MOLECULAR DOCKING AND ADMET STUDIES OF 3-PHENYL COUMARIN DERIVATIVES FOR THEIR ANTI-CANCER ACTIVITY

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ABSTRACT

Cancer is one of the most prevalent diseases worldwide. Research community is untirely working on prevention and control of this disease by targeting various enzymes and proteins via synthetic and natural products. In the present study novel 3-phenyl coumarin derivatives were designed. All the compounds of this series were subjected to molecular docking studies for inhibition of 5 different proteins. The molecular docking study results revealed that, all the designed ligands showed binding energy (ranging from -13.4 to -44.0) and docking score (ranging from -3.27 to -9.56). ADME-Toxicity prediction reported that all novel coumarin derivatives were in the acceptable range of various pharmacological parameters. Total seven novel ligands were studied on 10 pdb ID's of five different proteins. Out of seven ligands five ligands i.e. 3, 4, 5, 6 and 7 have shown excellent docking score ranging from -9.56 to -8.21 on two proteins i.e. Amine Oxidase and Protein kinase. All these five ligands showed good affinity than reference compound capecitabin and R-(-) –deprenyl. These in silico results can thus serve as a template for further *invitro* and *invivo* studies to have novel drug for cancer with minimum toxicity.

KEYWORD: Molecular Docking, **ADME-Toxicity, Amine oxidase, Protein Kinase, Cancer, 3-phenyl coumarin derivatives.**

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INTRODUCTION

Cancer is a genetic disease, it is caused by certain changes on genetic level and result of multiple mutation.[1] Genes are made up of DNA which acts as an instructor to make proteins that controls the way our cells function, especially how they grow and divide. Protein molecules are responsible for almost all biological functions in cells.[2-3] In cancer the structure of protein becomes irregular and there is uncontrolled growth of cell. Protein is over expressed in several tumor cells which are considered as a target to control cancer. In the present study, five proteins such as NQO1, cytochrome P4502A6, Protein kinase, Amine oxidase and Epidermal growth factor receptor (EGFR) were considered as targets NAD(P)H:quinone oxidoreductase1(NQO1) a cytoplasmic homodimer that reduces oxidative stress and neoplastic lesion in cells.[4] NQO1 functions as a tumor suppressor[5], it stabilize the tumor suppressor gene p53 and interacts with p53 in a protein-protein interaction where it prevents various types of cancer formation.[6] NQO1[7-8] can be inhibited by dicoumarol analogues and series of 4-hydroxycoumarin derivatives. Cytochrome P450 2A6[9-11] catalyses 7-hydroxylation of coumarin analaogues and plays a major role in metabolism.[12-15] Binding of P450 2A6 with coumarin and methoxsalen are also reported in literature[16] and causes lung cancer risk. Oxidative deamination of mono-, di- and poly amines is catalyzed by the amine oxidases (AOs) that belong to a heterogeneous family of enzymes. AOs fall into two classes based on the chemical nature of the cofactors present in them: AOs that contain flavin adenin dinucleotide (FAD) as a cofactor, and AOs that contain copper II-2, 4, 5-trihydroxyphenylalanine quinone as a cofactor.[17] Monoamine oxidases (MAOs; EC1.4.3.4) falls in the first categories and hence are FAD's containing enzyme which are tightly bounded to the outer membranes of mitochondria through a cysteine residue. MAOs are present in several living organisms. Cell proliferation in animal models is increased by MAO-AIs and its inhibition increases cancer risk.[18-21] Coumarin derivatives have been recognized as potential MAO inhibitors.[22-27] In cancer Protein kinase C is overexpressed leading to activation of transcription.[28] Kinase inhibition delivers kinase inhibitor drug with good potency and pharmacokinetic properties.[29-32] Furo-coumarinsulfonamides acts as protein kinase C inhibitors particularly for cancer tumors[33]. A number of cancers such as lung cancer, anal cancers are associated with upregulation or overactivity of EGFR. The activity of EGFR enhances tumor growth, metastasis and invasion.[34] It also acts as a key factor in epithelial malignancies. In the tumor microenvironment production of EGFR ligands are sustained, which causes stimulation of EGFR in cancer.[35-40] Coumarin derivatives like Daphnetin have been identified as EGFR-PTK inhibitors.[41] Anticancer properties.[42-43] of

coumarin derivatives are associated with its low toxicity and antioxidant activities. Coumarin compounds can be used not only to treat cancer, but to treat side effects caused by existing drugs radiotherapy and surgery.[44] These reports from literature showed a strong correlation of all these 5 selected proteins and coumarin with cancer, and also possibility of coumarin derivatives to have minimum side effects. Molecular docking is as an optimization tool in which ligand binds to a particular binding site of receptors and is used to predict the orientation that maximizes the "interaction" while minimizing total energy of the intermolecular complex to get desired biologically activity.[45] Docking study overcomes the limitation of conventional methods which are expensive and time consuming. It uses computational chemistry which are simple, non expensive and used to discover biologically active molecules. The docking is a rational approach used to predict whether a given molecule will bind to a target. In docking, Glide module (Schrodinger suite) used for finding favorable interactions between designed ligands and a receptor molecule. While docking usually a protein or cofactor binds with a single ligand molecule. These observation prompted authors to design various novel coumarin based ligands that have not been studied till now and to get their molecular docking results with 5 proteins. All designed ligands were docked with 10 pdb files of all 5 proteins to get best fit. In present study we analysed anticancerous activity of various novel 3-phenyl coumarin derivatives by molecular docking studies. ADME properties of designed ligads were also determined by using the Schrodinger software 9.0

MATERIALS AND METHODS

Protein preparation
Amongst number of enteries from server based files best proteins were selected. Number of disallowed regions along with Ramachandran plot was main criteria for selecting protein[46]. 10 pdb files of best 5 proteins having resolution of 2.20 were docked with prepared ligands. First step of docking analysis was performed using Preparation Wizard, which involved the optimization and refinement of these pdb files. Grid calculations were performed for the protein active site by generating default size of grid box x = 33.8893 Å, y = 22.068 Å, and z = 10.424 Å. 3D structures along with Ramchandran plots of all 10Pdb files for five selected proteins i.e. 2f10 for NQO1 (Figure 1,2), 4rui for Cytochrome P450 2A6 (Figure 3,4), 2ya3 for Protein kinase (Figure 5,6), 2pwb for Protein kinase (Figure 7,8), 2qc6 for Protein kinase (Figure 9,10), 2v5z for Amine Oxidase (Figure 11,12), 2v60 for Amine Oxidase (Figure 13,14), 2v61for Amine Oxidase(Figure 15,16) and 1m17 for EGFR (Figure 17,18) are shown in Figure (1-18).
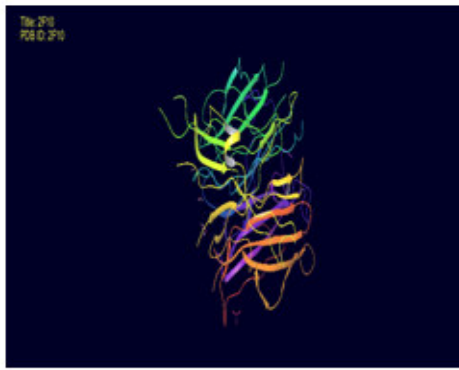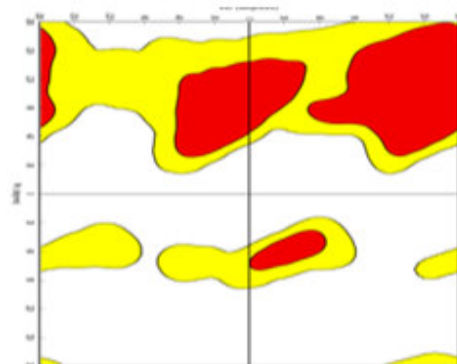
Figure 1
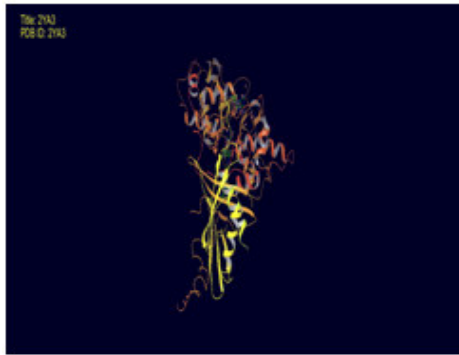*Protein NQO1 pdb 2f10*
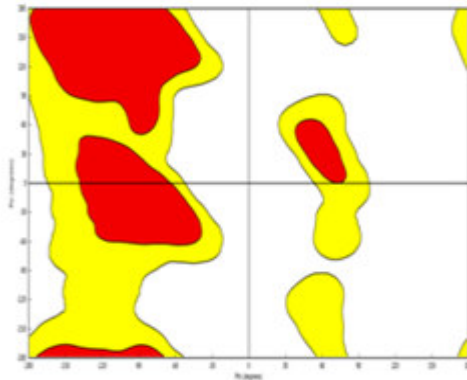
Figure 2
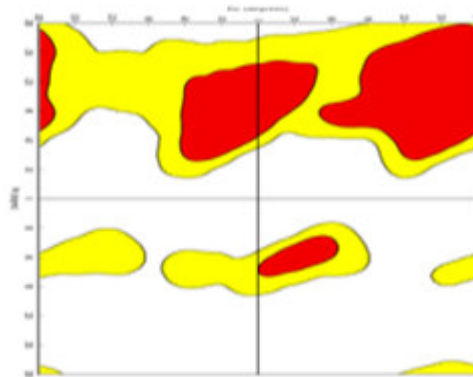*Ramchandran plot of 2f10*

Figure 3
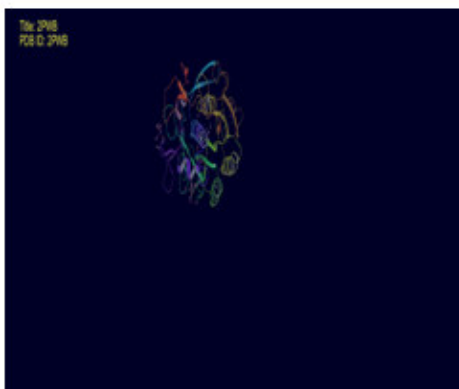*Protein P450 pdb 4rui*
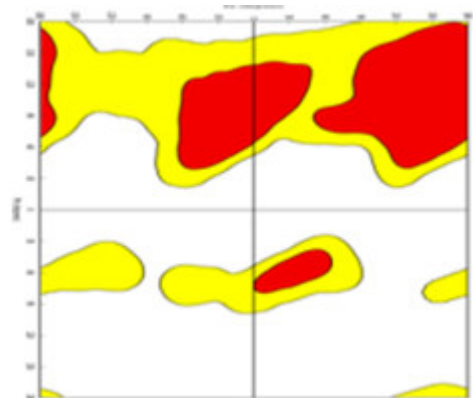

Figure 4
Ramchandran plot of 4rui

Figure 5
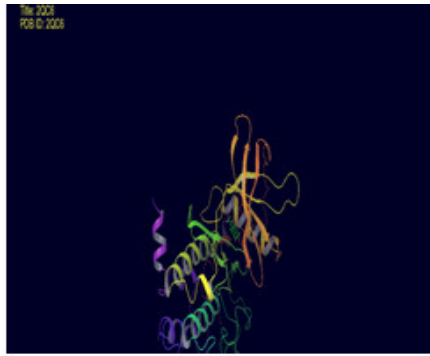*Protein Kinase pdb 2ya3*

Figure 6
Ramchandran plot of 2ya3

Figure 7
Protein Kinase pdb 2pwb

Figure 8
Ramchandran plot of 2pwb

Figure 9
Protein Kinase pdb 2qc6
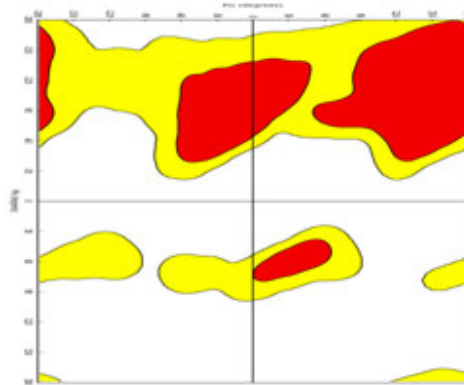
Figure 10
Ramchandran plot of 2qc6

Figure 11
Protein Amine Oxidase pdb 2v5z

Figure 12
Ramchandran plot of 2v5z
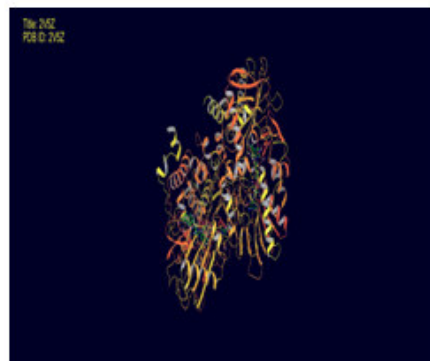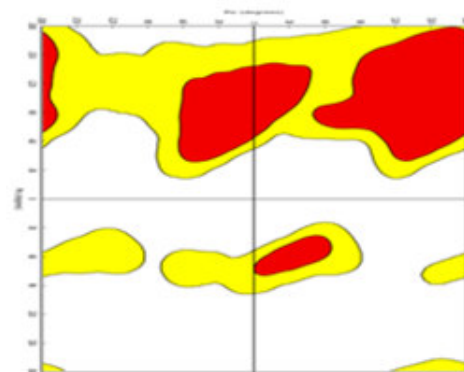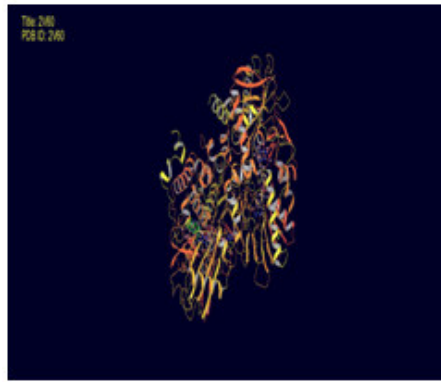
Figure 13
Protein Amine Oxidase pdb 2V60
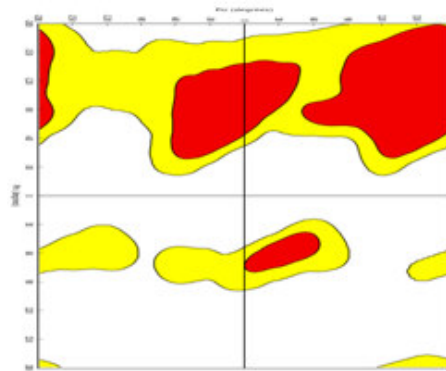
Figure 14
Ramchandran plot of 2V60
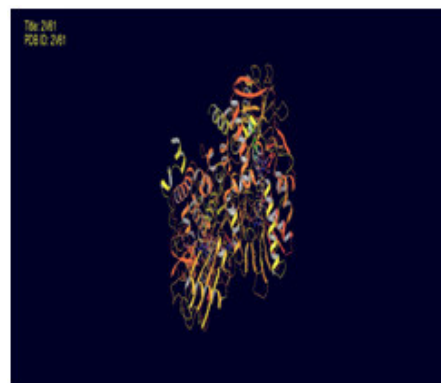
Figure 15
Protein Amine Oxidase pdb 2V61
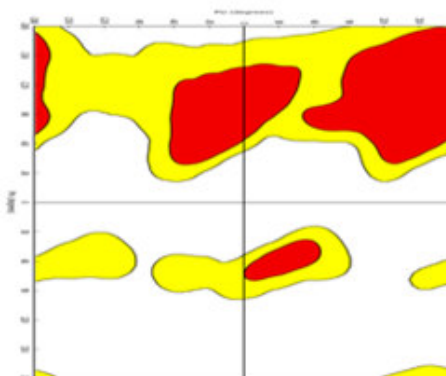
Figure 16
Ramchandran plot of 2V61
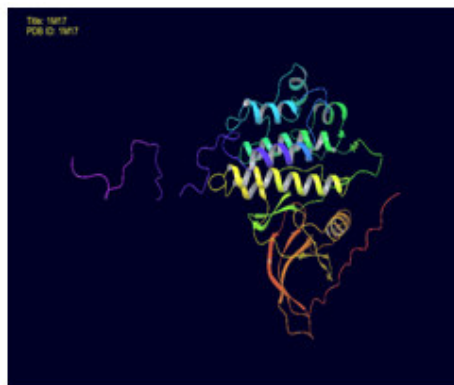
Figure 17
Protein EGFR pdb 1M17
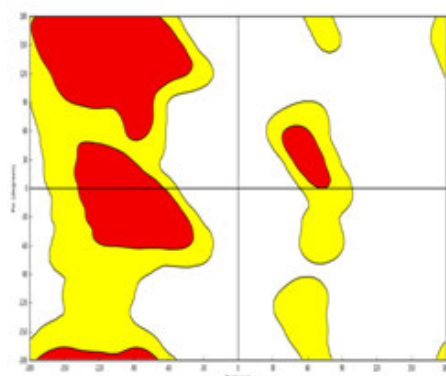
Figure 18
Ramchandran plot of 1M17

Ligand preparation
The structure of the ligands i.e.3-phenyl coumarin derivatives were drawn by using Chem bio draw ultra (12.0) and converted to MOL-SD file. Once imported the .mol file into the maestro workspace, LigPrep were used for preparing the ligands (LigPrep is a utility of Schrodinger software that generates 3D structures from 2D). Structure of docked ligands and reference compounds are mentioned in Figure 19 (1-9).
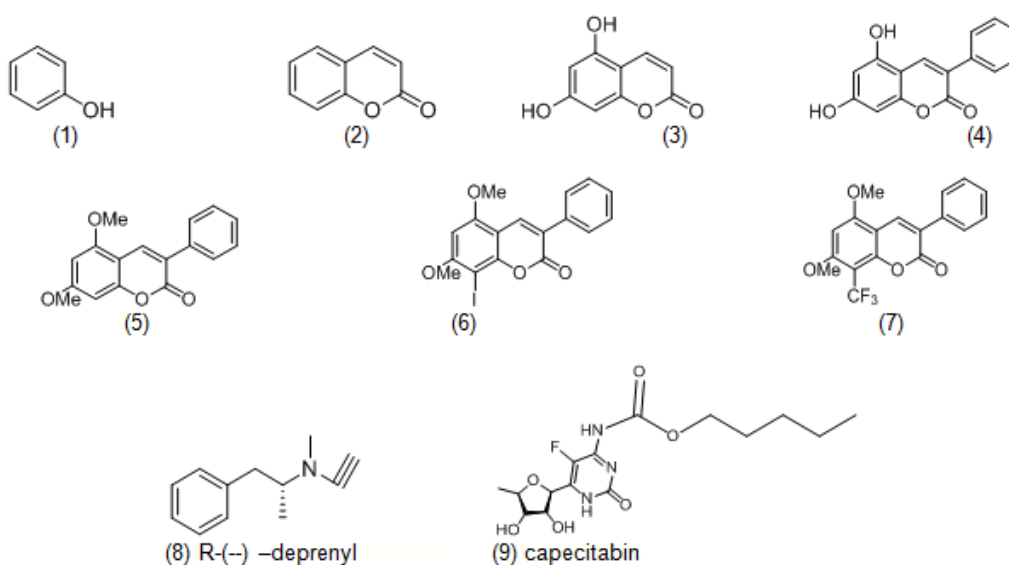
Figure 20 (1-9)
Structure of docked ligands and reference compounds

DOKING STUDIES

Molecular docking studies were conducted in order to get best docking scores amongst all designed ligands. The ligand molecules were drawn and analyzed using Chem BioDraw Ultra 12.0 and converted to MOL-SD file. 3D, coordinates were prepared using dock server. Computational studies for molecular docking were performed with various novel 3-phenyl coumarin derivatives. All the designed ligands (1-7) along with reference compounds 8, 9 were docked with the Molecular modeling software, Schrödinger. Docking studies for calculation of binding energy and docking score were done for all ligands within active sites of 5 proteins using ligPrep. A grid was generated with prepared ligands and proteins (default value 1.0 ^{0}A), module 5.5 (Glide Version 5.5, 2009) (XP) in extra precision mode using MCSA based minimization. [47]The binding energy and docking scores of all prepared ligands and reference compounds with 10 pdb of selected 5 proteins are mentioned in Table 1 & 2.

Table 1
Doking score and Binding energies of selected ligands and the reference compounds with Protien (Amine Oxidase, Protien Kinase)

| S.N.of Ligands | Protein Amine Oxidaze | | | | | | Protien Kinase | | | | | |
| | 2V5Z | | 2V60 | | 2V61 | | 2PWB | | 2YA3 | | 2QC6 | |
	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)
1	-6.22	-20.01	-6.15	-20.57	-6.45	-22.34	-5.65	-18.71	-4.72	-16.22	-6.83	-20.10
2	-7.96	-31.08	-7.14	-27.32	-7.32	-28.30	-5.12	-18.98	-4.43	-24.90	-7.54	-27.56
3	-8.21	-36.11	-7.41	-30.05	-7.24	-32.58	-5.45	-24.92	-6.38	-32.09	-7.62	-32.59
4	-9.56	-42.42	-8.38	-34.62	-8.82	-29.24	-3.81	-22.74	-5.54	-38.39	-8.15	-23.27
5	-9.53	-44.96	-7.07	-21.27	-9.18	-33.61	-3.16	-20.44	-4.65	-38.52	-5.72	-13.35
6	-8.88	-44.09	-8.05	-33.15	-8.95	-33.02	-1.99	-20.07	-4.36	-35.97	-6.30	-19.19
7	-9.35	-34.21	-6.94	-13.48	-8.23	-24.89	0.00	-20.49	-3.65	-34.49	-6.46	-17.31
*8	-6.41	-25.07	NA	NA	-6.15	-22.85	-2.68	-16.85	-2.50	-20.38	-5.44	-21.08
*9	-8.21	-50.53	-6.07	-23.89	-7.38	-24.95	-4.33	-37.32	-5.32	-52.28	-5.53	-37.53

***8*9-Reference compounds where *8 is R-(-) –deprenyl and *9 is capecitabin**

Table 2
Docking score and Binding energies of selected ligands and the reference compounds with Protien (P450, EGFR, NQO1)

| S.Nof Ligands | Protein P450 | | Protein EGFR | | Protein NQO1 | | | |
| | 4RUI | | 1M17 | | 2F10 | | 3JSX | |
	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)	docking score	glide energy (kcal/mol)
1	-6.12	-19.96	-4.80	-19.38	-4.07	-14.03	-5.25	-18.64
2	-7.65	-29.72	-6.49	-22.76	-4.29	-17.45	-6.96	-28.40
3	-7.74	-31.39	-6.00	-32.26	-4.84	-25.52	-7.71	-32.68
4	-6.04	-33.90	-5.99	-37.52	-4.47	-29.68	-6.57	-41.28
5	-6.32	-35.96	-6.31	-37.45	-4.51	-30.27	-6.42	-37.82
6	-4.46	-39.69	-5.15	-39.22	-3.27	-30.17	-6.20	-41.40
7	-5.14	-40.23	-5.98	-39.04	-3.80	-28.06	-7.16	-43.63
*8	-6.88	-29.71	-3.97	-25.66	-3.60	-27.08	-4.55	-24.88
*9	NA	NA	-6.29	-48.27	-4.47	-41.87	-6.76	-51.82

***8*9-Reference compounds where *8 is R-(-) –deprenyl and *9 is capecitabin**

ADME/T property analysis by Qik Prop 3.2
ADME (Absorption, Distribution, Metabolism and Excretion) properties were calculated by using Qik Prop designed by Professor William L. Jorgensen. With this utility a range of properties of a particular molecule can be compared with 95% of known drugs. It can also detect 30 functional groups that cause false positive results in high-throughput screening (HTS) assays. [48] In present study Qik Prop was used to predict Weakly polar component of SASA(WPSA)(acceptable range 0.0-175), total solvent-accessible volume in cubic Å radius (Volume) (acceptable range 500.0 – 2000.0), number of hydrogen bonds that would be donated(donor HB) (acceptable range 0.0-6.0), number of hydrogen

bonds that would be accepted(*accptHB*) (acceptable range 02-20.0), aqueous solubility log S in mol dm^{-3} (QPlogS)(acceptable range -6.5-0.5), IC50 value for blockage of HERG K+ channels (QPlogHERG) (acceptable range below –5), Caco-2 cell permeability in nm/sec, non-active transport(QPPCaco) (acceptable range <25 poor, >500 great), brain/blood partition coefficient(QPlogBB) (acceptable range –3.0 – 1.2), MDCK cell permeability in nm/sec(QPPMDCK)

(acceptable range <25 poor,>500 great), skin permeability log Kp(QPlog Kp)(acceptable range –8.0 – 1.0) human oral absorption on 0 to 100% scale(Percent Human Oral Absorption) (acceptable range <25% is poor>80% is high)and number of violations of Lipinski's rule of five. All values for above mentioned pharmacological parameters to determine ADME properties of designed ligands and reference compounds are given in Table-3.
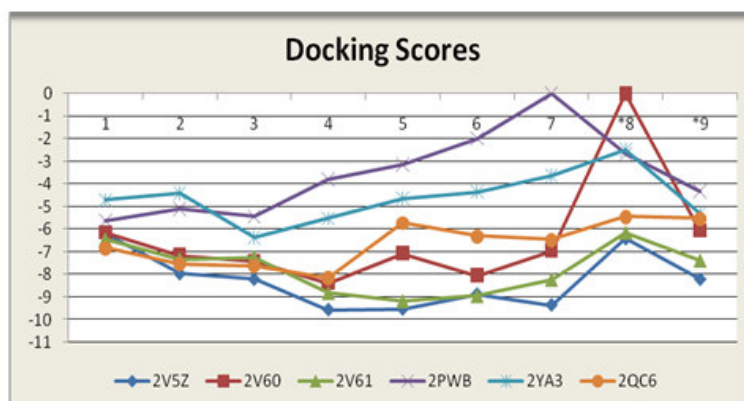
Table 3
***ADME and pharmacological parameters prediction for the ligands and
the reference compounds using QikProp***

| S.N	WPSA	Volume	donorHB	accptHB	QPlogS	QPlogHERG	QPPCaco	QPlogBB	QPPMDCK	QPlogKp	PercentHumanOralAbsorption	RuleOfFive
1	0	403.04	1.00	0.75	-0.05	-3.44	3002.27	0.10	1623.37	-1.64	100.00	0
2	0	516.26	0	2.50	-1.86	-3.88	2036.93	0.01	1067.38	-1.93	94.31	0
3	0	558.95	2.00	4.00	-1.33	-3.68	222.22	-0.92	97.34	-3.89	69.58	0
4	0	792.14	2.00	4.00	-3.03	-5.14	320.92	-1.00	144.82	-3.01	81.93	0
5	0	906.07	0	4.00	-3.75	-5.38	2940.52	-0.13	1587.31	-1.23	100.00	0
6	105.95	1012.50	0	4.00	-5.25	-5.50	3224.82	0.14	6673.76	-1.30	100.00	0
7	76.60	976.56	0	4.00	-4.80	-5.46	2942.23	0.04	4173.99	-1.35	100.00	0
*8	21.52	1140.31	4.00	11.10	-3.79	-5.08	48.54	-2.44	24.66	-5.29	57.16	0
*9	0	718.28	0.50	1.00	-3.37	-4.54	8855.31	0.28	5225.95	-0.40	100.00	0

RESULTS AND DISCUSSION

Docking simulation technique resulted with very interesting results. Results indicated that binding of all designed ligands with 3 pdb files 2v5z , 2v60, 2v61of Amine oxidase showed docking scores ranging from (-6.22 to -9.56), (-6.15 to -8.38) (-6.45 to -9.18) and binding energies ranging from (-20.01 to -44.96), (-13.48 to -34.62), (-22.34 to -33.61) respectively. Docking score of ligands with 3pdb files of Protein kinase 2pwb, 2ya3, 2qc6, were ranging from (-0.0 to -5.65), (-18.71 to -24.92), (-3.65 to -6.38) and binding energies ranging from (-16.22 to -38.52), (-5.72 to -4-8.15), (-13.35 to -32.59) respectively. Similarly docking score and binding energy of 1 pdb file 4rui of P450 was found to be in range of (-5.14 to -7.74) and (-19.96 to -

40.23) respectively. Docking score and binding energy of ligands with 1 pdb file 1m17 of EGFR was found to be (-4.80to -6.49), (-19.38 to -39.22) respectively. Likewise binding of ligands with 2 pdb files 2f10, 3jsx of NQO1 showed docking score ranging from (-3.27 to -4.84), (-5.25 to -7.71) and binding energies ranging from (-14.03 to -30.27) , (-18.64 to -43.63) respectively. Our results on 3 pdb files (2v5z,2v60,2v61) of Amine oxidase , 3 pdb files (2pwb,2ya3, 2qc6) of protein kinase,1 pdb file (1m17) of EGFR and 2 pdb files(2f10and 3jsx)of NQO1 proteins revealed that designed ligands had shown moderate to excellent binding with all pdb files. In most of cases they are showing better binding than reference drugs. Details of analysis of docking score and docking energy are well depicted in graphical representation (Figure-20, 21, 22, 23).
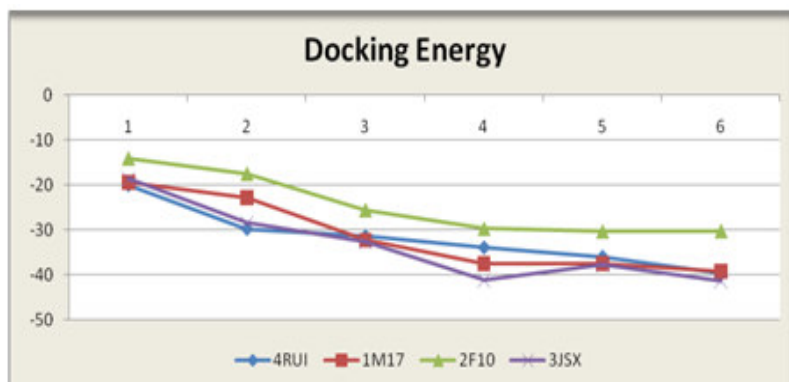

Figure 21
Graphical representation of docking score-Table-1
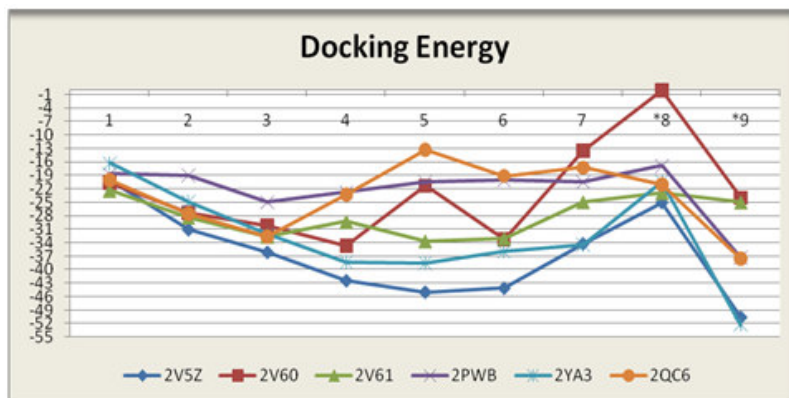
Figure 22
*Graphical representation of docking energy-Table-1*
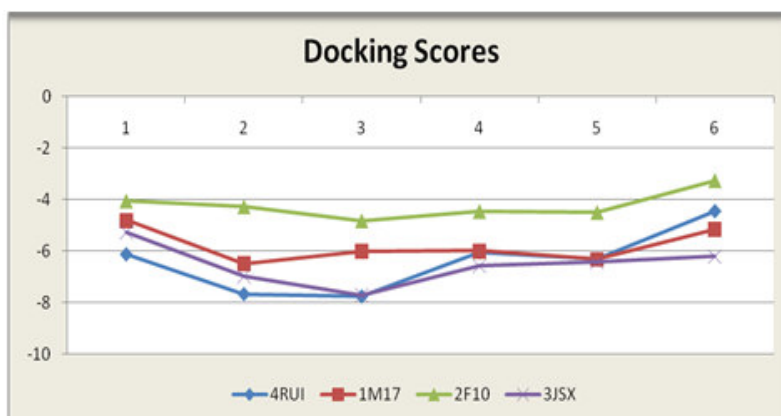
Figure 23
*Graphical representation of docking score-Table-2*

Figure 24
Graphical representation of docking energy-Table-2

Analysis of molecular docking studies revealed that out of seven designed ligands, five ligands showed excellent docking score. Ligand 5 showed best docking score and binding energy with two pdb files of Amine Oxidase as compared to other ligands. Values of docking score and docking energy of both pdb files i.e. 2v5z and 2v61 were found to be (-9.53), (-44.96) and (-9.18),(-33.61) respectively. Ligand 4 had shown second best docking score and docking energy with pdb files 2qc6 of protein kinase and 2v5z of Amine oxidase (docking score, -8.15 and docking energy, -23.27 kcal/mol) and (docking score, -9.56 and docking energy, -42.42) respectively. Pdb file 3jsx of Protein NQO1 showed good binding with ligand 7(docking Score, -7.16 and docking energy, -43.63). 3 D structures of binding of above mentioned best five ligands with proteins are given in (Figure 24, 25, 26,27, 28).
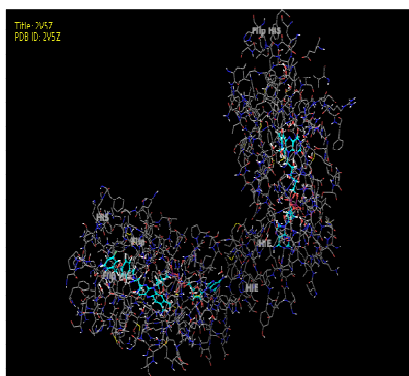
Figure 24
Binding of ligand 5 with 2v5z
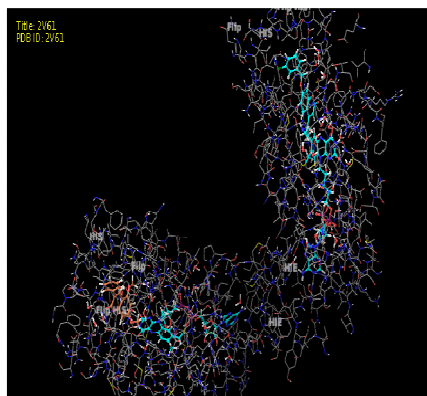
Figure 25
Binding of ligand 5 with 2v61
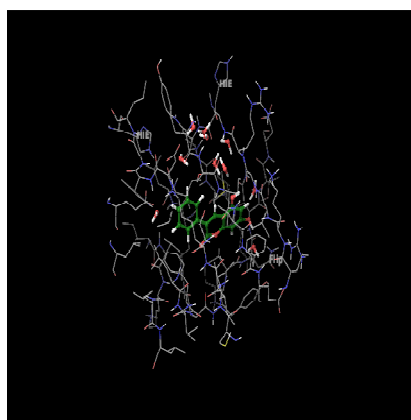
Figure 26
Binding of ligand 4 with 2qc6

Figure 27
Binding of ligand 4 with 2v5z

Figure 28
Binding of ligand 7 with 3jsx

By comparing the result of 3-Phenyl coumarin derivatives with known inhibitors (reference compounds) it was found that ligands 4, 5, 6 and 7 showed better docking interaction with pdb files 2v5z, 2v61 and 2qc6 than that of reference compounds Table-4.

Table 4
Comparison of best ligand score of 3- phenyl coumarin derivatives and known inhibitors

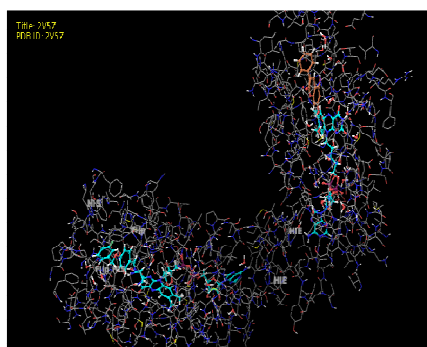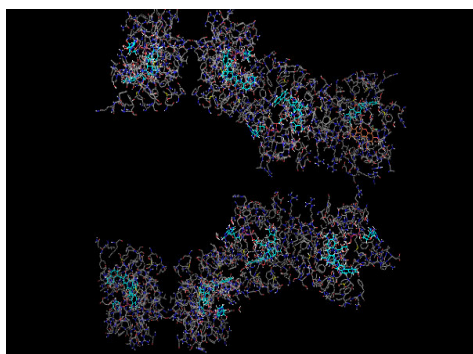| PDB Id | 3-Phenyl Coumarin Derivative | | | | | | | | Known Inhibitors | | | |
| | 4 | | 5 | | 6 | | 7 | | *8 | | *9 | |
	docking score	docking energy	docking score	Docking energy	docking score	docking energy	docking score	docking energy	docking score	docking energy	docking score	docking energy
2V5Z	-9.56	-42.42	-9.53	-44.96	-8.88	-44.09	-9.35	-34.21	-8.21	-50.53	-6.41	-25.07
2V60	-8.38	-34.62			-8.05	-33.15			-6.07	-23.89	Not Found	Not Found
2V61	-8.82	-29.24	-9.18	-33.61	-8.95	-33.02	-8.23	-24.89	-7.38	-24.95	-6.15	-22.85
2QC6	-8.15	-23.27							-5.53	-37.53	-5.44	-21.08

ADME-toxicity analysis
Various pharmacological properties for ADME toxicity analysis were calculated using QikProp (Schrödinger 2012) mentioned in Table- 3. The results indicated that all docked ligands were in acceptable range for WPSA, donorHB, accpt HB, QPCaco, QPlogS, QPlogK$_p$ and Rule of five. Ligands 2 to 7 are in acceptable range for Volume. Ligands 4, 5, 6 and 7 are in acceptable range for QPlogHERG. Ligands 1, 2, 5, 6 and 7 are in the acceptable for QPPMDCK. The best docking ligands 5, 6, 7 showed 100% percent Human-Oral absorption in comparison to capecitabin (57.16%).

CONCLUSION

The interaction of proteins with ligand molecules plays a major role in structural based drug designing. In the present work authors designed and docked seven ligands of differently substituted 3-Phenyl coumarin derivatives including two others i.e. phenol and 7-hydroxycoumarin (possible precursors of 3-phenyl derivatives) for better comparative docking score, binding energy and ADME/T properties. Analysis of docking studies showed that ligand 3, 4, 5, 6 and 7 showed best inhibition of enzyme activity with Amine oxidase and Protein Kinase. Most of the ligands showed moderate to excellent binding with selected proteins. These ligands showed better results than reference compounds. ADME-Toxicity prediction indicated that docked compounds had shown better pharmacological parameters than capecitabin and R-(-) –deprenyl. Therefore we conclude that these compounds can be developed as excellent lead for anticancer activity, as they are showing interaction with mostly all selected proteins. Authors wanted to generate these compounds as novel anticancerous drug with minimum side effect as coumarin derivatives are associated with antioxidant properties also. The study is further continued for the development of lead molecules in our laboratory.

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CONFLICTS OF INTEREST

Conflicts of interest declared none.

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