Research Article

In Silico Drug Design and Analysis of 4-Phenyl-4*h*-Chromene Derivatives as Anticancer and Anti-Inflammatory Agents

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ABSTRACT

Cancer constitutes the second main mortality cause in the world. 4*H*-Chromene derivatives are an attractive template for the identification of potential anticancer agents. In recent years, there has been much interest in this class of compounds and their potential utility as anti-cancer drugs along with anti-inflammatory activity. An attempt has been made to control such life threatening diseases by synthesizing chromene based compounds. In this study we have computationally investigated the binding interaction of 4-phenyl-4*H*-chromene analogues with some known anticancer and anti-inflammatory targets (1TUB, 1UOM and 1TNR) by using ArgusLab and AutoDock programs. Nine of these ligands showed high docking scores than the unsubstituted 4*H*-chromene and exhibited better drug-likeliness parameters as compared to the reference drugs.

Keywords: Anticancer and Anti-inflammatory targets, Oestrogen receptor, TNF-α receptor, Tubulin, 4-Phenyl-4*H*-chromene.

INTRODUCTION

Chromene (Benzopyran) is one of the privileged medicinal scaffold which appears as an integral part in natural compounds and generated great attention because of their interesting biological activity. Chemically, it is a heterocyclic ring system consisting of a benzene ring fused to a pyran ring. Chromene composes the basic back bone of various types of polyphenols and widely found in natural alkaloids, flavonoids, anthocyanins and tocopherols.[1]

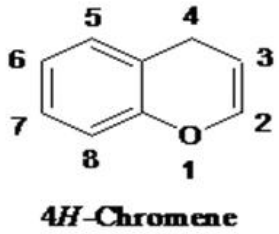


Figure 1: Structure of 4*H*-chromene

Presence of the chromene-based structure in a molecule is associated with its capacity to prevent diseases.[2] It is known that certain natural and synthetic chromene derivatives possess diverse biological activities such as antitumor, antivascular[3], antimicrobial[4], antioxidant[5], TNF-α inhibitor[6], antifungal[7], anticoagulant, antispasmolytic, estrogenic[8], antiviral[9], anti-helminthic, anticancer[10], anti-HIV[11], antitubercular[12], anti-inflammatory[13], herbicidal, analgesic and anticonvulsant[14] activity. A key aspect is that the lipophilic nature of the benzopyran derivatives helps to cross the cell membrane more easy way.[15]

Out of diverse array of chromenes, 4*H*-chromenes are of interest to present research workers. The potency of these clinically useful pharmacophore in the treatment of cancer and inflammation and other activities encouraged the development of some more potent and significant compounds. In recent years, there has been much interest in this class of compounds and their potential utility as anti-cancer drugs. The anti-inflammatory activity of 4*H*-chromene derivatives helps to decrease the intensity of pain related to carcinomas. In this research project, *in silico* designing of a group of novel 4-phenyl-4*H*-chromene with potential application as oestrogen receptor and tubulin receptor targeting ligands for is undertaken because these derivatives are currently in clinical trials for the treatment and prevention of breast cancer. Along with this, anti-inflammatory activities of the same 4-phenyl-4H-chromene analogues were evaluated for their *in silico* anti-inflammatory activity against TNF-α receptor target.

MATERIALS AND METHODS

Group of compounds were designed by substituting at 4th and 7th positions of 4*H*-chromene. Nine substitutions were selected to complete the group. The 4th position was designed by substituting 3-substituted phenyl ring and 7th position with electron donating groups. These compounds are evaluated against targets like tubulin, oestrogen receptor (ER) and TNF-α receptor (TNFR). In this study, tamoxifen and ibuprofen were used as reference drugs for cancer and inflammation respectively.

4-Phenyl-4*H*-chromene derived compounds were indicated in the whole study by using code numbers. Name and code of the analogues were listed in the table 1.

Designing of ligand structure

The structure of the ligands were generated by using ChemSketch which is a chemically intelligent drawing interface freeware developed by Advanced Chemistry Development. The ligands were drawn and the smile

notations were generated. These smile notations were used for the development of the 3D structure of the ligands by using another online server called CORINA.

Preparation of protein structure

The crystal structures of the proteins (1TUB, 1UOM, 1TNR) were obtained from the Protein Data Bank (PDB).

Active site identification

All the targets were possessing natural ligand and so active site residue identification was carried out taking advantage of the same. The protein was loaded in SWISS PDB Viewer. Proteins which have many chains were cleaned and a single chain of interest was selected. Using the control panel of this software, natural molecules were selected. All the residues surrounding this ligand which comes in 6.00A^0 were identified and selected. These molecules were checked in previous literature to confirm the selection and also their hydrophobic properties were checked to confirm its presence in the binding pocket. The same procedure was carried out for all the targets.

Preparation of active site

Explicit Hydrogen atoms missing in the PDB structure were added using ArgusLab. Furthermore the atom list of the molecules were prepared, which represents numbers of all the atoms of the active site residues involved.

Energy minimization

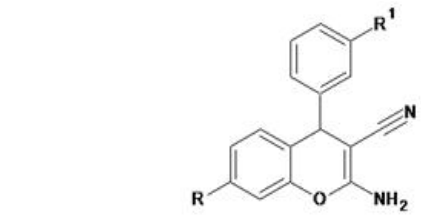
Hydrogen added clean files of proteins were loaded in online software, CHIRON. Chiron performs rapid energy minimisation of protein molecules using molecular dynamics with an all-atom representation for each residue in the protein. The conformations and energy states of the newly added hydrogen were fixed and corrected by minimizing the energy.

Molecular docking

The docking was performed to study the binding efficacy of the compounds and the target. The docking was done by using two softwares, ArgusLab and AutoDock. Preliminary docking was performed using ArgusLab molecular modelling that was quick enough than AutoDock. The preliminary screening helps to compare the docking score of unsubstituted chromene with those ligands. Then the pre-screened ligands were validated using AutoDock version 4.0 which is more efficient and flexible. The score obtained was more accurate if it takes much longer time for docking than ArgusLab. The more negative binding energy values were considered as the binding affinity value of the ligands for each docking. These values and their interaction with the protein were tabulated.

Drug-Likeness screening

The ligands were further submitted for drug-likeness by calculating total polar surface area (TPSA) and the Lipinski parameters using online softwares like molsoft and molinspiration.



2-amino-3-cyano-4-(3'-substituted phenyl)-7-substituted-4*H*-chromene

R^1 = -Br, -Cl, -F

R = -OH, $-OCH_3$

Figure 2: General structure of designed compounds

Table 1: Compound codes of 4-Phenyl-4*H*-chromene analogues

Compound name	Compound code
2-amino-3-cyano-4-(3'-bromophenyl)-7-hydroxy-4*H*-chromene	CS1
2-amino-3-cyano-4-(3'-chlorophenyl)-7-hydroxy-4*H*-chromene	CS2
2-amino-3-cyano-4-(3'-bromophenyl)-7-methoxy-4*H*-chromene	CS3
2-amino-3-cyano-4-(3'-chlorophenyl)-7-methoxy-4*H*-chromene	CS4
2-amino-3-cyano-4-(3'-fluorophenyl)-7-hydroxy-4*H*-chromene	CS5
2-amino-3-cyano-4-(3'-fluorophenyl)-7-methoxy-4*H*-chromene	CS6
2-amino-3-cyano-4-(3'-chlorophenyl)-7-dimethylamino-4*H*-chromene	CS7
2-amino-3-cyano-4-(3'-bromophenyl)-7-dimethylamino-4*H*-chromene	CS8
2-amino-3-cyano-4-(3'-fluorophenyl)-7-dimethylamino-4*H*-chromene	CS9

Table 2 : ArgusLab docking for the designed 4*H*-chromene derivatives

Compound Code	Docking Score (kcal/mol)		
	Tubulin	ER	TNFR
CS1	-11.29	-12.11	-9.65
CS2	-11.14	-13.16	-9.52
CS3	-10.91	-12.23	-9.17
CS4	-11.07	-10.62	-8.89
CS5	-10.97	-12.22	-9.38
CS6	-10.47	-10.79	-8.26
CS7	-9.31	-12.46	-9.12
CS8	-9.63	-11.20	-8.64
CS9	-9.32	-10.37	-8.51
Unsubstituted 4-phenyl-4*H*-chromene	-9.27	-10.19	-8.21
Colchicine	-7.59		
Tamoxifen		-9.71	
Ibuprofen			-10.24

RESULTS AND DISCUSSION

The docking scores obtained from the preliminary docking program by using ArgusLab were listed in the table 2.

All the compounds were docked against the three known targets. Tubulin (PDB: 1TUB) and oestrogen receptor (PDB: 1UOM) were anticancer targets and TNF-α receptor (PDB: 1TNR) was the anti-inflammatory protein target. In the case of binding interactions of protein targets, all the generated ligands showed higher docking score than their unsubstituted chromene scaffold. This can be assured by the pre-screening program of ArgusLab.

The inhibition of the tubulin and oestrogen receptors helps to suppress the rapid progression of the cancer. In the case of tubulin, these derivatives were intended to bind at the colchicine binding site of tubulin.[9] So the docking score of the compounds targeted tubulin were evaluated against the docking score of colchicine. All the compounds showed better binding interaction with target than colchicine. Then the docking scores of the compounds targeted oestrogen receptor were compared with the score of tamoxifen (-9.71 kcal/mol) which is used as a potent drug for the treatment of breast cancer. All the compounds have showed decent binding interaction with ER and higher docking score than Tamoxifen. In addition, all these derivatives were targeted with TNF-α receptor for its anti-inflammatory activity. It showed less docking score when compared with the standard drug ibuprofen, but tends to have anti-inflammatory activity.

4-Phenyl-4*H*-chromene analogues listed above was subjected to docking with the crystallized structure of targets by AutoDock version 4.0 screening program. The bound analogues were examined for their binding energies and hydrogen bonding, which is shown in the table 3. The conformations with the highest binding energy and greater number of hydrogen bonds in all the ligands were taken in consideration for ranking the analogues.

All the derived analogues showed higher docking scores than the unsubstituted 4-phenyl-4*H*-chromene. AutoDock program that typically yields lower scores when compared to ArgusLab docking score, but it makes more accurate values. All the cancer targeted compounds showed higher docking score than their standard drug. CS4 has the highest docking score against tubulin (-8.17 kcal/mol) and CS5 has the highest docking score against oestrogen receptor (-6.76 kcal/mol). TNFR targeted compounds showed better docking score but it is lower when compared to the reference drug. The main observation obtained from these data that the values of ArgusLab and AutoDock were not comparable. One of the examples was that the ArgusLab values of CS1 and CS2 targeted against ER were -12.11 kcal/mol and -13.16 kcal/mol but the scores after AutoDock program were -6.18 kcal/mol and -5.97 kcal/mol. This indicates the higher docking score for a ligand in ArgusLab does not necessarily occur in the AutoDock results. So this confirms that there is no correlation between the scores obtained from ArgusLab and AutoDock.

Table 3: AutoDock Docking Scores

Compound Code	Docking Score (kcal/mol)		
	Tubulin	ER	TNFR
CS1	-7.54	-6.18	-5.82
CS2	-7.87	-5.97	-5.12
CS3	-7.92	-6.29	-5.87
CS4	-8.17	-6.4	-5.89
CS5	-7.78	-6. 76	-5.79
CS6	-7.34	-6.21	-5.5
CS7	-7.32	-6.32	-5.59
CS8	-7.44	-5.71	-5.37
CS9	-7.65	-6.08	-5.42
Unsubstituted 4-phenyl-4*H*-chromene	-7.27	-4.85	-4.93
Colchicine	-5-41		
Tamoxifen		-3.86	
Ibuprofen			-6.45

Table 4: Ligand-target hydrogen bonding interactions

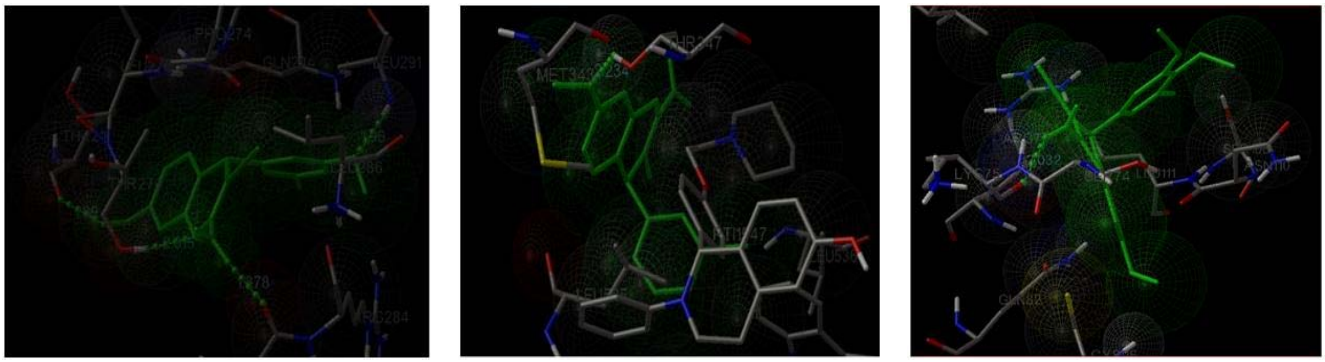
Compound Code	Hydrogen Bonding Interactions		
	Tubulin	ER	TNFR
CS1	TYR283 THR216 THR276	ASP351	LYS75
CS2	TYR283 THR276 THR216	THR347	ASN110
CS3	LYS19 HIS229	ASP351	SER74 LEU159
CS4	LEU291 TYR283 THR216 THR276	THR347	CYS73
CS5	THR276 TYR283 THR216	Nil	LEU159 PRO37
CS6	TYR283 THR276	Nil	LYS75
CS7	Nil	THR347	LEU159
CS8	Nil	ASP351	SER74
CS9	Nil	Nil	LYS75
Unsubstituted 4-phenyl-4*H*-chromene	TYR283 THR276	Nil	CYS76

AutoDock screening program also helps to know about the hydrogen bonding interactions of all the derived compounds. It shows in the table 4.

Number of hydrogen bonding will considerably increase the affinity of ligand-target interaction. AutoDock results shows that most of the chromene derivatives have hydrogen bonding between the ligand-target interactions. Some of them have more than one hydrogen bond that is, most of the tubulin targeted derivatives shows more than one hydrogen bond commonly TYR283, THR276 and TR216. CS4-tubulin complex has the highest docking score (-8.17 kcal/mol) which shows four hydrogen bonding interactions. All analogues targeted the other two proteins shows one or two hydrogen bonding interactions. This hydrogen bonding interactions helps to increase the binding energy of ligand-protein interactions.

The derived analogues were evaluated for their drug-likeness. It was done by calculating the parameters like Lipinski rule of five[16] and some of their extension parameters like number of rotatable bonds and TPSA. The drug-likeness assessments of the compounds were shown in the table 5.

This result shows that the value of all the derivatives relies within the optimal range. All the compounds have the molecular weight less than 500 daltons and possess number of hydrogen bond donors and hydrogen bond acceptors of all the analogues below 5 and 10 respectively. All the values of partition coefficient and number of rotatable bonds were coming under the limit of 5. Moreover, none of the analogues exhibited TPSA greater than 140 Å². All these data indicates that it has no more violations likely to be an orally active drug.



CS4-1TUB **CS4-1UOM** **CS1-1TNR**

Figure 3: Ligand-target complexes with hydrogen bonding interactions

Table 5: Drug-likeness assessments of the 4-phenyl-4*H*-chromene derivatives

Compd Code	Molecular Formula	Mol.Wt (g/mol)	No. of HBA	No. of HBD	Mi LogP	No. of rot. b	TPSA (Å²)
CS1	$C_{16}H_{11}BrN_2O_2$	343.18	3	3	3.023	1	79.277
CS2	$C_{16}H_{11}ClN_2O_2$	298.72	3	3	2.892	1	79.277
CS3	$C_{17}H_{13}BrN_2O_2$	357.20	3	2	3.559	2	68.283
CS4	$C_{17}H_{13}ClN_2O_2$	312.75	3	2	3.428	2	68.283
CS5	$C_{16}H_{11}FN_2O_2$	282.27	3	3	2.377	1	79.277
CS6	$C_{17}H_{13}FN_2O_2$	296.30	3	2	2.913	2	68.283
CS7	$C_{18}H_{16}ClN_3O$	325.79	2	2	3.473	2	62.287
CS8	$C_{18}H_{16}BrN_3O$	370.25	2	2	3.604	2	62.287
CS9	$C_{18}H_{16}FN_3O$	309.34	2	2	2.959	2	62.287

CONCLUSION

Structure based drug designing is a wide area in rational drug designing that offers a valuable alternative to the costly and time consuming process of random screening. In this approach, docking protocol was applied which ensure us to find the prospective ligands that can bind to the active sites of the anticancer and anti-inflammatory (1TUB, 1UOM and 1TNR) targets. Softwares like ArgusLab and AutoDock 4.0 were used to prove the binding affinity of the derivatives. 4-Phenyl-4*H*-chromene was used as the lead compound and their nine analogues showed higher docking score against the selected targets. All of them have docking score higher than their unsubstituted 4-Phenyl-4*H*-chromene. In the case of anticancer targets, derivatives have higher docking score than the standard drug tamoxifen. These analogues have better but not higher docking score against the target 1TNR which is a TNF-α receptor. This indicates that these 4-phenyl-4*H*-chromene analogues have significant cytotoxicity with medium level of anti-inflammatory activity which helps to decrease the pain related to the cancer. The final assessment of drug-likeness and its related parameters helps to confirm the oral activity of the compounds. Thus we strongly hope that this molecular docking approach will lead to novel insight into these classes of ligand molecules, which could further enhance our

understanding to design novel compound and to identify the more efficient compounds to be synthesized. These informations about the cytotoxicity and anti-inflammatory activity of the 4-phenyl-4*H*-chromene derivatives will be more strongly proven through the synthesis and in-vitro studies of these analogues.

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