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Novel Coumarin Derivatives as Potential HDAC2 Inhibitors : *in silico* Study

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ABSTRACT

Designing of selective HDAC2 inhibitors will have less adverse effects and better safety profiles. For HDAC2 selectivity, the coumarin derivatives were designed according to the structural requirement of HDAC2 inhibitors. The designed derivatives were then subjected to docking studies and ADME screening by *in silico* approach. Results showed that compounds had good binding affinity towards HDAC2 and also had drug likeness property. The results of the study can be used for further structural modifications, synthesis and biological evaluation of selective HDAC2 inhibitors.

Key words : ADME, coumarin, drug likeness, HDAC2 inhibitors, molecular docking

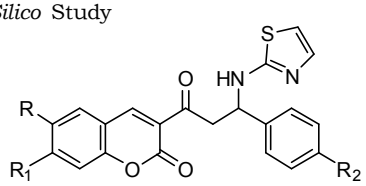
INTRODUCTION

Cancer is a disease caused not only by genetic mutations but also by epigenetic changes. These epigenetic changes include DNA methylation and post-translational histone acetylations that change DNA accessibilities and chromatin structures. There are atleast eight different types of histone post-translational modifications, namely, methylation, acetylation, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, deamination and proline isomerization. The acetylation and deacetylation of histones are controlled by two enzymes : histone acetyltransferase (HAT) and histone deacetylases. The balance between acetylation and deacetylation of histones regulate gene expression through chromatin modifications (Li and Seto, 2016). An excessive level of histone acetylation causes transcriptional activation and results in apoptotic cell death, whereas deacetylation promotes transcriptional repression by silencing of genes and results in cancer pathologies by repressing tumor regulatory genes. Thus, disruption in the activities of these enzymes results in various kinds of cancers (Mottamal *et al.,* 2015). In recent years, overexpression of HDACs had been reported in many cancer cells. Thus, in recent years HDAC had been identified as a validated target for cancer. In human genome, 18 HDAC family members have been recognized and are grouped in four classes. HDAC2 belongs to class I family and is a Zn^{+2} dependent metalloprotein. It had been reported to be overexpressed in many cancer cells. In recent years, it had become a therapeutic target for effective cancer therapy. In cancer pathological conditions where the HDAC2 is overexpressed, inhibitors of HDAC2 were found to be effective in reversing the malignant phenotype of tumor cells and have subsequently emerged as promising cancer therapeutic agents (Bondarev *et al.,* 2021). HADC2 inhibitors have the potential to inhibit multiple signalling pathways to inhibit tumor growth and induce apoptosis. They not only targets histones but have the ability to influence a variety of processes such as cell angiogenesis, cell cycle arrest, immune modulation and apoptosis by targeting non-histone proteins. Disruption of multiple pathways and lack of specificity result in major side effects like bone marrow depression, adverse effects to gastrointestinal tract, fatigue, vomiting, diarrhea and nausea (McClure *et al.,* 2018). Thus designing of specific HDAC2 inhibitors is need of the hour. Till date six HDAC inhibitors have been approved by FDA. They are vorinostat, romidepsin, belinostat (PXD101), pracinostat, panobinostat and chidamide for the treatment of hematological malignancies.

MATERIALS AND METHODS

Coumarin based HDAC2 inhibitors were designed as coumarin has immense anticancer activity (Al-Warhi *et al.,* 2020). Coumarin derivatives are unique in nature and they readily interact with diverse enzymes and receptors. Hence, coumarin is a highly privileged pharmacophore for the development of targeted anticancer drugs. Forty coumarin derivatives (Table 1) were designed and then docking studies were conducted with HDAC2 enzyme. The physiochemical descriptors, ADME parameters, pharmacokinetic properties, drug like nature and medicinal chemistry friendliness were measured by using SwissADME (Daina *et al.,* 2017).

Table 1. Coumarin compounds (P1-P40) selected for *In Silico* Study



Compound	R	R_1	R_2
P1	H	H	H
P2	H	H	OCH_3
P3	H	H	CH_3
P4	H	H	Cl
P5	H	H	Br
P6	H	H	NO_2
P7	H	H	F
P8	H	H	OC_2H_5
P9	Cl	H	H
P10	Cl	H	OCH_3
P11	Cl	H	CH_3
P12	Cl	H	Cl
P13	Cl	H	Br
P14	Cl	H	NO_2
P15	Cl	H	F
P16	Cl	H	OC_2H_5
P17	NO_2	H	H
P18	NO_2	H	OCH_3
P19	NO_2	H	CH_3
P20	NO_2	H	Cl
P21	NO_2	H	Br
P22	NO_2	H	NO_2
P23	NO_2	H	F
P24	NO_2	H	OC_2H_5
P25	H	OCH_3	H
P26	H	OCH_3	OCH_3
P27	H	OCH_3	CH_3
P28	H	OCH_3	Cl
P29	H	OCH_3	Br
P30	H	OCH_3	NO_2
P31	H	OCH_3	F
P32	H	OCH_3	OC_2H_5
P33	H	$N(C_2H_5)_2$	H
P34	H	$N(C_2H_5)_2$	OCH_3
P35	H	$N(C_2H_5)_2$	CH_3
P36	H	$N(C_2H_5)_2$	Cl
P37	H	$N(C_2H_5)_2$	Br
P38	H	$N(C_2H_5)_2$	NO_2
P39	H	$N(C_2H_5)_2$	F
P40	H	$N(C_2H_5)_2$	OC_2H_5

All calculations were performed using the various modules of Schrodinger (Kashyap and Kakkar, 2021). The crystal structure of HDAC2 (PDB ID : 4LXZ) was reported from Protein Data Bank. The protein was refined with the help of protein preparation wizard, where all the bond orders were fixed and water molecules beyond 5 Å were deleted. This was followed by optimization of the hydrogen bond network and the resultant structure was minimized using OPLS2005 force field.

LigPrep module was used to prepare the ligands. The missing hydrogen atoms were added and the resulting structures were desalted. This was followed with generation of all possible ionization states. Since HADC contains a Zn^{+2} ion, metal binding sites were also added.

Receptor grids were generated with a constant spacing of 1Å, centered at the centroid of co-crystallized ligands by using receptor grid generation panel of glide. Prior to docking of the hybrid ligands, the docking method was validated by re-docking the co-crystal ligand in the respective binding site by extra precision Glide docking protocols. Low RMSD value indicates a good docking strategy. After validation, the ligands obtained in ligand preparation step were docked into the HDAC2 binding site.

The drug likeness properties of the derivatives were evaluated by computing their absorption, distribution, metabolism and excretion with the help of SwissADME.

RESULTS AND DISCUSSION

All the designed coumarin derivatives as HDAC2 inhibitors had good docking score ranging from -7.5 to -4.3 (Table 2). Compound P12 had a highest docking score of -7.5 and compound P30 had a lowest docking score of -4.3. Compound P12 had the highest docking score as it formed one hydrogen bond interaction with the nitrogen of 2-ylamino thiazole group and ASP104 amino acid unit (Fig. 1). Hydrogen bonding stabilized the ligands at the target site and helped in altering binding affinity and drug efficacy (Chen *et al.*, 2016).

Table 2. Docking score and binding interactions with amino acid residues of coumarin compounds (P1-P40)

Compounds	Docking score (Kcal/mol)	Hydrogen bond	Coordination bond with Zn^{+2} ion	Pi-Pi stacking interactions	Halogen bond
SAHA	-9.6	ASP104,TYR308	O of OH	PHE155, HIE183	-
P12	-7.5	ASP104	-	PHE155, HIE183	TYR308
P10	-7.4	ASP104	-	PHE155, HIE183	-
P2	-7.4	ASP104	-	PHE155, HIE183	-
P13	-7.4	ASP104	-	PHE 155	TYR308
P23	-7.3	ASP104	-	PHE155, HIE183	-
P18	-7.3	ASP104	-	PHE 155	-
P4	-7.3	ASP104	-	PHE155, HIE183	TYR308
P14	-7.3	ASP104,TYR308	O of NO_2	PHE 155	-
P26	-7.2	ASP104	-	PHE155, HIE183	-
P15	-7.2	ASP104	-	PHE155, PHE210	TYR308
P20	-7.1	ASP104	-	PHE155, PHE210 HIE183	-
P31	-7.1	ASP104	-	PHE155, HIE183	-
P5	-7.0	ASP104	-	PHE155	TYR308
P7	-7.0	ASP104	-	PHE155, PHE210	-
P19	-7.0	ASP104	-	PHE155, HIE183	-
P32	-6.9	ASP104	-	PHE155	TYR308
P9	-6.9	ASP104	-	PHE155, HIE183	-
P28	-6.9	ASP104	-	PHE155, PHE210	TYR308
P27	-6.9	-	-	PHE155, HIE183	-
P21	-6.8	ASP104	-	PHE155	TYR308
P29	-6.8	ASP104	-	PHE155, PHE210	TYR308
P8	-6.8	ASP104	-	PHE155, HIE183	TYR308
P37	-6.8	ASP104	-	PHE155, PHE210	TYR308
P25	-6.8	ASP104, TYR308	-	PHE155, HIE183 PHE210	-
P24	-6.6	ASP104,TYR308	-	PHE155, HIE183 PHE210	-
P33	-6.5	ASP 104	-	PHE155, HIE183, PHE210	TYR308
P11	-6.5	ASP 104	-	PHE155, PHE210, PHE155	-
P6	-6.4	ASP104, TYR308	O of NO_2	PHE 155	-
P34	-6.3	ASP104	-	PHE155, PHE210 HIE183	-
P16	-6.3	ASP 104, HIE183	-	PHE155, PHE210	-
P35	-6.3	ASP104	-	PHE155, PHE210, HIE183	-
P17	-6.3	ASP104	-	PHE155, PHE210, HIE183	-
P3	-6.2	ASP104	-	PHE155, HIE183	-
P1	-6.0	ASP104	-	PHE155, HIE183 PHE210	-
P22	-5.9	TYR308, ASP104	O of NO_2	PHE210, PHE155, HIE183	TYR308
P38	-5.7	ASP104	O of NO_2	PHE155, HIE183	-
P40	-5.3	-	-	PHE155	-
P36	-5.1	-	-	PHE155	TYR308
P39	-5.0	-	-	PHE155, PHE210, HIE183	-
P30	-4.3	TYR308	O of NO_2	PHE155	-

The compound also formed the pi-pi stacking interaction between the benzene ring of chlorophenyl group and amino acid residues HIE183 and PHE155. The thiazole ring of the compound also formed the pi-pi stacking interactions with the PHE155 amino acid residue. Pi-pi stacking was non-covalent interactions formed between the pi bonds of aromatic rings and stabilized the ligands in the active site pocket of the receptor thereby contributing to docking score (Brylinski, 2018). Apart from this, a halogen bond was also formed between ASP308 amino acid and chloro group of chlorophenyl ring. All these interactions were responsible for increasing the interaction between the HDAC2 and P12, thus a high Glide score. Compounds P14, P25, P24, P6 and P22 formed two hydrogen bond interactions with ASP104 and TYR308. Whereas all other compounds formed one hydrogen bond interaction with ASP104, exception to above was compound P30, which formed single hydrogen bond interaction with TYR308. Glide XP based docking method revealed two pi-pi

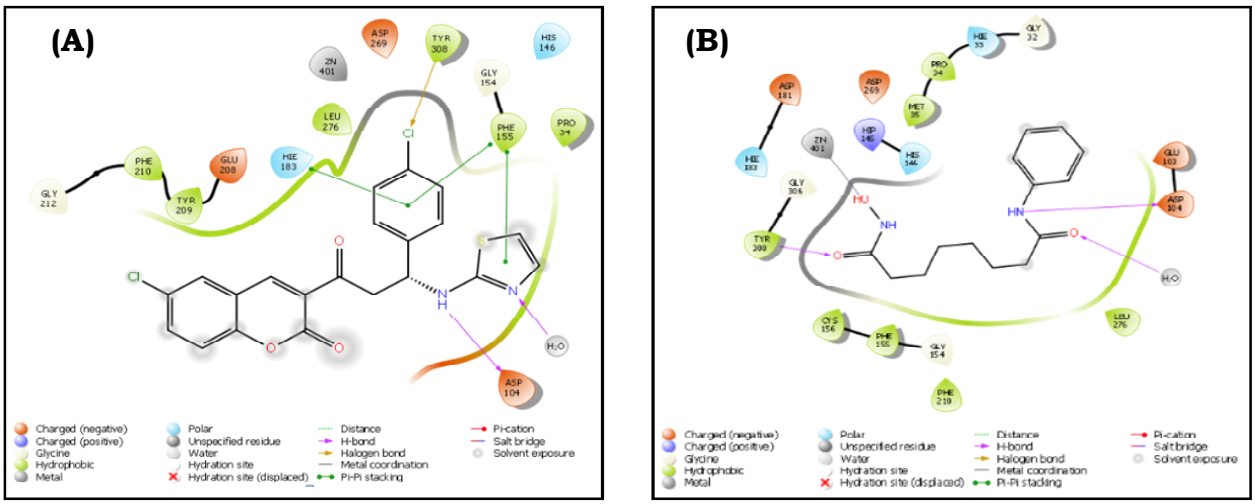


Fig. 1. Docking poses of P12 (A) and SAHA (B) in catalytic pocket of HDAC2.

stacking interactions with amino acids PHE155 and HIE183 by compounds P2, P3, P4, P8, P9, P10, P12, P19, P23, PP26, P27, P31 and P38. Compounds P7, P15, P16, P28, P29 and P37 also formed two pi-pi stacking interactions but with PHE155 and PHE210 amino acid residue. Some compounds like P1, P11, P17, P22, P24, P25, P33, P34, P35 and P39 formed three pi-pi stacking interactions with PHE155, HIE183 and PHE210 residues. Compounds like P5, P6, P13, P14, P18, P21, P30, P32, P36 and P40 formed one pi-pi stacking interactions with amino acid residue PHE155. In addition to all these interactions, the compounds also formed halogen bonds with the enzyme. Halogen bonds are the favourable interactions in molecular recognition and enhance affinity of leads towards active site (Cavallo *et al.*, 2016). Compounds P4, P5, P8, P12, P13, P15, P21, P22, P28, P29, P32, P33, P36 and P37 interacted with the TYR308 residue of the active site with these bonds.

After molecular docking, the coumarin compounds were then subjected to ADME analysis. ADME is an abbreviation for "absorption, distribution, metabolism and excretion". These criteria influence, the drug concentration and kinetics in tissue and thus further affect the pharmacological activity of the compounds as drug candidate. SwissADME was used to predict the ADME, physicochemistry, druglikeness, pharmacokinetics, and medicinal chemistry friendliness properties of the compounds, P1 to P40 (Table 3). Lipinskis rule of five, links the physicochemical properties of the compounds to the oral bioavailability of drug and hence the druglikeness property. It states that an orally active drug has no more than one violation from the ranges it described. All the compounds showed no violation except compounds P21, P22 and P37, which showed one violation each (molecular weight above 500 daltons). So, all the compounds followed the lipinskis rule as the HBD (hydrogen bond donors) was less than 5, HBA (hydrogen bond acceptors) was less than 10 and logP values less than 5. TPSA (topological polar surface area) characterizes the transport properties of the drug. The TPSA values should be between 20 to 130 Å^2. Compounds with TPSA value above 140 Å^2 are poor at permeating cell membranes. For a compound to cross the blood brain barrier it should have value less than 90 Å^2. All the compounds fell within the range, except compounds P6, P14, P17, P18, P19, P20, P21, P23, P24, P30 and P38. The clogP values are helpful in estimating the distribution of drugs within the body. All the compounds were within the acceptable range of 2.00 to 5.00, except compounds P36 and P37. The bioavailability score of all the compounds was found to be 0.55. The pharmacokinetic parameters of the compounds showed that most of the compounds had high passive gastrointestinal absorption but none of them may penetrate the blood brain barrier. Compounds P34, P35, P36, P37, P39 and P40 were found to be substrate for P-glycoprotein. P-glycoprotein is a transmembrane efflux pump that pumps its substrate from inside to outside cell. Cytochrome P450 isoforms are involved in metabolism of various drugs. Most of the therapeutic molecules are found to be substrate of five major isoforms CYP1A2,

Table 3. SwissADME predictions of physiochemical properties and bioavailability of compounds (P1-P40)

Compound	TPSA	Consensus Log $P_{O/W}$	GI absorption	Lipinski's violations	Bioavailability score
P12	100.44	4.86	High	0	0.55
P10	109.67	4.28	High	0	0.55
P2	109.67	3.78	High	0	0.55
P13	100.44	4.86	High	0	0.55
P23	146.26	3.42	Low	0	0.55
P18	155.49	2.95	Low	0	0.55
P4	100.44	4.26	High	0	0.55
P14	146.26	3.75	Low	0	0.55
P26	118.90	3.83	High	0	0.55
P15	100.44	4.71	High	0	0.55
P20	146.26	3.62	Low	0	0.55
P31	109.67	4.21	High	0	0.55
P5	100.44	4.35	High	0	0.55
P7	100.44	4.06	High	0	0.55
P19	146.26	3.46	Low	0	0.55
P32	118.90	4.18	High	0	0.55
P9	100.44	4.36	High	0	0.55
P28	109.67	4.44	High	0	0.55
P27	109.67	4.23	High	0	0.55
P21	146.26	3.73	Low	1	0.55
P29	109.67	4.44	High	0	0.55
P8	109.67	4.24	High	0	0.55
P37	103.68	5.12	Low	1	0.55
P25	109.67	3.78	High	0	0.55
P24	155.49	3.33	Low	0	0.55
P33	103.68	4.51	High	0	0.55
P11	100.44	4.62	High	0	0.55
P6	146.26	3.35	Low	0	0.55
P34	112.91	4.52	High	0	0.55
P16	109.67	4.65	High	0	0.55
P35	103.68	4.86	High	0	0.55
P17	146.26	3.12	Low	0	0.55
P3	100.44	4.17	High	0	0.55
P1	100.44	4.73	High	0	0.55
P22	192.08	2.50	Low	1	0.55
P38	149.50	3.81	Low	0	0.55
P40	112.91	4.83	Low	0	0.55
P36	103.68	5.03	Low	0	0.55
P39	103.68	4.82	High	0	0.55
P30	155.49	3.10	Low	0	0.55

CYP2C19, CYP2D6 and CYP3A4. Inhibition of these enzymes results in toxic or adverse effects due to accumulation of drug or its metabolites. Almost each compound was substrate for one or more isoform, but majority of the compounds were substrate for CYP1A2, CYP2D6 and CYP3A4. So, the compounds had good physiochemical and pharmacokinetic properties to be lead for therapeutic molecules. HDAC2 became promising therapeutic target for the treatment of cancer. In search of selective HDAC2 inhibitors, 40 coumarin derivatives were designed. These derivatives were screened for activity against cancer by docking them into the active site pocket of HDAC2. The derivatives had good docking score and interacted with the receptors through various interactions. The interactions responsible for good docking score were hydrogen bond, pi-pi stacking and halogen bond. These active compounds were further selected for ADME prediction by *in silico* approach. Molecular docking results and ADME calculations revealed that these compounds could lead for further structural modification, *in vitro* and i*n vivo* studies, in search of selective HDAC2 inhibitors as anticancer drugs.

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