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COMPARATIVE INSILICO DOCKING STUDY INVOLVING ANTAGONISTIC ACTIVITY OF COUMARINDERIVATIVES ON EGFR AND CDK2

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Abstract

Epidemiological evidence suggests that about 25% of cancer occurs due to chronic inflammation, thus it is clear that cancer and inflammation are related. Piroxicam is the one of the drug that is used in the treatment of both cancer and inflammation, but it is having some side effects like constipation, blurring of vision ,skin rashes etc. Coumarin is having both anti-inflammatory and anti-cancer activity so the purpose of this study is to screen the best target among EGFR and CDK2. Docking analysis was carried out using AutoDock4. From the study it was found that EGFR showed better result compare to CDK2. Also methyl substitution at 8th position and chlorine substitution at 5th position of coumarin showed better activity than standard drug piroxicam and phytoconsitutents *isofraxidin* and*scopoletin*.

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Introduction

Inflammation is the body's response to internal and external environment in order to eliminate unwanted agents from body and thus restore the tissue physiology. Chronic inflammatory conditions in selected organs increase the risk of cancer. EGFR (epidermal growth factor) plays an important role in inflammation as well as cancer. EGFR belongs to HER family of receptors, in which EGFR is activated by binding to EGF which cause receptor dimerization and tyrosine autophosporylation leading to cell proliferation [4, 5].

CDK2 belongs to family protein kinases, it is also known as cell division protein kinase-2. Initially it was discovered for its action in regulating cell cycle later it was found that inhibition of this protein lead to variety of action like anti-cancer, anti-inflammatory action etc. CDKs require cyclin for its activation. CDK2 inhibitors produce anti-inflammatory activity by inhibiting MAPK, NF-Kb and PI3K signalling pathways. In the case of cancer CDK2 inhibitors prevent the stimulation of the cell to enter in to s phase of cell cycle and reduce cell proliferation [6, 7]

Computer –aided drug design (CADD) uses computational chemistry to discover, enhance or to study drug and related biologically active molecules. The problems associated with the conventional method of drug designing are overcome by the CADD. Two methods in CADD are structural based and ligand based drug design. Structural based drug design depends on the three dimensional structure of biological target whereas ligand based drug design depends on molecules that bind to biological target [8]. In this work we have carried out the study on coumarin derivatives and the standard drug selected for the study was piroxicam [9] .The phytoconstituents selected for the study are *scopoletin* and *isofraxidin* [10].

Materials and methods

Twenty eight lead molecules were designed by using chemsketch by giving substitution on 5th, 6th, 7th and 8th position of the compound having coumarin nucleus as shown in fig: 1

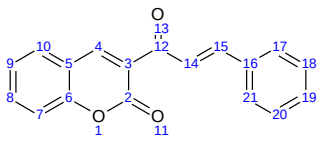

Fig.13-[(2*E*)-3-phenylprop-2-enoyl]-2*H*-chromen-2-one

Selection of target

Primary and Secondary Structure Analysis

Targets were selected from PDB after carrying out the primary and secondary structure analysis. PDB is a crystallographic database or the three-dimensional structural data of large biomolecules such as proteins and nucleic acids.

Primary structure analysis was done by using protparam, which computes various physico-chemical properties from protein sequences. Various parameters studied using protparam are molecular weight, theoretical pi, half-life, GRAVY, aliphatic index, instability index [11, 12], results are given in table 1and 2. Secondary structure analysis was done using sopma, which indicates whether a given amino acid lies in a helix, strand or coil, results are computed in table 3 and 4.

Table 1. Primary Structure Analysis of EGFR

SL.NO	PDB ID	Molecular weight	Theoretical pi	Half life (hr.)	Aliphatic index	Extinction coefficient m-1cm-1	GRAVY	Instability index
1	1M14	37827.7	5.67	30	94.26	52745	-0.221	43.79
2	1M17	37827.7	5.67	30	94.26	52745	-0.221	43.49
3	1XKK	40269	5.88	30	91.11	52725	-0.315	42.52
4	2GS2	37516.4	5.59	30	95.12	52745	-0.208	43.47
5	2GS6	41363.4	5.10	30	90.27	54360	-0.305	45.47
6	2JSF	37257.1	5.59	30	95.69	52745	-0.220	44.04
7	2J6M	37257.1	5.59	30	95.69	52745	-0.220	44.04
8	2ITY	3707.9	5.70	4.4	96.28	52745	-0.210	44.48
9	2ITX	37257.1	5.59	30	95.69	52745	-0.220	44.04
10	2ITW	37257.1	5.59	30	95.69	52745	-0.220	44.04

Table.2 Primary structure analysis of CDK2

SL.NO	**PDB ID**	**Molecular weight**	**Theoretical pi**	**Half life (hr.)**	**Aliphatic index**	**Extinction coefficient m-1cm-1**	**GRAVY**	**Instability index**
1	2KW6	7421.4	9.43	30	81.23	2980	-0.729	56056
2	2M1L	7631.7	9.42	1.9	70.87	2980	-0.577	55.48

Table.3 Secondary structure analysis of EGFR

SL.NO	PDB ID	Alpha helix	Extended strand	Beta turn	Random coil
1	1M14	157	57	35	81
2	1M17	157	57	35	84
3	1XKK	157	62	33	100
4	2GS2	157	55	32	86
5	2GS6	157	55	32	86
6	2JSF	157	55	32	83
7	2J6M	157	55	32	83
8	2ITY	157	55	32	81
9	2ITX	157	55	32	83
10	2ITW	157	55	32	83

Table.4 Secondary structure analysis of CDK2

SL.NO	PDB ID	Alpha helix	Extended strand	Beta turn	Random coil
1	2KW6	50	1	1	13
2	2M1L	45	4	3	17

From primary and secondary structure analysis parameters like half-life and random coil coefficient were considered for the selection of targets. Targets that show highest value in both the parameters were selected as targets for docking analysis.

Preparation of ligands

The ligands were designed from chemsketch and saved in PDB format, their smiles notation were also obtained from same. Chemsketch is chemically intelligent drawing interface software developed by Advanced Chemistry Department.

Validation of ligands

Drug likeness is a parameter that helps to determine the various molecular properties of compound in conjugation with the pharmacophore. This was determined using an online software Molinspiration , using this software molecular properties based on Lipinski rule of five and drug ADME profile was also checked.Various parameter are determined which include log p, number of hydrogen bond donor or acceptors which is necessary for eliminating non-drug like molecules[13].The results are compiled in table 6 and 7.

Docking study:

Molecular docking

Docking is a method, which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.In this study docking was carried out usingAutoDock4. After the energy minimisation of ligand and protein, water molecules are removed and docked with the lead molecules to get the docking score. The results are computed in table 8 and 10. The selected targets were also docked with the phytoconstituents having coumarin nucleus and the standard drug used in the treatment of inflammation as well as the cancer and the results are computed in table 9 and 11.

Results and Discussion

Table.6 Analysis of Lipinski rule of five for novel proposed analogues of coumarin

Sl.No	Name of the compound	Molecular Weight	No.of Hba	No.of Hbd	C log p	No of Rot.b	No.of Violations
1	5-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	310.74	3	0	4.45	3	0
2	6-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	310.74	3	0	4.47	3	0
3	7-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	310.74	3	0	4.47	3	0
4	8-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	310.74	3	0	4.45	3	0
5	5-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	355.19	3	0	4.58	3	0
6	6-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	355.19	3	0	4.6	3	0
7	7-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	355.19	3	0	4.6	3	0
8	8-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	355.19	3	0	4.58	3	0
9	5-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	294.28	3	0	2.98	3	0
10	6-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	294.28	3	0	3.96	3	0
11	7-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	294.28	3	0	3.96	3	0
12	8-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	294.28	3	0	3.93	3	0
13	5-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	292.29	4	1	3.55	3	0
14	6-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	292.29	4	1	3.31	3	0
15	7-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	292.29	4	1	3.31	3	0
16	8-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	292.29	4	1	3.55	3	0
17	5-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	290.32	3	0	4.22	4	0

The results for validation of ligands shows that values of all twenty eight compounds based on molecular weight is less than 500 Daltons,no.of hydrogen bond donors and acceptors are below 5 and 10, partition coefficient is within the limit ,this shows that there is no violation of Lipinski rule of 5.

Table.7 Bioactivity results of proposed analogues of coumarin

Sl.No	GPCR Ligand	Ion channel modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1	-0.42	-0.49	-0.54	-0.23	-0.37	-0.12
2	-0.4	-0.53	-0.56	-0.22	-0.34	-0.11
3	-0.37	-0.48	-0.62	-0.19	-0.35	-0.08
4	-0.49	-0.67	-0.6	-0.21	-0.35	-0.09
5	-0.44	-0.66	-0.5	-0.27	-0.41	-0.13
6	-0.54	-0.65	-0.6	-0.38	-0.47	-0.17
8	-0.21	-0.68	-0.66	-0.3	-0.4	-0.1
9	-0.33	-0.51	-0.42	-0.14	-0.33	-0.08
10	-0.36	-0.54	-0.51	-0.16	-0.35	-0.07
11	-0.29	-0.47	-0.47	-0.22	-0.31	-0.06
12	-0.41	-0.49	-0.5	-0.07	-0.34	-0.03
13	-0.37	-0.45	-0.52	-0.14	-0.26	-0.03
14	-0.33	-0.48	-0.46	-0.02	-0.31	-0.01
15	-0.36	-0.51	-0.5	-0.03	-0.33	0
16	-0.37	-0.47	-0.5	-0.13	-0.23	0.06
17	-0.46	-0.75	-0.55	-0.21	-0.42	-0.14
18	-0.45	-0.61	-0.6	-0.24	-0.38	-0.14
19	-0.44	-0.61	-0.62	-0.26	-0.4	-0.15
20	-0.51	-0.71	-0.64	-0.22	-0.46	-0.12
21	-0.37	-0.6	-0.54	-0.14	-0.32	-0.07
22	-0.35	-0.5	-0.58	-0.15	-0.26	-0.07
23	-0.35	-0.49	-0.6	-0.16	-0.28	-0.07
24	-0.32	-0.66	-0.59	-0.14	-0.4	-0.09
25	-0.43	-0.61	-0.44	-0.04	-0.45	-0.11
26	-0.46	-0.54	-0.55	-0.12	-0.45	-0.12
27	-0.46	-0.54	-0.57	-0.14	-0.46	-0.12
28	-0.42	-0.62	-0.51	-0.14	-0.47	-0.13

Docking Analysis

Docking scores for coumarin derivatives against EGFR is given in table.8 and the docking score for phytoconstituents having coumarin pharmacophore is given in table.9

Table.8 Docking scores for novel proposed analogues of coumarin against EGFR

SL.No	Substitution	Docking Score (kcal/mol)
1	5-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.5563
2	6-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.02525
3	7-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.50823
4	8-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.07289
5	5-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.97647
6	6-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.50701
7	7-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-5.73848
8	8-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.57724

9	5-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.00148
10	6-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.38909
11	7-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.80107
12	8-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.5179
13	5-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.57281
14	6-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.35864
15	7-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.79347
16	8-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.36727
17	5-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.24347
18	6-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.28572
19	7-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.48994
20	8-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-10.0089
21	5-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.07182
22	6-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.92836
23	7-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.9066
24	8-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.90994
25	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-5-carbaldehyde	-8.78985
26	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-6-carbaldehyde	-7.6281
27	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-7-carbaldehyde	-9.69306
28	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-8-carbaldehyde	-8.43019

Table.9 Docking scores of phytoconstituents having coumarin pharmacophore against EGFR

SL.NO	Phytoconstituents	Docking score (kcal/mol)
1	*Isofraxidin*	-6.608
2	*Scopoletin*	-6.219

Docking score for coumarin derivatives against CDK2 is given in table.10 and the docking score of phytoconstituents against CDK2 is given in table.11

Table.10 Docking scores for novel proposed analogues of coumarin against CDK2

SL.No	Substitution	Docking Score (Kcal/mol)
1	5-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-9.08063
2	6-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.17984
3	7-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.23813
4	8-chloro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.17164
5	5-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.86579
6	6-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.25594
7	7-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.2153
8	8-bromo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.37073

9	5-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.24518
10	6-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.05296
11	7-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.63018
12	8-fluoro-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.2655
13	5-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.59957
14	6-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.93164
15	7-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.27102
16	8-hydroxy-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.91668
17	5-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.24409
18	6-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.40065
19	7-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.12575
20	8-methyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.45836
21	5-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-7.10218
22	6-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.49001
23	7-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-8.49001
24	8-ethyl-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one	-6.01942
25	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-5-carbaldehyde	-6.59915
26	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-6-carbaldehyde	-7.13007
27	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-7-carbaldehyde	-6.66722
28	2-oxo-3-[(2E)-3-phenylprop-2-enoyl]-2H-chromene-8-carbaldehyde	-6.12156

Table.11 Docking scores of phytoconstituents having coumarin pharmacophore against CDK2

SL.NO	Phytoconstituents	Docking score (kcal/mol)
1	*Isofraxidin*	-6.369
2	*Scopoletin*	-6.03

Docking analysis of coumarin derivatives with methyl substitution at 8th position shows highest score against EGFR.(Fig.2)

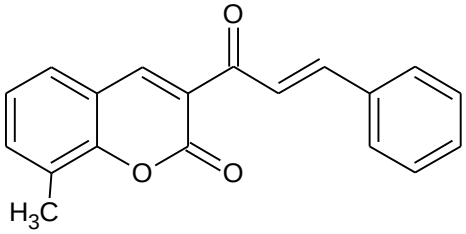

Fig.2 8-methyl-3-[(2*E*)-3-phenylprop-2-enoyl]-2*H*-chromen-2-one

Docking analysis of coumarin derivatives with chlorine substitution at the 5th position shows highest score against CDK2.(Fig.3)

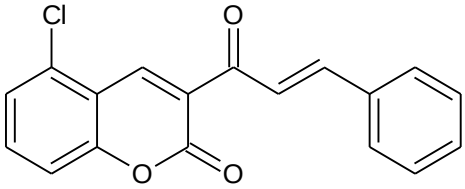

Fig.3 5-chloro-3-[(2*E*)-3-phenylprop-2-enoyl]-2*H*-chromen-2-one

Among twenty eight compounds fifteen of them show good score against EGFR and seven of them show good score against CDK2 as compared to the standard drug piroxicam.

Conclusion

Docking studies conducted in coumarin derivatives against CDK2 and EGFR for anti-inflammatory as well as anti-cancer activities was successful and it was found that among the two targets, EGFR showed good affinity towards the fifteen proposed analogues, and in that methyl substitution at the 8th position of the coumarin scaffold showed the best docking score as compared to the standard drug piroxicam and the two phytoconstituents whereas CDK2 showed good affinity towards seven proposed analogues among them chlorine substitution at 5th position shows highest score as compared to standard and two phytoconstituents. From this study we came to a conclusion that, among twenty eight ligands the best ligand for anti-inflammatory and anti-cancer activity was obtained when the coumarin derivative is substituted with methyl group at 8th position and chlorine atom at 5th position.

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