Original Article

Designing And *In Silico* Screening Of Novel Umbelliferone-Imines for Anti-HIV And Anti-Cancer Activities

Akshat Uniyal[1], Jitender Gairolla[2], Anuj Saini[3], Deepak Rana[4], Amit Anthwal[5]*

[1] Alpine Institute of Management and Technology, Dehradun, Uttarakhand, 248007.
[2] Department of Microbiology, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India.
[3, 4, 5]*School of Applied and Life Sciences, Uttaranchal University, Dehradun, Uttarakhand, 248007, India.

***Corresponding author: -** Dr. Amit Anthwal

*School of Applied and Life Sciences, Uttaranchal University, Premnagar, Dehradun 248007, India,
E-mail:- anthwalamit1984@gmail.com
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Abstract

HIV is a viral infection which is to be addressed with great concern. Another fatal disease is cancer responsible for high mortality. In present work we herein report designing of Umbelliferone-imine **(1-16)** and their *in-silico* screening on HIV 1 protease and Cell division protein kinase 2 enzymes using online software SwissDock. Also, theoretical molecular properties and biological activities of newly designed compounds were predicted with help of free online screening engines namely Osiris and Molinspiration. The predicted value of ΔG for HIV 1 protease were in range of -9.34 Kcal/mol to -8.40 Kcal/mol which indicated very high binding affinities of target and ligand. On basis of binding of ligand to protein residue and from literature it was concluded molecules bind at site of anti-HIV drugs and hence may act as potent HIV 1 protease inhibitors. The Cell division protein kinase 2 inhibition was also predicted in terms of ΔG which ranged from-8.24 Kcal/mol to -7.43 Kcal/mol.

Keywords: HIV 1 protease, Umbelliferone, docking, Cell division protein kinase 2.

INTRODUCTION:

Umbelliferone or hydroxycoumarin are reported to show wide range of biological activities. Coumarins are precursor of many pharmaceutical reagents such as anticoagulants beside this coumarin derivatives are reported to show antioxidant, antibacterial, anti-inflammatory, anticoagulant, anticancer, antiviral and antifungal activities [1-7]. Viruses are great threat to globe and it is very much clear from present Covid-19 pandemic. Owing to their high tendency to undergo mutation and develop new strain treatment of viral disease is challenge for us. Due to this there is thrust for new antiviral agent. HIV is one the major cause of mortality and morbidity worldwide. At present number of approved drugs for HIV is around 20 and most of them act by either inhibiting HIV reverse transcriptase or protease [8]. Due to mutant nature of virus it very easily develops drug resistance. Replication of HIV virus involves nearly 10 steps and it give opportunity to researcher to develop chemotherapeutics which curbs the virus multiplication at one of the steps. The most important steps among these are preparation of the proviral DNA from viral RNA by process of reverse transcription and HIV protease catalysed precursor proteins proteolysis. The Protease and Reverse Transcriptase enzyme inhibitor are still best choice for developing HIV drugs but major issue with these drugs is toxicity and drug resistance hence to improve high antiretroviral therapy there is need for development of more potent and safer antiviral agent. In research carried out by various groups it was proved that coumarin derivatives are emerging anti-HIV agents [9-11]. Many natural and synthetic coumarin derivatives are reported to show anti-HIV activity [12]. One of the major causes of death worldwide is cancer. Coumarin derivatives owing to their diverse biological activity are reported to show anti-cancer activity. Cell division protein kinase 2 which is also known by name Cyclin-dependent kinase-2 (CDK2) is crucial for cell division. CDK2 inhibitor are considered to be very important class of antineoplastic agents. In thrust to develop new hybrid molecule having diverse biological activitivities [13-18], we here in report designing and *in silico* anti-cancer and anti-HIV screening of Umbelliferone-imines. For docking studies of designed compounds Hiv1 protease and Cell division protein kinase 2 were selected as target proteins. SwissDock online software was used for *in silico* screening of compounds and molecular properties were predicted using Molinspiration and Osiris software.

MATERIALS AND METHODS:

Molinspiration

Molinspiration molecular properties explorer online software was used for predicting various molecular properties of compounds [19]. Using this software various physical properties were theoretically predicted like molecular weight, log P, N-violation and TPSA (total polar surface area) and values of biological properties in terms of G-protein-coupled receptors abbreviated as GPCR, kinase inhibitors abbreviated as KI, ion channel modulators abbreviated as ICM, protease

inhibitors abbreviated as PI, nuclear receptor ligands abbreviated as NRL, and enzyme inhibitor abbreviated as EI were predicted. The predictions of software are presented in tabular form in Table 2.

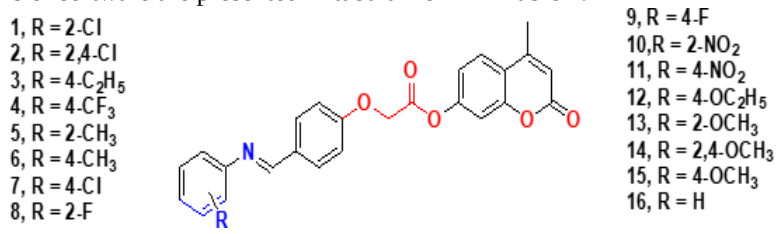

Figure 1. Structure of Umbelliferone-imines

Table 1. IUPAC names of designed compounds

S.No.	IUPAC name compounds
1	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((2-chlorophenyl)imino)methyl)phenoxy)acetate
2	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((2,4-dichlorophenyl) imino) methyl) phenoxy) acetate
3	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4-ethylphenyl)imino)methyl)phenoxy)acetate
4	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4-(trifluoromethyl) phenyl) imino) methyl) phenoxy) acetate
5	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-((o-tolylimino)methyl)phenoxy)acetate
6	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-((p-tolylimino)methyl)phenoxy)acetate
7	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4-chlorophenyl)imino)methyl)phenoxy)acetate
8	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((2-fluorophenyl)imino)methyl)phenoxy)acetate
9	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4fluorophenyl)imino)methyl)phenoxy)acetate
10	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((2-nitrophenyl)imino)methyl)phenoxy)acetate
11	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4-nitrophenyl)imino)methyl)phenoxy)acetate
12	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4-ethoxyphenyl) imino) methyl) phenoxy) acetate
13	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((2-methoxyphenyl) imino)methyl) phenoxy) acetate
14	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((2,4-dimethoxyphenyl) imino) methyl)phenoxy) acetate
15	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-(((4-methoxyphenyl) imino)methyl) phenoxy) acetate
16	(E)-4-methyl-2-oxo-2H-chromen-7-yl 2-(4-((phenylimino)methyl)phenoxy)acetate

Table 2. Molinspiration predictions of Umbelleferone-imines (OH-NH interaction value is zero for all compounds)

Molecular properties Data Predictions						Drug-likeness Properties Data Predictions					
S.No.	M.W	*mi*logp	TPSA	N violation	Vol.	GPCR	ICM	KI	NRL	PI	EI
1	447.87	5.55	78.11	1	378.52	-0.62	-0.94	-0.48	-0.34	-0.63	-0.33
2	482.32	6.21	78.11	1	392.05	-0.60	-0.91	-0.48	-0.34	-0.62	-0.33
3	441.48	5.84	78.11	1	398.34	-0.61	-0.87	-0.48	-0.24	-0.58	-0.28
4	481.43	5.82	78.11	1	396.28	-0.54	-0.78	-0.36	-0.14	-0.53	-0.27
5	427.46	5.32	78.11	1	381.54	-0.63	-0.88	-0.45	-0.25	-0.59	-0.28
6	427.46	5.37	78.11	1	381.54	-0.65	-0.93	-0.46	-0.29	-0.62	-0.32
7	447.87	5.60	78.11	1	378.52	-0.64	-0.91	-0.46	-0.29	-0.64	-0.33
8	431.42	5.04	78.11	1	369.91	-0.67	-0.93	-0.45	-0.29	-0.61	-0.28
9	431.42	5.09	78.11	1	369.91	-0.63	-0.92	-0.42	-0.26	-0.62	-0.31
10	458.43	4.83	123.94	0	388.31	-0.69	-0.96	-0.62	-0.47	-0.73	-0.37
11	458.43	4.88	123.94	0	388.31	-0.71	-0.89	-0.53	-0.32	-0.67	-0.35
12	457.48	5.36	87.35	1	407.33	-0.64	-0.88	-0.47	-0.28	-0.61	-0.32
13	443.45	4.93	87.35	0	390.53	-0.62	-0.97	-0.51	-0.28	-0.62	-0.35
14	473.48	4.96	96.58	0	416.07	-0.59	-0.94	-0.47	-0.25	-0.60	-0.33
15	443.45	4.98	87.35	0	390.53	-0.63	-0.91	-0.44	-0.29	-0.61	-0.31
16	413.43	4.92	78.11	0	364.98	-0.65	-0.94	-0.46	-0.28	-0.62	-0.31

OSIRIS

Osiris is free online molecular property explorer available online for predicting theoretical properties of molecules[20]. Using software biological properties like possibility of molecule to show mutagenic, tumorogenic, irritant behaviour and effects on reproductive system are predicted. Besides, physical properties like molecular weight, log P value, TPSA etc and biological property indicators like drug likeness and drug score were also predicted. The values of above-mentioned properties are tabulated in table 3. In table 3, G stands for No toxicity risk, Y stands for Low Toxicity Risk, R stands for High Toxicity risk MUT abbreviation of Mutagenic; TUMO abbreviation of Tumorogenic; IRRI abbreviation of Irritant; REP abbreviation of Reproductive effective. Mol. Wt abbreviation of Molecular weight in g/mol; clogP corresponds to octanol/water partition coefficient; S abbreviation of Solubility; DL abbreviation of Drug likness; D-S abbreviation of Drug-score. TPSA abbreviation of total polar surface area

Table 3. Osiris predictions of Umbelleferone-imines derivatives

S.No.	Toxicity Risk Prediction Data				Molecular Properties Data Predictions					
	MUT	TUMO	IRRI	REP	M.W.	cLog P	S	D-L	D-S	TPSA
1	G	G	G	R	447.87	4.56	-5.63	-17.12	0.20	74.19
2	G	G	G	R	482.32	5.17	-6.36	-15.13	0.17	74.19
3	R	G	G	R	441.48	4.71	-5.39	-17.98	0.12	74.19
4	R	G	G	R	481.42	4.80	-5.67	-26.42	0.11	74.19
5	G	G	G	R	427.45	4.30	-5.23	-15.54	0.21	74.19
6	R	G	G	R	427.45	4.30	-5.23	-19.21	0.13	74.19
7	G	G	G	R	447..87	4.56	-5.63	-17.12	0.20	74.19
8	G	G	G	R	431.42	4.06	-5.20	-17.08	0.22	74.19
9	G	G	G	R	431.42	4.06	-5.20	-19.04	0.22	74.19
10	R	R	G	R	458.42	3.03	-5.35	-22.64	0.08	120.0
11	R	R	G	R	458.42	3.03	-5.35	-28.89	0.08	120.0
12	R	R	R	R	457.46	4.29	-5.21	-18.69	0.06	83.42
13	G	G	G	R	443.45	3.88	-4.91	-15.48	0.22	83.42
14	G	G	G	R	473.48	3.81	-4.93	-18.36	0.21	92.65
15	G	G	R	R	443.45	3.88	-4.91	-19.01	0.17	83.42
16	G	G	G	R	413.43	3.95	-4.89	-16.07	0.23	74.19

In silico anti-HIV activity

In silico anti-HIV activity was predicted using SwissDock online software [21]. For docking studies HIV 1 protease was selected having PDB code 1A30. The protein was uploaded from SwissDock data bank for docking and ligand file was uploaded on online portal in .mol2 format after which the docking results were obtained online. The binding affinity of new compounds in terms of ΔG (Kcal/mol) was predicted. The binding affinity data of compounds is presented in table 4. It has been reported that anti-HIV drug Indinavir and many other drugs bind to meta-pocket with Arg8, Leu23, Asp25, Asp29, Asp30, Gly27, Gly48, Gly49, Ile47, Ile50, Pro81 etc. residues[22]. Some of the compounds demonstrated H-bond with Ile50 and Asp29 residues which indicates that the designed compound interacts with HIV 1 protease at binding site of drugs.

Table 4. Docking studies of Umbelliferone-imines on HIV 1 protease (1A30)

Compound No.	ΔG (Kcal/mol)	No. of H-bonds	Residues Involved in Binding
	-9.31		Ile50
2	-9.00	0	-
	-8.92		
4	-8.97	0	-
	-8.55		
6	-8.97	0	-
	-9.07		Ile50
8	-8.4	0	-
	-9.34		Ile50
10	-9.13	1	Ile50
11	-8.56		
12	-9.18	2	Asp29, Ile50
13	-9.12		
14	-8.64	0	-
15	-8.68		Ile50
16	-9.00	0	-

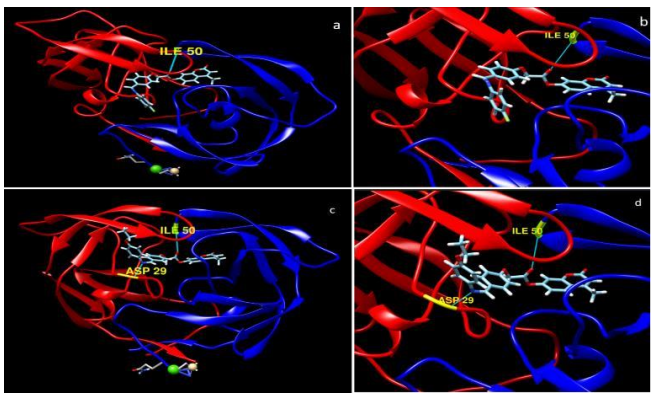

Figure 2. a & b Docking images of compound 9 with HIV 1 protease enzyme. c & d Docking images of compound 12 with HIV 1 protease enzyme

In silico anti-cancer activity

In silico anticancer activity of compound was screened using SwissDock online software [21]. For docking studies cell division protein kinase 2, PDB code 1B38, was considered as it interferes with process of cell division and helps to control cancer. The protein was used from SwissDock data bank and ligands used were the newly designed compounds. The binding affinity of new compounds in terms of ΔG (Kcal/mol) was predicted online. The binding affinity data of compounds is presented in table 5. It was found that none of the compound showed H-bonding with any of the residue of amino acids of cell division protein kinase 2.

Table 5. Docking studies of Umbelliferone-imines on cell division protein kinase 2 PDB code 1B38

Compound No.	ΔG (Kcal/mol)	Compound No.	ΔG (Kcal/mol)
	-7.92		-7.51
2	-8.16	10	-7.78
	-7.71	11	-7.78
4	-7.87	12	-7.57
	-7.65	13	-8.24
6	-7.74	14	-8.17
	-7.80	15	-8.09
8	-8.19	16	-7.43

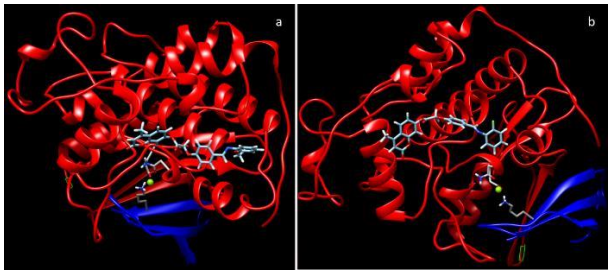

Figure 3. a) Docking image of compound 13 with cell division protein kinase 2. b) Docking image of compound 8 with cell division protein kinase 2.

RESULTS & DISCUSSION:

The molinspiration data reveals that six compounds don't have any violation of rule of 5. In case of Osiris predictions most of the molecules may not show mutagenic, tumorigenic or irritant effect but may have reproductive effects. The drug score is summation of all the properties of molecules, all the designed molecules have positive value for drug score. The results of *in silico* anti-HIV activity in terms of docking score (ΔG value) with HIV 1 protease enzymes were very inspiring. Compound 9 with -F group at 4-position gave highest binding score. It may be due to possibility hydrogen bonding between Fluorine and amino acid residue at site of enzyme. Eight compounds had ΔG values greater than -9.0 Kcal/mol which can be attributed to excellent target-ligand interaction. The tentative mechanism of action of designed compounds may be by inhibiting HIV protease enzyme which will in turn prohibit replication of virus. This is in confirmation with literature where coumarin derivatives are reported to show good anti-HIV activity. Besides, the binding of molecules with Ile50 and Asp29 residues, which are among residues reported to have interaction with drug, proves that the compounds bind at site where anti-HIV drugs are binding for inhibition of activity of enzyme. The designed compounds also demonstrated good binding score with CDK2 enzyme which hints towards capability of compounds to exhibit good anti-cancer activity. The compound 13 with -OCH_3 group at ortho position gave highest binding score of -8.24 Kcal/mol whereas placing -OCH_3 group at para position reduces value to -8.09 Kcal/mol. Besides, compound 8 with -F group at 2-position stands second in terms of binding energy with value -8.19 Kcal/mol. The binding scores with CDK2 reveals that compounds may exhibit good anti-cancer activity. None of the compound formed H-bond with protein residues present at binding site.

CONCLUSIONS:

It may be concluded from above studies that if synthesized and tested for *in-vitro* anti-HIV activity the designed compounds may demonstrate excellent activity. These compounds may be further modified and docked for in-depth QSAR studies which may give a lead molecule for further research. These compounds are expected to show good anti-cancer activity as per the docking score of the compounds with CDK2 enzyme. If we compare the docking scores the compounds anti-HIV activity is expected much better than anti-cancer. The designed compounds after docking with other enzyme targets for anti-HIV and anti-cancer activity may give some interesting results.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding current research work**.**

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