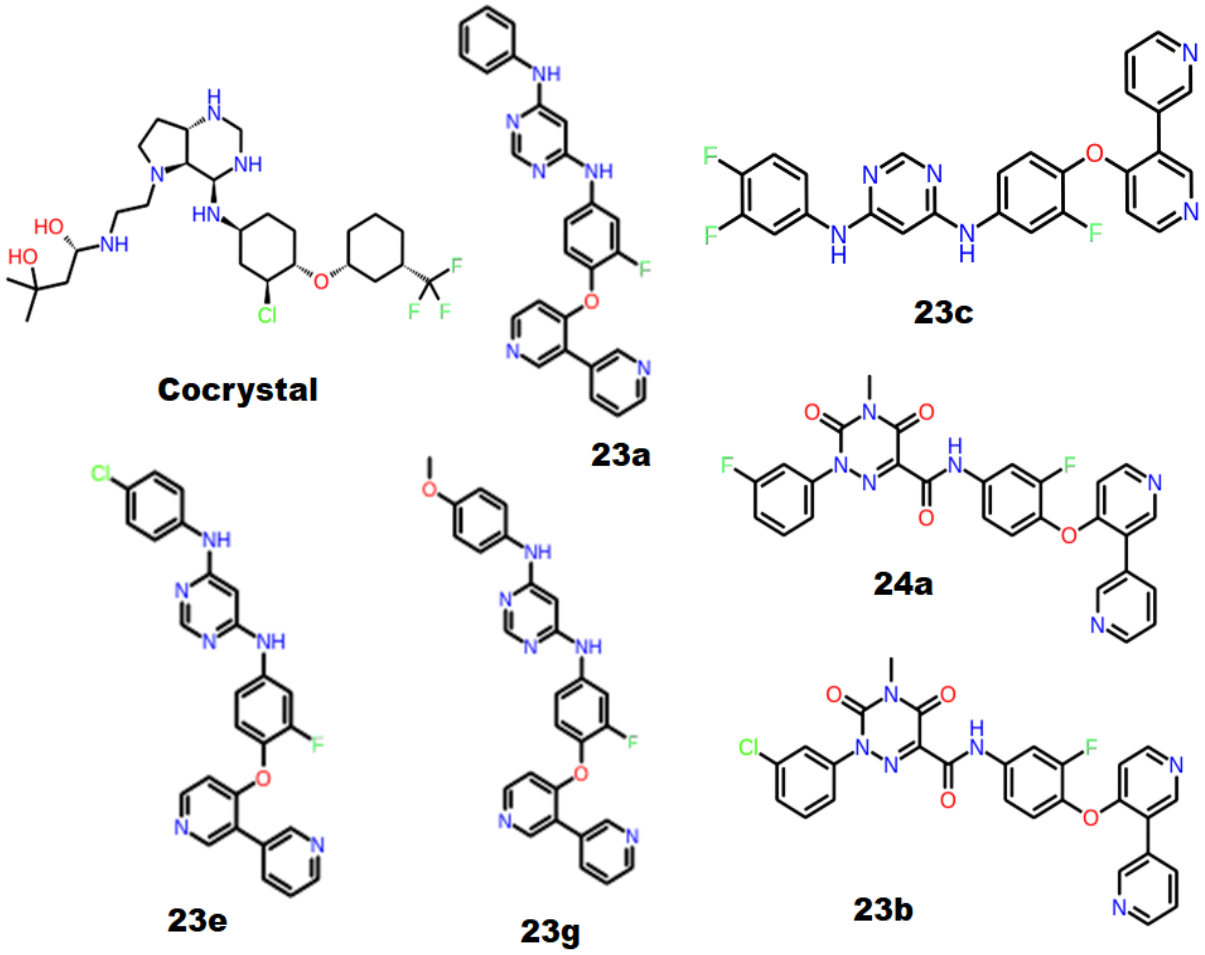

Figure: 2 Two dimensional representation of cocrystal and bi-pyridine analogs

This protein has important active site regions such as Met769, Gln767, Leu694, Ala719, Lys721, Leu764, Thr766, Leu768, Pro770, Phe771, Gly772, Leu820, Thr830, and Asp831. The result from docking native ligand of cocrystal (4-anilinoquinazoline erlotinib) compound has three hydrogen interaction with active site amino acids (crystal the amino group interact with the oxygen atom of Asp855 at a distance of 2.4 Å. Similarly tri methyl oxygen atoms directly interact with oxygen atom of Leu718 at a distance of 2.0Å in addition to that amide group (NH_2) of Cys 797 made hydrogen bond with oxygen atom of crystal compound at a distance of 2.1Å) as well as it has several hydrophobic interactions such as Leu844, Ala843, Val726, Ala722, and Leu799. Although cocrystal anilinoquinazoline docking score and glide energy were found to be -8.95 and -60.86 kcal/mole. Bipyridine derivatives all compounds among them **24b** shows better docking score and glide energy as well as interaction profiles also. Bipyridine 24b has two hydrogen bond interactions on active site of EGFR target protein. Hydrogen bond interaction was found to be an NH2 group of Asp855 interact with amino group Nitrogen atom of 24b at a distance of 2.3 Å similarly Thr854 methoxy oxygen atom interact with Oxygen atom of the compound 24b at a distance of 2.0 Å (Figure: 3). 24b compound has several hydrophobic interactions such as Met766, Cys755, Leu777, Leu778, Cys797, Leu792, Met793, Ala743, Ile748, and Phe997 although 24b

docking score and glide energy were found to be -9.68 and -73.46 kcal/mole.

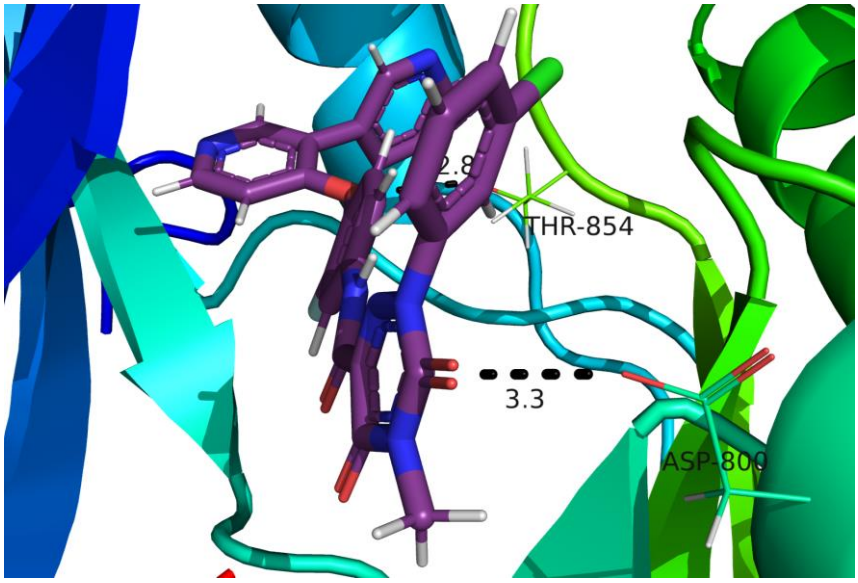

Figure 3: Representation of compound 24b interaction for pymol view

Second best compound for bipyridine derivatives 24a. The 24a compound having three hydrogen bond interactions with active site regions (NH_2 Nitrogen atom Asp855 directly interact with benzo nitrogen group of 24a compound at a distance of 2.3 Å in addition to that Thr854 oxygen atom were made hydrogen bond with oxygen atom of the compound 24a at a distance of 2.0 Å most strong interaction of Lys745 amide nitrogen atom interact with oxygen atom of 24a compound at a distance of 2.0 Å in terms of hydrophobic interaction also were observed such as Val726, Ile744, Met766, Cys797, Phe997, Ile789 and Ile744. 24a compound docking score and glide energy were found to be -9.60 and -73.59 kcal/mole. Compound 23a also having better binding affinity against EGFR drug target protein. It is having two major hydrogen bond interaction of important functional residue of the protein. The 23a compound nitrogen atom directly interact with the methoxy oxygen atom of the Asp 855 at a distance of 2.0 Å additionally one more hydrogen bond for Cys 747 Amide group was making interaction with the 23a compound benzo nitro group at a distance of 2.2Å as well as various hydrophobic interaction were observed like Ala722, Leu844, Cys775, Met766, Leu777, Leu858, Leu788, Ile789 and Ala743. 23a compound docking score and glide energy were found to be -8.76 and -72.06 kcal/mole. The compound 23g has good binding interaction with the particular catalytic sites. This 23g compound was having three hydrogen bond interactions such as Cys775 nitrogen atom were made interaction with nitrogen group of compound 23g at a distance of 2.3Å as well as Compound 23g has hydrogen bond with nitrogen atom interact with oxygen atom of Asp855 at a distance of 2.1Å similarly Ser720 nitrogen atom made interaction with the amide group of NH2 nitrogen atom of the compound 23g at a distance of 2.5 Å although it's having several hydrophobic interactions viz.. Phe997, Ala722, Leu833, Phe856, Met766, Cys755, Ala743 and Ile744. 23g compound docking score and glide energy were

found to be -8.91and -71.16 kcal/mole. Compound 23c also having good binding affinity and two hydrogen bond interaction with the catalytic sites. The nitrogen group of compound 23c interacts with the oxygen atom of Asp855 at a distance of 2.0Å similarly Cys797 amide group interacts with the benzo amide nitrogen group of compound 23c at a distance of 2.7 Å. The hydrophobic interaction was found Leu718, Leu844, Val726, Leu777, Ala743, Leu788, Leu858 and Met766. Compound 23c docking score and glide energy were found to be -9.45 and -69.88 kcal/mole. The least binding affinity in the bipyridine in the series is compound 23e. It's having similar hydrogen bond interaction for compound 23c. Although all compounds having common hydrogen bond interaction of important active site amino acid **Asp855.** This all compounds and cocrystal interaction profile was given in (Figure: 4). These molecular docking results suggest that all bipyridine derivatives were having a good binding affinity as well as in terms of scoring (Table: 1) comparing with known erlotinib anti-cancer inhibitor. The ADME toxicity profiles also were employed all synthesized compounds its shows that obey the Lipinski rule of five (Table: 2).

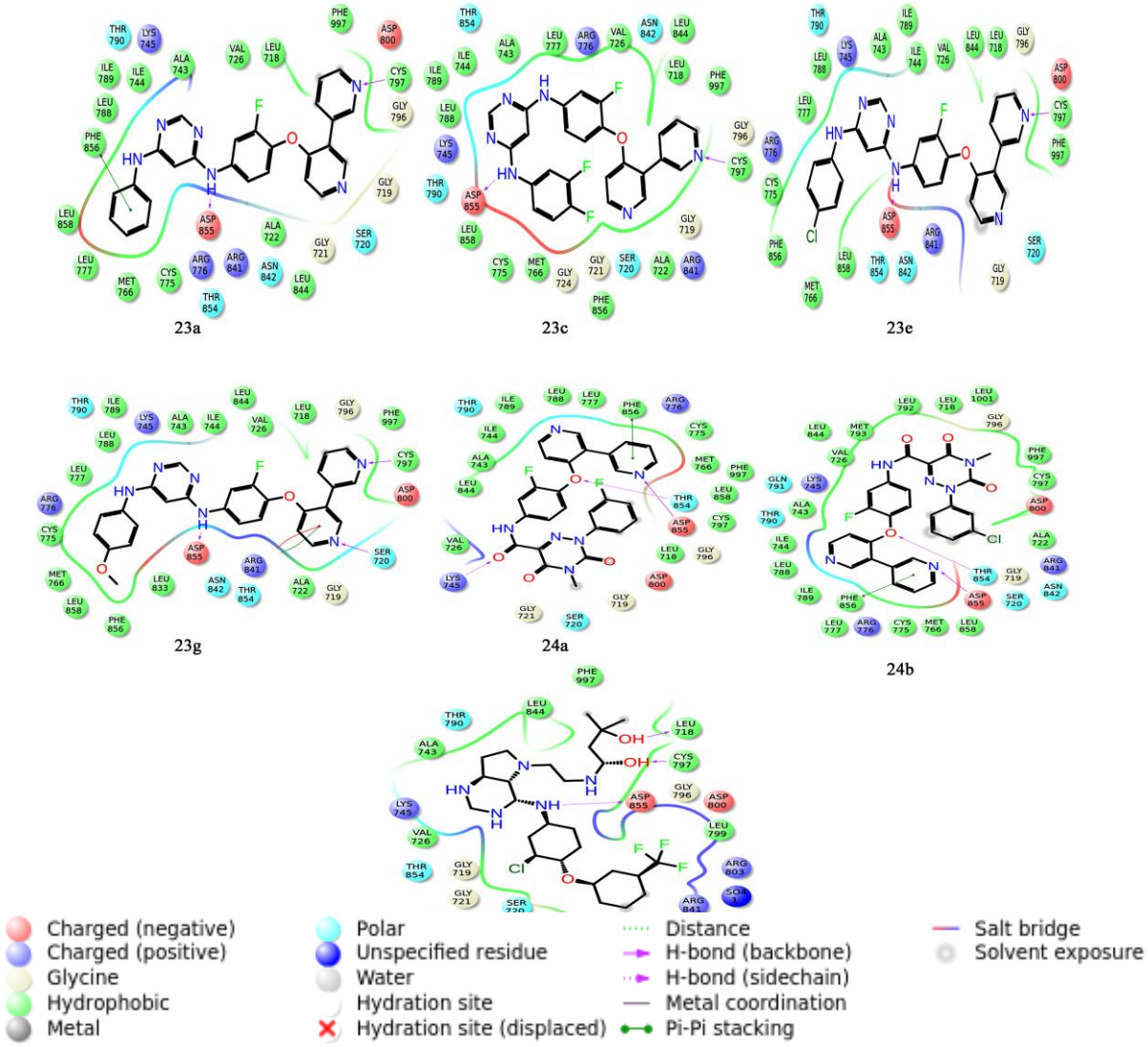

Figure 4: Ligand interaction of synthesized Bipyridine derivatives against EGFR

Table: 1 24b seems better by interactions as well as glide energy and score. Asp855 is catalytic residue and Cys797 is a member of the active site

Compounds	Docking score (Kcal/mol)	Glide energy (Kcal/mol)	Hydrogen bond interactions	Hydrophobic interactions
23a	-8.76	-72.06	**Asp855,** Cys797.	Ala722, Leu844, Cys775, Met766, Leu777, Leu858, Leu788, Ile789, Ala743.
23c	-9.45	-69.88	**Asp855,** Cys797.	Leu718, Leu844, Val726, Leu777, Ala743, Leu788, Leu858, Met766.
23e	- 7.97	-67.80	**Asp855,** Cys797.	Leu858, Met766, Cys755, Ala745, Val726, Leu718.
23g	-8.91	-71.16	**Asp855,** Cys797, Ser720.	Phe997, Ala722, Leu833, Phe856, Met766, Cys755, Ala743, Ile744.
24a	-9.60	-73.59	**Asp855**, Thr854, Lys745.	Val726, Ile744, Met766, Cys797, Phe997, Ile789, Ile744.
24b	**-9.68**	**-73.46**	Thr854, **Asp855**.	Met766, Cys755, Leu777, Leu778, Cys797, Leu792, Met793, Ala743, Ile748, Phe997.
Cocrystal	-8.95	-60.86	**Asp855**, Cys797, Leu718.	Leu844, Ala843, Val726, Ala722, Leu799,

3.1 Docking Method validation

The docking method validation is most important for drug designing research area. Here we had done molecular docking studies epidermal growth factor receptor native crystal structure inhibitor. From that docking result, we took on specific docked conformation and simultaneously took the crystal structure conformation from protein data bank. Thus both docked and crystal structure conformation poses should me superposition analysis has been carried out (Figure: 4). The superposition analysis both structural conformations should be similar as well as ligand binding with the same orientation at the active sites of the target protein[25]. Superposition analysis calculated the RMSD structural deviation for both conformations. The overall structural deviation is RMSD of 0.140Å.

3.2 ADME properties

All the synthesized compounds were showed significant values for the properties analyzed and

exhibited drug-like characteristics based on Lipinski's rule of 5. The ADME values of newly synthesis designed compounds are given in (Table 2). The first three properties are based on Lipinski's rule of five, molecular weight (mol.MW). All synthesized bi compounds showed ADME properties in an acceptable range.

Table: 2 ADME properties analysis for synthesized compounds and cocrystal

Compounds	mol MW	donorHB	accptHB	QPlogPoct	PSA
Cocrystal	570.137	6	12.15	33.345	101.426
23a	450.474	2	6.5	24.698	72.493
23c	486.455	2	6.5	25.61	72.49
23e	484.919	2	6.5	25.426	72.493
23g	480.5	2	7.25	25.35	80.783
24a	528.474	0	8	24.403	133.425
24b	544.928	0	8	25.088	133.419

3.3 Free Energy calculation

Binding free energy calculation results show that synthesized compound 24b has a good binding energy similar to the cocrystal (erlotinib). As we discussed given in (Table: 3) cocrystal binding free energy of (ΔG_{Total} = -105.28 kcal/mol) although similar best binding free energy was observed synthesized bipyridine 24b compound (ΔG_{Total} = -93.09 kcal/mol)[26]. Vander walls interactions to high 24b compound compared with the co-crystal compound. Results reveal similar binding free energy for best lead compound 24b and cocrystal.

Table 3. Free energy calculations (MM-GBSA) of best synthesized best 24b compound and Cocrystal

Compounds	Solv GB	vdW	Coulomb	Covalent	Hbond	ΔG_{Total}
Cocrystal	30.748	-52.839	-53.016	32.905	-8.076	-105.285
24B	19.955	-74.892	0.561	8.841	3.912	-93.095

4. CONCLUSION

In this present study was carried out molecular docking simulation of synthesized bipyridine series against anticancer drug target of epidermal growth factor in lung cancer. From the docking simulation results suggest that all series of 4-(2-fluorophenoxy)-3, 30- bipyridine derivatives better binding affinity and interaction with important catalytic sites. Among the all compound **24b** has a show high docking score and glide energy in terms of interaction pattern also shows that strong binding affinity. Free energy calculation reveals that **24b** compounds have similar binding free energy compared to the cocrystal native inhibitor. All compounds are accepting the Lipinski's rule of five. Hence this study of molecular docking and free energy calculation showed these

compounds treat as the anti-cancer inhibitor against lung cancer.

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CONFLICT OF INTEREST

No conflict of interest related to the study.

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