Annals of Biology **38** (2) : 274-279, 2022

Impact Score : 0.32 (Scopus)

In Silico Designing of Coumarin Derivatives as Potential Histone Deacetylase2 Inhibitors (HDAC2)

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(Received : March 30, 2022; Accepted : May 5, 2022)

ABSTRACT

Histone deacetylases are a class of enzymes that regulates the expression of tumor suppressor genes by removal of acetyl group from histones. Forty coumarin derivatives were designed as a privileged scaffold with potential anticancer activity, proven in literature. The inhibitors were designed according to the structural requirements of inhibitors given in the literature. The classical inhibitors had a CAP group, a linker and zinc binding group. The designed forty coumarin compounds were subjected to molecular docking against HDAC2 enzyme. The HDAC2 inhibitors were then analyzed through SwissADME to calculate their drug likeness properties. It was found that the coumarin compounds had good binding affinity towards HDAC2 and showed good ADME properties. The study showed that the designed coumarin compounds were good leads for the HDAC2 inhibition. The result of this work can pave the way for further designing, synthesis and biological evaluation of potent HDAC2 inhibitors.

Key words : Histone deacetylases, HDAC2 inhibitors, molecular docking, drug design, ADME

INTRODUCTION

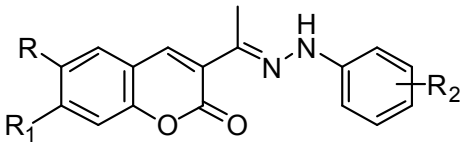
Histone deacetylases (HDACs) are responsible for deacetylating the lysine residues of histones and other protein substrates. This results in more compact chromatin and compromised DNA accessibility. A balance of acetylation and deacetylation is important for proper gene expression. Disturbance of this balance in important chromatin region such as tumor suppressor gene results in malignant states. HDAC2 belongs to zinc dependent class I family of HDACs. In the last few decades HDAC2 have shown promise as a therapeutic agent due to its ability to induce cell apoptosis by restoring tumor suppressor gene expression in various cancer cell lines. These properties make HADC2 a suitable target for developing anticancer agents. Since it has been proved that, HADC2 is a drug gable target for the treatment of various kinds of cancer (Jones *et al.*, 2016); important HDAC inhibitor classes had been developed, which include hydroxamic acids, carboxylic acids, cyclic peptides, thiols and benzamides. Of these, FDA has approved six HDAC inhibitors as anticancer drugs (Poole, 2014; Fenichel, 2015; Gao *et al.*, 2017; Schlenk *et al.*, 2018). Since these inhibitors are pan HDAC inhibitors they are associated with many side effects like thrombocytopenia, cardiac and gastrointestinal toxicities (Chakrabarti *et al.*, 2016). These side effects can be overcome by the development of selective HDAC inhibitors. They all have common structural features consisting of Zn binding group (ZBG), the linker and the CAP moiety. The CAP group is involved in surface recognition and forms bonds with surface amino acids. The linker sits into the tunnel of the active site and forms a bond with the amino acids present there which are mostly hydrophobic in nature. Lastly is the ZBG, which chelates the Zn^{+2} ion present at the end of the tunnel.

MATERIALS AND METHODS

Coumarin based HDAC2 inhibitors were designed as coumarins are one of the promising scaffolds that exhibit significant anticancer activity along with other diverse biological activities. It has immeasurable anticancer potential with minimum side effects depending on the substitutions on the basic nucleus. The antitumor activity of natural and synthetic coumarin derivatives was extensively explored by many researchers and it was found that coumarin was a highly privileged pharmacophore for the development of targeted anticancer drugs. The designed

coumarin compounds had (i) coumarin CAP that occupied narrow tubular pocket of HDAC2 and contained one H-bond acceptor. This H-bond acceptor coordinated with Zn+2 ion, (ii) an amide linker which sat in the linker region and (iii) a hydrophobic group, which stabilized the inhibitor and checked their potential as anticancer activity by using histone deacetylase2 (HDAC2) enzyme inhibition as the target site. 40 coumarin derivatives were designed (Table 1) and docked into the active site pocket of HDAC2 enzyme and also calculated there drug likeness properties through SwissADME.

Table 1. Coumarin compounds (C1-C40) selected for *in silico* study



Compound	R	R_1	R_2
C1	H	H	H
C2	H	H	4-Cl
C3	H	H	4-CN
C4	H	H	2-CH_3
C5	H	H	2-NO_2
C6	H	H	3-NO_2
C7	H	H	4-Br
C8	H	H	2,4-di Cl
C9	Cl	H	H
C10	Cl	H	4-Cl
C11	Cl	H	4-CN
C12	Cl	H	2-CH_3
C13	Cl	H	2-NO_2
C14	Cl	H	3-NO_2
C15	Cl	H	4-Br
C16	Cl	H	2,4-di Cl
C17	NO_2	H	H
C18	NO_2	H	4-Cl
C19	NO_2	H	4-CN
C20	NO_2	H	2-CH_3
C21	NO_2	H	2-NO_2
C22	NO_2	H	3-NO_2
C23	NO_2	H	4-Br
C24	NO_2	H	2,4-di Cl
C25	H	OCH_3	H
C26	H	OCH_3	4-Cl
C27	H	OCH_3	4-CN
C28	H	OCH_3	2-CH_3
C29	H	OCH_3	2-NO_2
C30	H	OCH_3	3-NO_2
C31	H	OCH_3	4-Br
C32	H	OCH_3	2,4-di Cl
C33	H	$N(C_2H_5)_2$	H
C34	H	$N(C_2H_5)_2$	4-Cl
C35	H	$N(C_2H_5)_2$	4-CN
C36	H	$N(C_2H_5)_2$	2-CH_3
C37	H	$N(C_2H_5)_2$	2-NO_2
C38	H	$N(C2H5)_2$	3-NO_2
C39	H	$N(C_2H_5)_2$	4-Br
C40	H	$N(C_2H_5)_2$	2,4-di Cl

Molecular docking is the binding orientation of small molecules to their target proteins in order to predict the affinity and activity of small molecules. Hence, docking plays an important role in drug designing. All molecular docking studies were performed using the Schrodinger interface (Maestro version 9.6) LLC, New York software. The process of molecular docking included the following steps :

Ligand preparation : All the ligands were drawn using 2D sketcher in Maestro 9.6 and then converted to 3D structures. All the molecules were prepared using LigPrep module of Schrodinger Suite. The process of ligand preparation consisted of several steps i. e. conversion of 2D structure of ligands into 3D structure, generation of ionization state of ligands at pH 7.0±2, removal of non-compliant structures, addition of hydrogen atoms, removal of counter ions and energy minimization of ligand with OPLS_2005 force field. The preparation parameters were kept identical for all the ligands.

Protein preparation : The X-ray crystal structure of the HDAC2 was taken from RCSB protein data bank (*https://www.rcsb.org/*) with PDB ID : 4LXZ. The protein structure was prepared using the protein preparation wizard of Schrodinger suite. In the first step, missing hydrogens were added to crystal structure and proper bond orders were assigned. All the molecules beyond 5 Å were deleted. In the next step, the redundant protein chains and hetero atoms were deleted. As HDAC2 required Zn for its catalytic function, so it was kept intact.

Grid generation : The grid generation was done by using, "Receptor grid generation panel". The grid generation was performed to define the active site of the protein. Grid generation was performed by selecting co-crystallized ligand as centroid.

The molecular docking was performed using the Glide module (Grid-based Ligand Docking with Energetics) of Schrödinger package. The prepared ligands were docked against grid generated receptors HDAC2 in an extra precision (XP) flexible mode. The GlideScore (GSore) representing affinity of ligands against receptors was obtained from pose viewer file

of docked complexes. A total of 40 coumarin derivatives were docked against the active site pocket of HDAC2 (Table 1).

Pharmacokinetic profile : The drug likeness of coumarin derivatives were evaluated by computing their absorption, distribution, metabolism and elimination (ADME) properties with the help of SwissADME.

RESULTS AND DISCUSSION

Redocking is the most important validation method to evaluate the accuracy of the docking studies. It determines how closely the lowest binding energy pose resembles the experimental binding mode determined by X-ray crystallography. In this method, docking results were validated by removing the crystal ligand from the binding site and redocking it to the binding site of HDAC protein and root mean standard deviation (RMSD) was calculated (Fig. 1). RMSD between the predicted and the observed confirmation for crystal ligands SAHA was 0.93. RMSD less than 2Å showed the reliability of Glide XP docking mode in reproducing the experimentally observed binding mode for HDAC inhibitor. Thus, all the docking studies were performed using extra-precision flexible docking protocol.

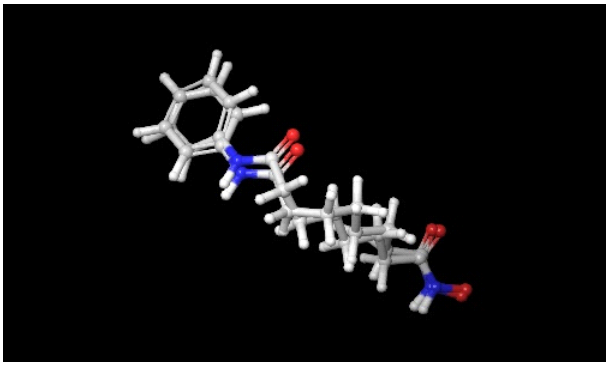


Fig. 1. Redocking pose between docked ligand and crystal ligand.

Designed coumarin derivatives as HDAC2 inhibitors had high docking score ranging from -7.78 to -2.2. The XP docking score and binding interactions with amino acids of coumarin compounds with standard SAHA in the active site pocket of HDAC2 are given in Table 2. HDAC2 belonged to class I of HDACs, so it was a zinc containing enzyme. HDAC2 inhibitors are known to bind to Zn^{+2} in the catalytic active site. Compound 32 exhibited highest binding affinity of -7.78 (Kcal/mol) among all the compounds. The high binding affinity of compound 32 was due to the coordination bond formed between the Zn^{+2} ion and O of OCH3 group on 7th position of coumarin ring (Fig. 2). This coordination bond of the ZBG with the catalytic Zn^{+2} was essential for HDAC2 inhibition. Compounds C28, C25, C31, C26, C27, C29 and C30 like C32 formed coordination bond with Zn^{+2} through O of OCH3 group on 7th position of coumarin ring (Table 2). Likewise compounds C22, C20 and C21 formed the coordination bond with Zn^{+2} with their O of nitro group present on Coumarin ring. Whereas, compounds C3, C19, C11 and C35 formed coordination bond with Zn^{+2} through their CN group present on the 4th position of phenyl hydrazine ring. Similarly, compounds C6 and C14 formed the coordination bonds with their O of nitro group present on phenyl hydrazine ring. In addition to electrostatic

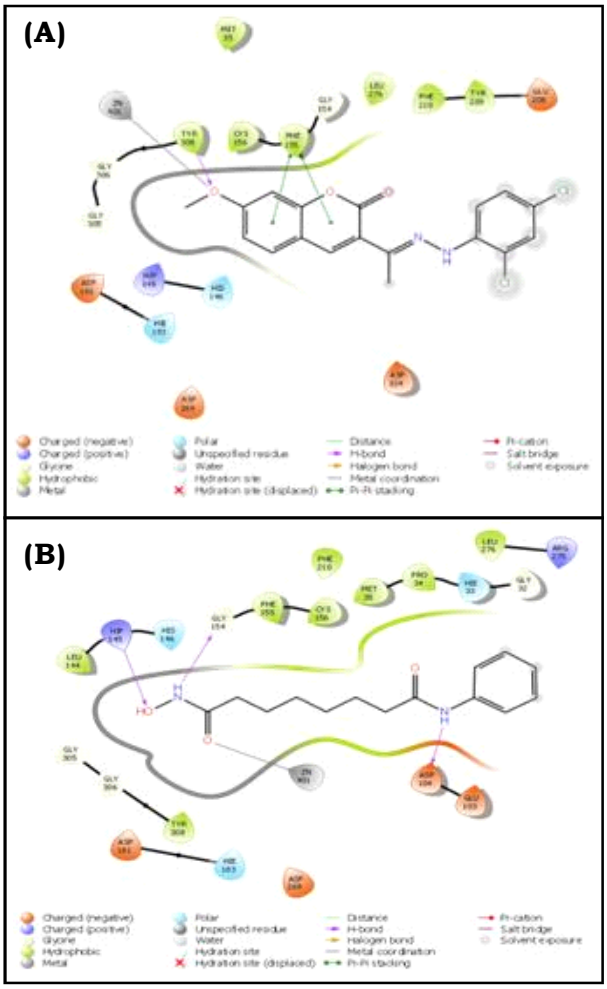


Fig. 2. Docking poses of C32 (A) and SAHA (B) in catalytic pocket of HDAC2.

Table 2. Docking score and binding interactions with amino acid residues of coumarin compounds

Compounds	Docking score (Kcal/mol)	Coordination bond with Zn^{+2} ion	Hydrogen bond	Pi-Pi stacking interactions	Halogen bond	Pi-cation interactions
SAHA	-9.568	with OH	TYR308, ASP308	-	-	-
C32	-7.788	with OCH_3	TYR308	PHE 155	-	-
C28	-7.661	with OCH_3	TYR308	PHE 155	-	-
C25	-7.642	with OCH_3	TYR308	PHE 155	-	-
C31	-7.533	with OCH_3	TYR308	PHE 155	-	-
C26	-7.229	with OCH_3	TYR308	PHE 155	-	-
C27	-7.095	with OCH_3	TYR308	PHE 155	-	-
C29	-6.918	with OCH_3	TYR308	PHE 155	-	-
C30	-6.888	with OCH_3	TYR308	PHE 155	-	-
C3	-6.866	with CN	-	PHE 155	-	-
C19	-6.728	with CN	HIP145	PHE 155	-	-
C11	-6.495	with CN	-	PHE 155, TYR 209	-	-
C35	-6.248	with CN	-	PHE 155	-	-
C33	-5.0	-	PHE210	PHE155 HIE183	-	Zn^{+2}
C16	-4.9	-	-	PHE155	TYR308	-
C18	-4.8	-	PHE210	PHE155	HIP145	-
C10	-4.8	-	PHE210	PHE155	HIP145	-
C6	-4.6	With O of NO_2	-	PHE155 PHE 210	-	-
C22	-4.6	With O of NO_2	TYR308	PHE155, PHE210	-	-
C4	-4.6		PHE210	PHE155	-	-
C20	-4.5	With O of NO_2	TYR308	PHE155, TYR 209, PHE210	-	-
C9	-4.5	-	PHE210	PHE155	-	-
C21	-4.3	With O of NO_2	-	PHE155, HIE183	-	-
C14	-4.3	With O of NO_2	HIE183 PHE210	PHE155, TYR 210	-	-
C40	-4.3	-	-	PHE155	TYR308	-
C36	-3.7	-	PHE210	TYR209 PHE155	TYR308	-
C8	-4.3	-	PHE210	PHE155	TYR308	-
C2	-4.2	-	-	PHE155	TYR308	-
C15	-4.2	-	-	PHE155	TYR308, HIP145	-
C37	-4.1	-	-	PHE155, HIE183	-	HIE183 PHE210
C5	-4.1	-	PHE210	PHE155, HIE183	-	HIE183, PHE155
C34	-4.0	-	-	PHE155, HIP145	TYR308, HIP145	-
C39	-4.0	-	-	PHE155	TYR308, HIP145	-
C17	-3.9	-	-	PHE155	-	-
C1	-3.9	-	-	PHE155	-	-
C24	-3.8	-	TYR308	HIE183	-	PHE155, HIE183
C23	-3.7	-	-	PHE155	-	-
C7	-3.7	-	PHE210	PHE155	-	-
C38	-3.5	-	PHE210	PHE155	-	-
C13	-3.5	-	-	PHE155, HIE183	-	HIE183, PHE210
C12	-2.2	-	-	-	-	-

interactions with the Zn^{+2}, the compound C32 formed hydrogen bond with TYR308 amino acid residue, explaining its high negative docking score (Fig. 2). Likewise compounds C28, C25, C31, C26, C27, C29, C30 and C24 formed hydrogen bonds with TYR308. Hydrogen bonds were also formed with HIP145 (C19), PHE210 (C33, C18, C10, C4, C9, C14, C36, C8, C5, C7 and C38). One compound C14 also formed hydrogen bond with HIE183 amino acid residue. Hydrogen bonds were weak non-polar interactions, but they played important role in stabilizing protein-ligand complexes (de Freitas and Schapira, 2017; Kashyap and Kakkar, 2021).

Other interactions of interest are pi-pi stacking interactions and pi-cation interactions. These interactions help in protein recognition and stabilization of ligand in the active site of protein. Compound C32 formed pi-pi stacking interactions with PHE155. Likewise these interactions were formed by all the compounds except C12, which did not show any kind of interactions and had

a docking score of -2.2. These pi-pi stacking interactions were formed with the PHE155, TYR209, PHE210, HIE183, TYR210 and HIP145 amino acid residues by different compounds. The pi-cation interactions were formed by C33, C37, C5, C24 and C13 with Zn^{+2}, HIE183, PHE210, PHE155 amino acid residues. The compounds, also exhibited halogen bond interactions, which were known to improve both selectivity and efficacy towards protein active site. These interactions were shown by C16, C18, C10, C40, C36, C8, C2, C15, C34, C5, C24 and C13 with amino acids TYR308 residues.

The therapeutic action of the compound depended upon the ADME (absorption, distribution, metabolism and excretion) properties. SwissADME gave the physiochemical properties, pharmacokinetics, and drug-likeness in a user friendly submission and straightforward result interpretation (Daina *et al.*, 2017). Lipinski's rule of five showed the probability of the drug to be orally active. All the compounds followed Lipinski's rule of five showing their drug likeness property (Table 3), except compounds C16 and C40 showing one violation each. All compounds had an acceptable range of clogP value of 2.00-5.00. Topological polar surface area (TPSA) is the surface sum of all the polar atoms in the molecule, the acceptable range is 20-130 Å and all compounds fell in the acceptable region, except C21 and C22. All the compounds were moderately soluble. The

Table 3. Swiss ADME predictions of physio-chemical properties and bioavailability of compounds

Compound	TPSA	Consensus Log $P_{O/W}$	GI absorption	Lipinski's violations	Bioavailability score
C32	63.83	4.39	High	0	0.55
C28	63.83	3.68	High	0	0.55
C25	63.83	3.27	High	0	0.55
C31	63.83	3.92	High	0	0.55
C26	63.83	3.89	High	0	0.55
C27	87.62	3.06	High	0	0.55
C29	109.65	2.7	High	0	0.55
C30	109.65	2.63	High	0	0.55
C3	78.39	3.08	High	0	0.55
C19	124.21	2.28	High	0	0.55
C11	78.39	3.67	High	0	0.55
C35	81.63	3.81	High	0	0.55
C33	57.84	3.93	High	0	0.55
C16	54.6	4.81	High	1	0.55
C18	100.42	3	High	0	0.55
C10	54.6	4.37	High	0	0.55
C6	100.42	2.53	High	0	0.55
C22	146.24	1.87	Low	0	0.55
C4	54.6	3.6	High	0	0.55
C20	100.42	2.77	High	0	0.55
C9	54.6	3.87	High	0	0.55
C21	146.24	1.97	Low	0	0.55
C14	100.42	3.15	High	0	0.55
C40	57.84	5.05	High	1	0.55
C36	57.84	4.27	High	0	0.55
C8	54.6	4.29	High	0	0.55
C2	54.6	3.84	High	0	0.55
C5	54.6	4.44	High	0	0.55
C37	103.66	3.39	High	0	0.55
C5	100.42	2.72	High	0	0.55
C34	57.84	4.51	High	0	0.55
C39	57.84	4.65	High	0	0.55
C17	100.42	2.46	High	0	0.55
C1	54.6	3.27	High	0	0.55
C24	100.42	3.47	High	0	0.55
C23	100.42	3.1	High	0	0.55
C7	54.6	3.91	High	0	0.55
C38	103.66	3.22	High	0	0.55
C13	100.42	3.25	High	0	0.55
C12	54.6	4.21	High	0	0.55

gastro-intestinal absorption of all the compounds was high except C22 and C21. So, the bioavailability score for all the compounds was 0.55. In summary, all the compounds had drug-likeness property and were good candidate for oral absorption.

HDAC2 became promising therapeutic target. In quest for selective HDAC2 inhibitors, forty coumarin derivatives were designed. These coumarin derivatives were then subjected to molecular docking in the active site pocket of HADC2. The results showed that almost all the compounds had a good docking score. The compounds also interacted with the enzyme with all the important interactions like hydrogen bonds, pi-pi stacking interactions, halogen bonds and pi-cation interactions. These interactions were responsible for strong binding of the compounds with the enzyme. The compounds were further subjected to ADME analysis and showed good pharmacological and pharmacokinetic properties. Since, these compounds had considerable affinity towards the targets and showed good ADME properties they may be considered as credible leads for potential HDAC2 inhibition. Therefore, further biological assays and *in vitro* studies can be performed in search of effective drugs.

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