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Molecular docking based screening of novel designed chalcone series of compounds for their anti-cancer activity targeting EGFR kinase domain

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Abstract:
Epidermal growth factor receptors (EGFR) are critical for the growth of many tumors and expressed at high levels in about one third of epithelial cancers. Hence, blockade of the binding sites for EGFR has been hypothesized as an effective anti-cancer therapy. Chalcone derivative compounds have been shown to be highly effective anti-cancer agents, however there are still so many novel derivatives possible, one of which might get us the best targeted EGFR inhibitor. In this effort directed towards the discovery of novel, potent anti-tumor agents for the treatment of cancer, in the present study a library of novel chalcone series of compounds has been designed and evaluated for their anti-cancer activity targeting EGFR kinase domain using various computational approaches. Among the twenty five novel designed chalcone series of compounds, all of them have found to be successfully docking inside the active binding domain of EGFR receptor target with a binding energy in a range of -6.10 to -9.25 Kcal/mol with predicted IC50 value range of 33.50 micor molar to 164.66 nano molar respectively. On the other hand, calculated 2DQSAR molecular descriptor properties of the compounds showed promising ADME parameters and found to be well in compliance with Lipinski's rule of five. Among all the twenty five compounds tested, compound 21 ((2E)-3-(anthracen-9-yl)-1-phenylprop-2-2n-1-one) was found to be the best lead like molecule with a binding energy of -9.25 kcal/mol with predicted IC50 value of 164.66 nano molar. Conclusively, novel designed compound 21 of the present study have shown promising anti-cancer potential worth considering for further evaluations.

Keywords: EGFR kinase domain, chalcones, docking, ADME, QSAR, anti-cancer.

Background:
Epidermal growth factor receptor (EGFR) is one of crucial role player in the process angiogenesis, a critical step for the survival of cancer cells. This growth factor receptor kinase play important role in the progression, aggressiveness, development and metastasis of many solid tumors, such as head and neck cancers, non small cell lung cancer (NSCLC) and glioblastomas. Particularly, the involvement of the EGFR family of tyrosine kinases in cancer proliferation suggests that blockade of the binding sites for EGFR has been hypothesized as an effective anti-cancer therapy **[1].** EGFR gene encodes protein containing 621 residues and 1186 amino acids, which

compromise the binding site for specific ligand amino acid residues and extra cellular domain, which server binding site for EGFR inhibitors **[2].** A number of small molecule inhibitors of EGFR tyrosine kinase are under clinical development besides monoclonal antibody based therapies **[3-4].**

Synthetically and pharmacologically chalcones series of compounds have been recognized as a unique class of small compounds with a wide range of applications. Chalcone derivative compounds have been shown to be highly effective anti-cancer agents, however there are still so many novel derivatives possible, one of which might get us the best targeted EGFR inhibitor. In this present study we have designed novel chalcones derivatives from di flouro acetophenones reacted with different aldehydes by clasein-Schmidt reactions **[5].** We have chosen to design derivatives for chalcones structure in specific based on their versatile nature and for their synthesis and pharmacological actions. In the present study a library of novel chalcone series of compounds has been designed and evaluated for their anti-cancer activity targeting EGFR kinase domain using various computational approaches in an effort directed towards the discovery of novel, potent target specific anti-tumor agents for the treatment of cancer.

Methodology:

Computational methods:

Software and program

Schrodinger's maestro visualization program v9.6 **[6]** is utilized to visualize the receptors, ligand structures, hydrogen bonding network, to calculate length of the bonds and to render images. Chemsktech was used to draw the ligand compounds. Autodock 4.0 **[7]** is the primary docking program used in this work for the semi-flexible docking studies. Preparation of the ligands and protein receptors in pdbqt file and determination of the grid box size were carried out using Auto-Dock Tools version 1.5.6. Molinspiration, Orissis property explorer program was used to study the ADMET properties of the compounds.

Preparation of protein receptor and Ligand

The crystal structure of the EGFR kinase domain [PDB: 1M17] was obtained from the Protein Data Bank (PDB) **[8].** The crystal structure contained many missing atoms which were supplemented by the repair commands module of AutoDock. Before docking, the protein crystal structure was cleaned by removing the water molecules. H-atoms were added to these target proteins for correct ionization and tautomeric states of amino acid residues. The modified structure so obtained was used for the semi-flexible dockings. The ligand molecules was drawn using chemsketch software. The energy of the ligand molecule and receptors were minimized in Steepest Descent and Conjugate Gradient methods using Accelrys Discovery Studio (Version 4.0, Accelrys Software Inc.) **[9].** The minimization methods were carried out with CHARMM force field **[10].**

Semi-flexible docking

Autodock Version 4.0 is used to predict binding pose with associated energy along with the IC50 value prediction of the compounds with drug target EGFR Kinase domain. Protocol followed for carrying out the docking studies using Autodock version 4.0 in order to predict binding pose and IC50 values along with associated binding energies is of default parameters similar to the protocol followed elsewhere **[11-13].** Briefly, the energy scoring grid box was set to 126, 126 and 126 Å (x, y, and z) centered at X = 0.041; Y = -0.068 and Z = 0.128 with 0.375 angstroms grid points spacing assigned with default atomic salvation parameters. The grid box was designed such that the active site of EGFR kinase domain was surrounded by the three dimensional grid box centered at its active ligand binding site location. Lamarckian Genetic Algorithm (LGA) **[14]** was selected as docking engine, with all the docking parameters set to default. After each LGA run, Autodock reports the best docking solution along with IC50 values for each docked complex, and the results are reported based on cluster analysis. Binding Gibbs free energy (ΔG) is calculated as a sum of six energy terms of dispersion/repulsion, electrostatic interactions, hydrogen bonding, deviation from covalent geometry, desolvation effects and internal ligand torsional constraints. From a total of 10 docking modes represented by LGA cluster analysis, the lowest energy docking mode with respective IC50 prediction was selected from each docking simulation. Each compound was allowed with active rotatable bonds making them flexible.

Pharmacological properties of the compounds:

Osiris Property Explorer (www. organicchemistry. org/prog /peo/) **[15]** online server along with data warrior software **[16]** was used to check the pharmaceutical fidelity of the drug candidates. Molecular descriptors, such as Log P, the number of hydrogen bond donors, the number of hydrogen bond acceptors, and the molecular mass of the compounds were analyzed. Osiris Property Explorer was also used in analyzing various attributes of the drugs, such as toxicity and drug score.

Results & Discussion:

Docking of the compounds with EGFR Kinase domain active site

We have performed the docking studies for the present studied twenty compounds with the EGFR Kinase domain protein targeting its active binding site in order to know the binding energy involved in this complex formation and to know the molecular interactions responsible for this target specific inhibition. Docking results are tabulated in **Table 1 (see supplementary material)**. All the twenty five compounds studied in this present work have shown to be successfully docking inside the active site of EGFR kinase domain with a binding energy in a range of -6.10 to -9.25 Kcal/mol. We have compared our docking results with some of the FDA approved drugs for EGFR, as per the literature it is evident that Erlotinib, gefitini, Doxorubicin and Lapatinib were showing binding energy of -8.43, -8.53, -8.86 and -8.33 Kcal/mol respectively **Table 2 (see supplementary material).** When these docking results for the control FDA drugs compared with our compounds docking results, it was identified that compound 21 and 22 are showing better binding energies than these controls by showing -8.80 and -9.25 Kcal/mol of binding energy respectively. As per the molecular docking results, it was revealed that Compound 21 has the best estimated -9.25 Kcal/mol of binding energy **Table 1 (see supplementary material)** for the EGFR kinase domain inhibited complex formation by forming a hydrogen bond with LYS721. Apart from hydrogen bonds, this compound was also found to be

forming Van der waals interactions with MET742; THR830; THR766; ALA719; CYS751; LEU820; GLN767; LEU768; MET769; LEU694; ILE720; VAL702; ILE765 and LEU764 residues **(Figure 1).**

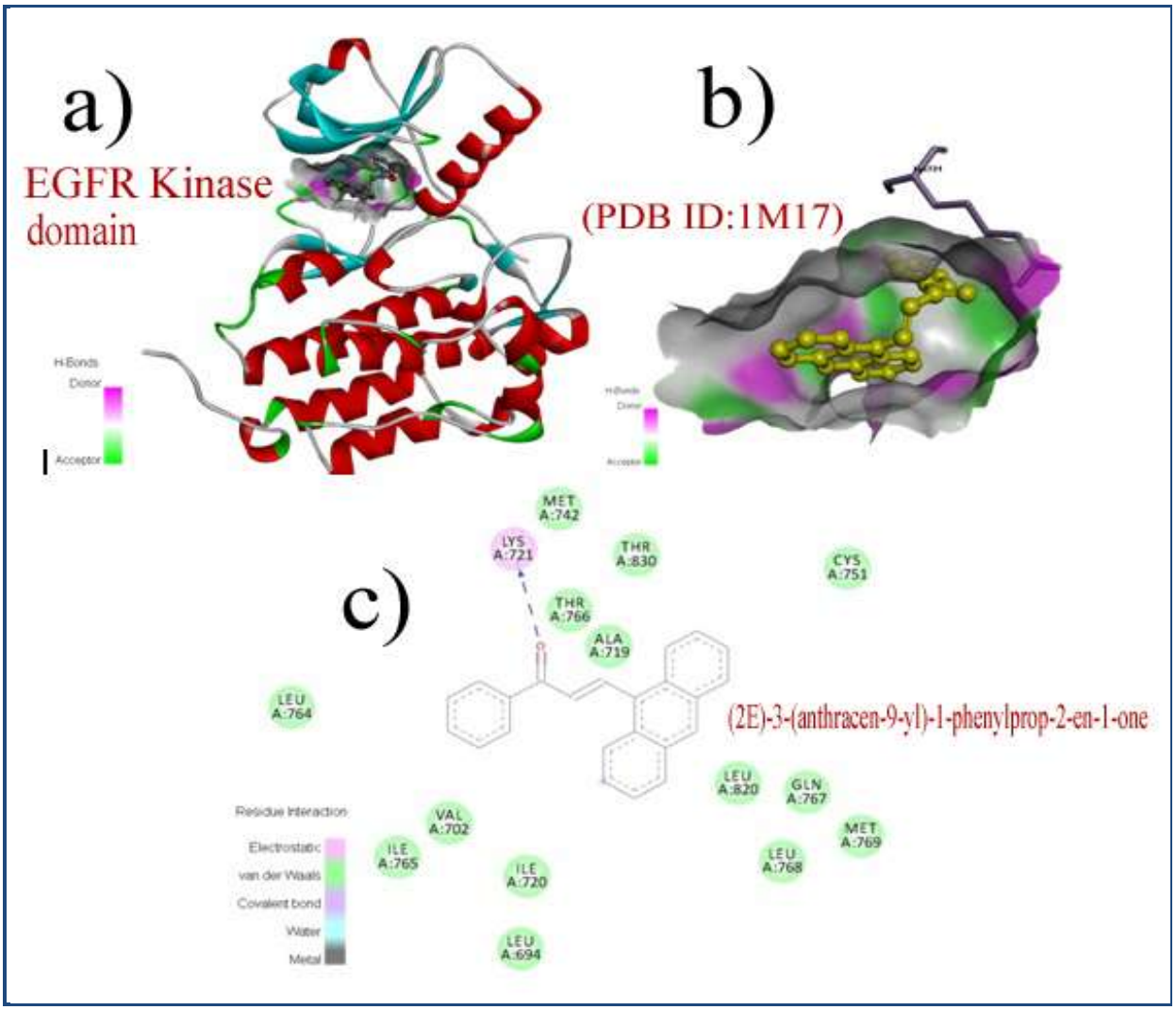

Figure 1: a) Docking snapshot of compound 21((2E)-3-(anthracen-9-yl)-1-phenylprop-2-2n-1-one) at the active binding site of EGFR kinase domain; **b)** 3D docking snapshot showing compound 21 ((2E)-3-(anthracen-9-yl)-1-phenylprop-2-2n-1-one) forming a hydrogen bond with LYS721 residue and **c)** represents the 2D interactions between compound 21 ((2E)-3-(anthracen-9-yl)-1-phenylprop-2-2n-1-one) and EGFR Kinase domain.

IC50 prediction

In order to understand the plausible experimental anti-cancer activity of the present studied compounds, we have carried out the half maximal inhibitory concentration (IC50) value predictions. IC50 value is a useful parameter to quantitatively measure the effectiveness of compound to inhibit a given biological process by half and is universally used to symbolize the inhibitory effect of compounds **[17]. Table 1 (see supplementary material)** shows the predicted IC50 value for the compounds 1-25 were in a range of 33.50 micro molar to 164.66 nano molar. We have compared our docking results with some of the FDA approved drugs for EGFR, as per the literature it is evident that Erlotinib, gefitini, Doxorubicin and Lapatinib were showing binding energy of 5.0, 0.08, 0.30, 779.60 µM respectively. When these IC50 values for the control FDA drugs compared with our compounds, it was identified that compound 21 and 22 are showing better inhibition constant than these controls by showing 164.66 and 351.87 nanomolar respectively **[20-22] Table 2 (see supplementary material).** Among which the compound 21 has shown the best possible inhibitory potential with 164.66 nano molar, whereas compound 20 with least predicted IC50 value of 33.50 micro molar. IC50 values obtained clearly demonstrated plausible high inhibitory potential of present studied compounds with kinase domain of EGFR.

Prediction of pharmacological properties:

Osiris Property Explorer was utilized to predict the pharmacological properties of the present studied compounds according to Lipinski's Rule of Five **[18]** and Oral Bioavailability. The pharmacological attributes prediction results are displayed in **Table 3 (see supplementary material).** Based on the experimental values, it was inferred that all the compounds successfully satisfied all the parameters of Lipinski's Rule of Five. The parameters of the Lipinski's rule are as follows: the molecular weight must be < 500 Da,

Log P < 5, the number of hydrogen donors must be < 5, the number of acceptor hydrogens must be < 10, and the refractivity molar range must be between 40–130. However, one parameter exception can be given out of above mentioned ones.

As per the veber's rule **[19]**, oral bioavailability of drugs could be measured by the molecular weight, number of rotatable bonds (n rotb), number of hydrogen bonds, and the expanse of the drug's polar surface (TPSA). The oral bioavailability was marked by small molecular weight (less than 500 Da); also, the number of rotatable bond must be less than 10, the number of hydrogen bond donors and acceptors must be less than 12, and TPSA values less than 140. **Table 3 (see supplementary material)** shows that all the compounds have a promising oral bioavaibility.

Screening for the best compound based on docking and drug likeliness results

Keeping in view of binding energies, IC50 values and ADME parameters of the present investigated compounds it was found that compound 21 ((2E)-3-(anthracen-9-yl)-1-phenylprop-2-2n-1-one) has the promising anti cancer drug like properties based on its ΔG binding energy and IC50 value. Based on Pharmacological properties, all the twenty five compounds showed good pharmacological attributes. These compounds were found to comply with Lipinski's rule, Veber's rule and oral bioavailability parameters. Whereas, compound 21 showed good pharmacological attributes, since it satisfied the Lipinski's Rule, Veber's Rule, Log P values with highest binding affinity and least half inhibitory potential.

Conclusion:

Our *In silico* studies provides a rationalization to the ability of present studied novel twenty five compounds as a valuable small ligand molecule with strong binding affinity towards EGFR Kinase domain for plausible anti-cancer activity involving large value of negative binding energy by forming various interactions with the residues, all or some of which fall under catalytic active site important residues consolidating their complex's thermodynamic stability. Moreover, predicted IC50 values further substantiated our hypothesis that these compounds have the potential to inhibit EGFR Kinase domain. The knowledge gained through this present study could be of high value for computational screening of target specific EGFR Kinase domain inhibitors by understanding the molecular interaction basis between ligand and receptor. On the other hand, promising ADME drug like profile for the present compounds especially compound 21 substantiates the need of further evaluating this compounds ability to inhibit cancer. The present investigated chalcone scaffold of compounds offers the possibility of expedient additional modifications that could give rise to lead structures with enhanced inhibitory activity and selectivity towards the drug receptor target like EFGR kinase.

References:

[1] Ciardiello F & Giampaolo Tortora, *Eur J Cancer* 2003 **10**: 1348 [PMID: 12826036]
[2] Francis M Saleshier *et al. Hygeia J D Med.* 2015 **7**: 46
[3] Busse D *et al. Semin Oncol.* 2001 **28**: 55 [PMID: 11706396]
[4] Shawver LK *et al. Cancer Cell*. 2002 **1:** 123 [PMID: 12086869]
[5] Chetana B Patil *et al. J Pharm Sci & Res*. 2009 **1:** 11
[6] Maestro (Version 9.6). 2013 New York, NY: Schrödinger, LLC
[7] Goodsell DS *et al. J Mol Recognit.* 1996 **9:** 1 [PMID: 8723313]
[8] Bernstein FC *et al. Arch Biochem Biophys.* 1977 **112**: 535 [PMID: 626512]
[9] http://accelrys.com/products/discovery-studio/visualization-download.php.
[10] Vanommeslaeghe K *et al. J Comput Chem.* 2010 **31**: 671 [PMID: 19575467]
[11] Reddy SV *et al. J Biomol Struct Dyn*. 2015 **11:** 1 [PMID: 25671592]
[12] SH Basha *et al. European Journal of Biotechnology and Bioscience*. 2014 **2**: 30
[13] SH Basha *et al. scientific reports*. 2012 **566**: 2
[14] Morris GM *et al. Journal of Computational Chemistry*. 1998 **19:** 1639
[15] Sander T *et al. J Chem Inf Model.* 2009 **49:** 232 [PMID: 19434825]
[16] Sander *et al. J Chem Inf Model*. 2015 **55:** 460 [PMID: 25558886]
[17] BT Burlingham *et al. Journal of chemical education* 2003 **2:** 214
[18] Lipinski CA, *Drug Discov Today Technol.* 2004 **1:** 337 [PMID: 24981612]
[19] Miles *et al. Drug Discov Today.* 2003 **8**: 876 [PMID: 14554012]
[20] Yuvasravana Roddam *et al. Int J Pharm Sci Rev Res.* 2014 **24:** 82
[21] Ohashi K *et al. Proc Natl Acad Sci U S A*. 2012 **31**: E2127 [PMID: 22773810]
[22] Aristatile Balakrishnan *et al. African Journal of Pharmacy and Pharmacology* 2013 **7**: 2499

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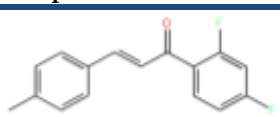
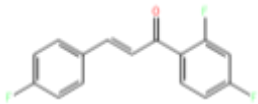
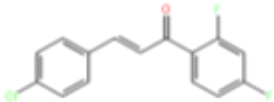
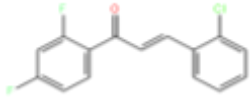
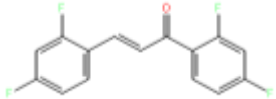
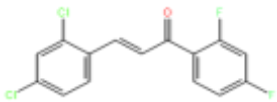
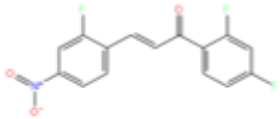
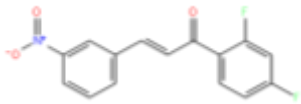
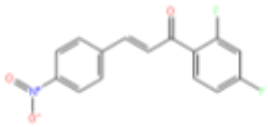
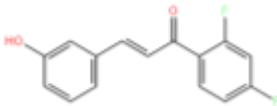
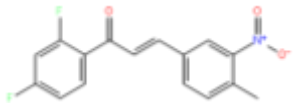
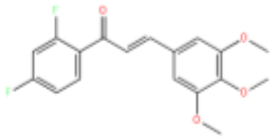
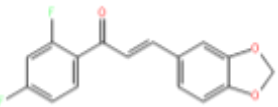
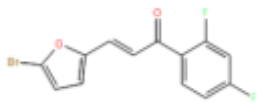
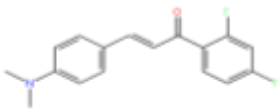
Supplementary material:

Table 1: Docking results of the present studied chalcone series of compounds with EGFR receptor domain

S.No	Compound	Docking energy (Kcal/mol)	pIC50 value (micromolar)
1.		-6.94	8.18
2.		-6.59	14.81
3.		-6.90	8.72
4.		-7.00	7.35
5.		-6.42	19.81
6.		-7.35	4.07
7.		-7.28	4.64
8.		-7.13	5.98
9.		-7.80	1.92
10.		-7.04	6.87
11.		-7.69	2.32
12.		-7.11	6.13
13.		-7.21	5.18
14.		-6.86	9.33
15.		-7.23	5.02

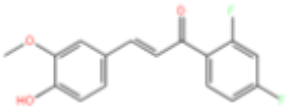
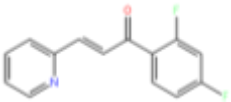
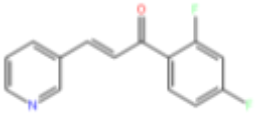
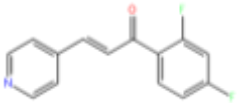
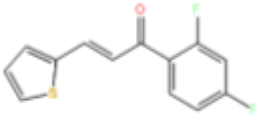
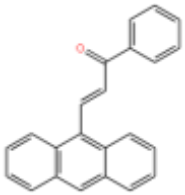
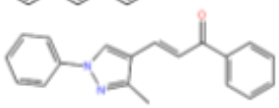
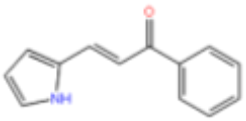
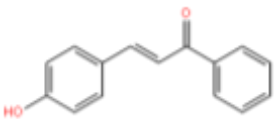
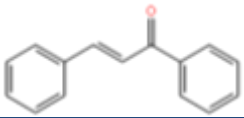
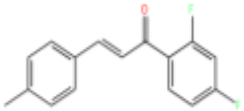
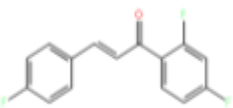
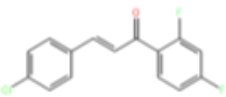
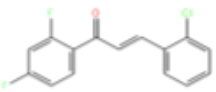
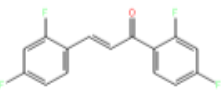
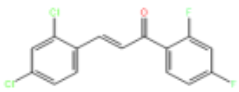
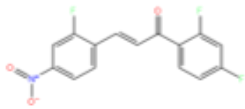
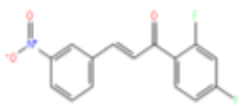
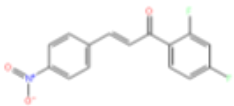
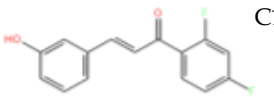
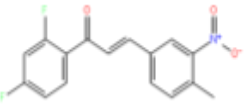
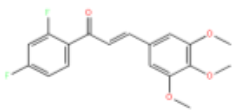
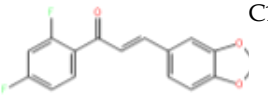
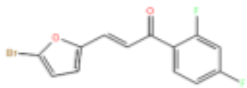
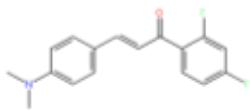
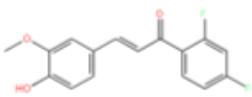
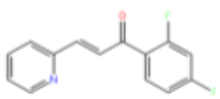
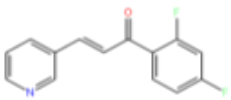
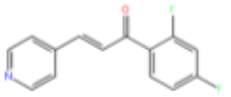
16.		-7.07	6.56
17.		-6.42	19.65
18.		-6.58	15.11
19.		-6.57	15.22
20.		-6.10	33.50
21.		-9.25	164.66 nanomolar
22.		-8.80	351.87 nanomolar
23.		-6.19	29.22
24.		-7.18	5.44
25.		-6.60	14.61

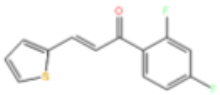
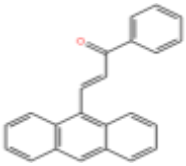
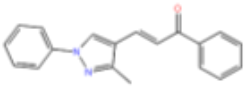
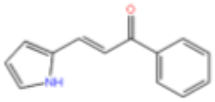
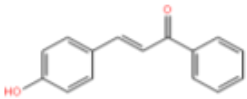
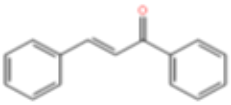
Table 2: Docking results of some of the FDA approved drugs for EGFR:

S.No	Ligand	Binding energy (Kcal/mol)	IC50 (micro molar)	Reference
1.	Erlotinib	-8.43	5.0 µM	20-21
2.	gefitinib	-8.53	0.08 µM	2
3.	Doxorubicin	-8.86	0.30 µM	2
4.	Lapatinib	-8.33	779.60 µM	22

Table 3: The molecular descriptor values of the chalcone series of compounds used in this study.

S.No	Compound	Molecular Formula	Mol. wt.	Log P	No. of H-bond donors	No. of H-bond acceptors	No. of rotatable bonds	TPSA	Drug likelines s
1.		C16 H12 F2 O	258.086	3.8493	0	1	3	17.07	-1.2839
2.		C15 H9 F3 O	262.061	3.6062	0	1	3	17.07	-1.2275

3.		C15 H9 Cl F2 O	278.031	4.1114	0	1	3	17.07	-1.1423
4.		C15 H9 Cl F2 O	278.031	4.1114	0	1	3	17.07	-1.1423
5.		C15 H8 F4 O	280.051	3.707	0	1	3	17.07	-1.2275
6.		C15 H8 Cl2 F2 O	311.992	4.7174	0	1	3	17.07	-1.1423
7.		C15 H8 F3 N O3	307.046	2.6846	0	4	4	62.89	-6.3876
8.		C15 H9 F2 N O3	289.055	2.5838	0	4	4	62.89	-6.3876
9.		C15 H9 F2 N O3	289.055	2.5838	0	4	4	62.89	-6.3876
10.		C15 H10 F2 O2	260.065	3.1597	1	2	3	37.3	-1.2064
11.		C16 H11 F2 N O3	303.071	2.9277	0	4	4	62.89	-6.4175
12.		C18 H16 F2 O4	334.102	3.2954	0	4	6	44.76	-1.155
13.		C16 H10 F2 O3	288.06	3.6168	0	3	3	35.53	-1.2825
14.		C13 H7 Br F2 O2	311.96	3.5167	0	2	3	30.21	-2.8876
15.		C17 H15 F2 N O	287.112	3.4018	0	2	4	20.31	0.31843
16.		C16 H12 F2 O3	290.075	3.0897	1	3	4	46.53	-1.121
17.		C14 H9 F2 N O	245.065	2.5585	0	2	3	29.96	-1.0514
18.		C14 H9 F2 N O	245.065	2.5045	0	2	3	29.96	-1.0514
19.		C14 H9 F2 N O	245.065	2.5045	0	2	3	29.96	-1.0514

20.		C13 H8 F2 O S	250.026	3.372	0	1	3	45.31	1.1089
21.		C23 H16 O	308.12	5.6926	0	1	3	17.07	0.1125
22.		C19 H16 N2 O	288.126	2.8705	1	3	4	37.79	3.9025
23.		C13 H10 N O	196.076	1.7121	0	2	3	29.43	1.5139
24.		C15 H12 O2	224.084	2.9581	1	2	3	37.3	0.13358
25.		C15 H12 O	208.089	3.3038	0	1	3	17.07	0.1125