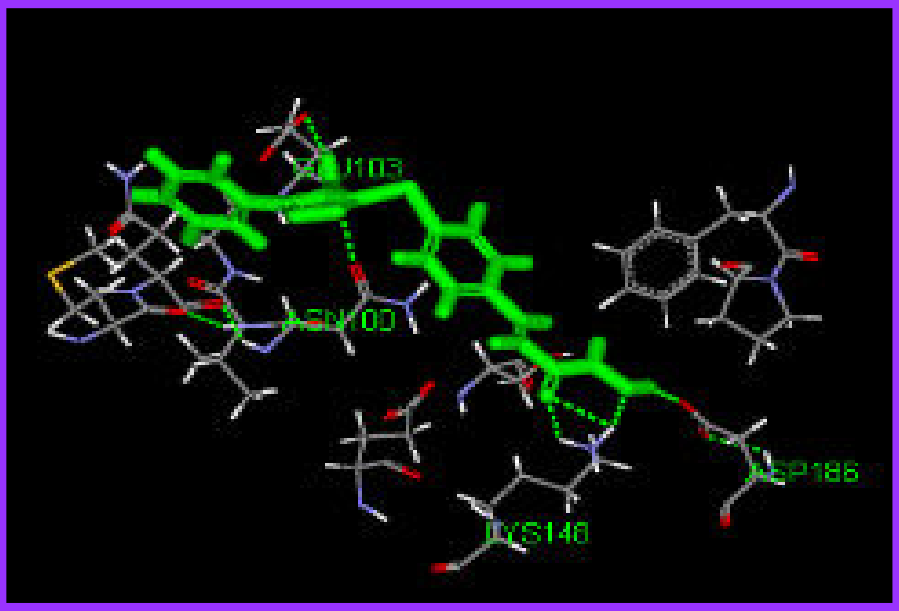

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In silco studies on modified hydroxamic acid and valporic acid as potential inhibitors for HDAC2

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Abstract

Histone deacetylases 2, class 1 HDAC family are emerged as an important therapeutic target for the treatment of various cancers. HDAC2 inhibitors are potent anti-cancer agents. Two inhibitors of HDAC2 are hydroxamic acid and valporic acid which are potent inducers of growth arrest, differentiation, and/or apoptotic cell death. Total 34 ligands optimized using triazole group substitution for the target protein histone deacetylase 2 on the basis of SAHA and valporic acid. All the ligands are docked with the target protein and results are compared with test compound SAHA. Eight ligands showed better binding affinity towards HDAC2.The binding affinity, free energy and drug scan screening of the above eight ligands have shown that P2, P6 and V6 molecules are best suitable to inhibit HDAC2.

Introduction

HDACs are the enzyme deacetylating the ε-amino groups of lysine located near the amino termini of core histone proteins (Mai et al., 2002 and Monneret, 2005). HDACs have been classified into class I-III, class I includes HDAC 1-3 and 8, class II includes HDAC 4-7, 9 -10 both classes operate by zinc-dependent mechanisms and class III includes Sir1-Sir 7 operated by NAD (Bieliauskas and Pflum, 2008). HDAC2 enzyme is greatly considered for developing anti-cancer drugs. HDAC inhibitors interact with chromosomes in the cancer cell and causes cancer cells to stop growing. Hydroxamic acid and valproic acid are potent inhibitors of HDAC. One of the hydroxamic acid derivatives in clinical phase is panobinostat (Prince et al., 2009). The first HDAC drug approved by U.S Food and Drug Administration is SAHA (suberoylanilide hydroxamic acid or vorinostat) for treating cutaneous T -cell lymphoma (Walkinshaw et al., 2008). SAHA inhibits the activity of class I & II HDACs (Marks et al., 2007). Present study involves *in silico* hydroxamic acid and valproic acid modification by utilizing triazole, in order to obtain a better inhibitor. Molecular docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Hence molecular docking is used to predict suitable ligand molecules for HDAC2 inhibition.

Material and Methods

Total 34 ligands optimized using triazole group substitution for the target protein histone deacetylase 2 on the basis of SAHA and valporic acid. This study is looking to compare the efficacy of SAHA with other types of inhibitors, by searching for the new ones or modifications of the existing ones. Triazole is known as a non-classical amide bioisostere compound and its biological activity, notably as antifungal, antimicrobials, and enzymatic inhibitors (Roffey, 1997). It is of interest to modify the SAHA and valporic acid structure by creating new ligands. The processes followed are adding replacing one of the amides group within the

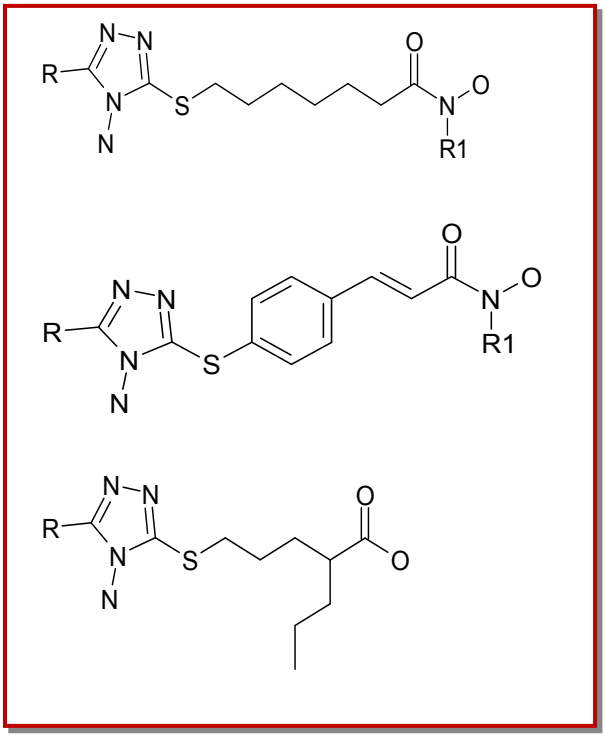

Figure 1: Triazole substituted hydroxamic acid derivative 1, 2 and valproic acid derivative 3

SAHA hydrophobic group with triazole linked with sulfur bond and styrene group then, conduct molecular docking with HDAC2, and testing its drug score and toxicity using computational tools, and finally compare the result with standard SAHA inhibitor. This structure -activity relationship (SAR) study is very important in uncovering novel inhibitors of HDAC2. The triazole bioisostere attributes on SAHA amide group could even -tually modify SAHA's properties. The hydrophobic tendency of triazole compared with the amide group on SAHA was expected to increase the binding affinity of modified ligands toward HDAC2. Thus, the binding of enzyme-ligand complex would be much stronger. Triazole could be treated as an additional functional group on SAHA, which could increase the hydrophobic attributes of SAHA cap group. The twelve alkyl groups for modified ligand variations are phenyl, biphenyl, napthyl, p-nitrobiphenyl, p-hydroxybiphenyl, amino-oxyphenyl, acetamidooxy phenyl, amine, acetamido-phenyl. The selections of those alkyl groups are based on hydrophobic attributes of those groups. Thus, this study would observe the influence of the cap group hydrophobicity of each modified ligand, in comparison with the SAHA standard ligand. Figure 1 shows modified hydroxamic acid derivative and modified valporic acid. All 34 Ligand showed in Table I and II. Docking studies carried out in LigandFit (Discovery Studio 2.0) (Venkatachalam et al., 2003). It is based on a cavity detection algorithm and Monte Carlo conforma-tional search algorithm for generating ligand poses

Table I

Different substitution used in hydroxamic acid derivatives

Compound	R	R1
1	H	H
2	C6H5	H
3	C6H5	CH3
4	C6H5	C6H5
5	C6H5C6H4	H
6	C10H13	H
7	P-NH2-C6H4-C6H4	H
8	P-OH-C6H4-C6H4	H
9	O-(NH2)-C6H4	H
10	O-(NHCOCH3)-C6H4	H
11	P-NH2-C6H4	H
12	P(NHCOCH3)C6H4	H

Table II

Different substitution used in valporic acid derivatives

Compound	R	R1
1	H	H
2	C6H5	H
3	C6H5	CH3
4	C6H5	C6H5
5	C6H5C6H4	H
6	C10H13	H
7	P-NH2-C6H4-C6H4	H
8	P-OH-C6H4-C6H4	H
9	O-(NH2)-C6H4	H
10	O-(NHCOCH3)-C6H4	H
11	P-NH2-C6H4	H
12	P(NHCOCH3)C6H4	H

consistent with the active site shape. All 34 ligands are drawn in Chemsketch and the hydrogen bonds were added and CHARMm force field was applied to all molecules. The 3D crystal structure of *Homo sapiens* HDAC2 (PDB ID: 3MAX) downloaded from protein database from the PDB structural database site (http://www.rcsb.org/pdb). After applying CHARMm force field macro molecule 3MAX was assigned as receptor. The receptor cavity was searched using flood filling algorithm and partition site was adjusted for the better fitments of molecule in the partition site of receptor. The comparative docking studies for all 34 molecules were performed. The determination of the ligand binding affinity was calculated using dock score were used to estimate the ligand-binding energies.

Drug scan

This was conducted in order to determine, whether the inhibitor has fulfilled the conditions as the drug candidate. It is done using Osiris Property Explorer,

Table III

Docking scores of SAHA

Chain	Site 2	Site 3	Site 6	Site 8
A	*	26.7	*	23.2
B	30.8	18.0	45.6	*

Table IV

Docking scores of compounds

Name of the compound	Site 2	Site 3	Site 6
P1	29.01 (-2.746)	**	48.00 (-2.832)
P2	**	**	64.70 (-4.578)
P3	24.40 (-2.261)	**	43.42 (-2.645)
P4	**	**	45,73 (-6.485)
P6	**	**	55.27 (-5.648)
P9	**	**	51.00 (-3.957)
V1	25.51 (0.004)	26.20 (0.610)	47.02 (-0.237)
V3	28.41 (-0.843)	19.67 (-5.88)	43.50 (-1.074)
V4	16.30 (-2.807)	**	44.63 (0.228)
V6	19.68 (-1.573)	**	47.27 (-4.001)
V9	21.75 (-4.573)	**	46.03 (-3.584)
V11	19.78 (-3.191)	**	44.378 (-2.07)
VAL1	18.13 (1.991)	19.71 (0.638)	35.48 (0.493)
VAL2	16.92 (-0.364)	**	36.68 (-1.070)
VAL3	**	**	32.62 (-1.684)
VAL4	0.07 (7.71)	**	33.12 (-2.785)
VAL6	**	**	26.83 (-2.653)
VAL8	4.81 (2.287)	**	42.36 (1.316)
VAL9	**	**	36.03 (-3.043)
VAL10	**	**	38.35 (-2.870)

and Lazar software's (Bhakat, 2012). The Osiris Property Explorer and Lazar calculated various attributes of the drugs, such as toxicity, drug likeness and drug score.

Table V

Interaction of modified ligands with Hdac2 (3maxb chain) *Homo sapiens* at site 6

Name of the compound	Hydrogen bond monitored
P1	B:GLU154:HN-Molecule-1:N3
P1	B:ASP186:OD1- Molecule-1:H35
P2	B:GLU103:OE1- Molecule-1:H28
P2	B:SER153:OG- Molecule-1:H34
P4	B:ASN100:HD21-Molecule-1:O17
P4	B:GLU103:0E2- Molecule-1:H35
P4	B:GLU151:OE1- Molecule-1:H40
P6	B:ASN100:HD21-Molecule-1:O17
P6	B:GLU103:0E2- Molecule-1:H35
P6	B:GLU151:OE1- Molecule-1:H40
P9	B:ASN100:OD1- Molecule-1:H30
P9	B:GLU103:OE1- Molecule-1:H35
P9	B:SER153:OG- Molecule-1:H41
V1	B:GLY154:HN-Molecule-1:ON14
V1	B:GLU103:0E1- Molecule-1:H19
V1	B:ASO104:OD2- Molecule-1:H33
V6	B:MET 96: E21-Molecule-1:03
V6	B:GLU103:0E2- Molecule-1:H35
V9	B:LYS171:H23-Molecule-1:04
V9	B:GLU103:OE1- Molecule-1:H25
V9	B: VAL101 :O- Molecule-1:H26
V9	B:ANS153:OD1- Molecule-1:H39

Results and Discussion

HDAC2 Contains three chains such as A, B and C, All the three chains are docked with test compound SAHA. It shows Chain B has good docking Score. The docking scores of SAHA with different chains of the target protein at different sites on HDAC2 are given in Table III. Chain C is inactive as it shows no results with the test compound.

All 34 ligands are docked on 3MAX B Chain the results shows triazole modified hydroxamic acids shows better docking score than the SAHA test compound. The docking score of the ligands are shown in Table IV and V. Results shows that P1, P2, P4, P6, P9, V1, V6 and V9 modified ligands shows more binding affinity than test compound SAHA (Figure 2-4).

Osiris property explorer used to find the *in silico* pharmacology features. The hydrophobicity of drugs

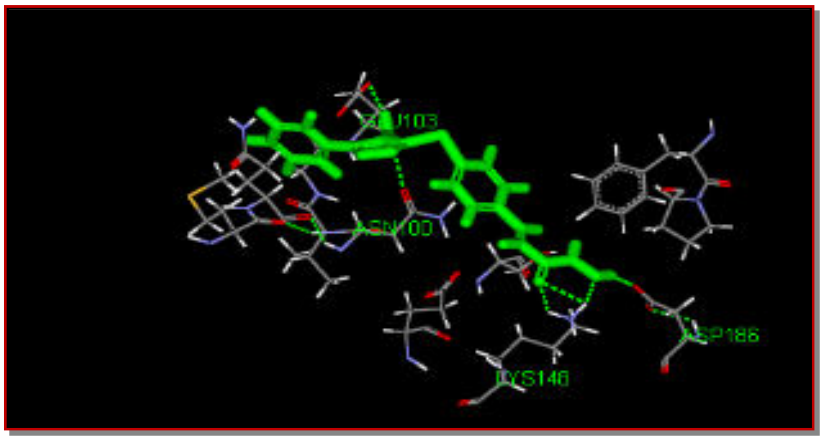

Figure 2: Docking of ligand p2 with 3 maxb at site 6

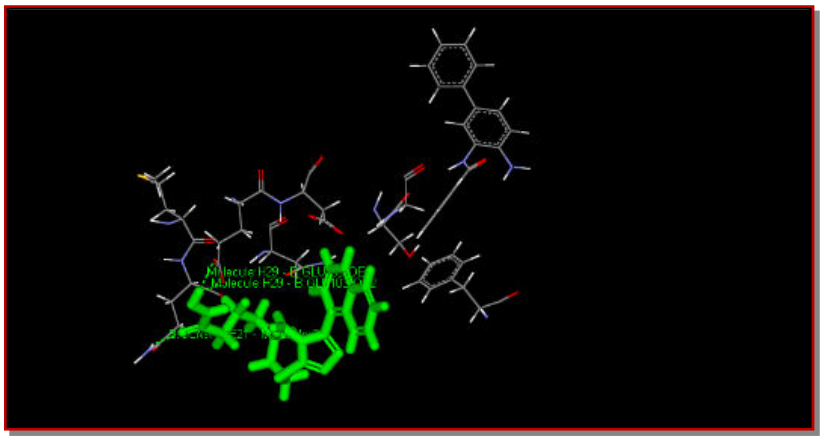

Figure 3: Docking of ligand V6 with 3 maxB at site 6

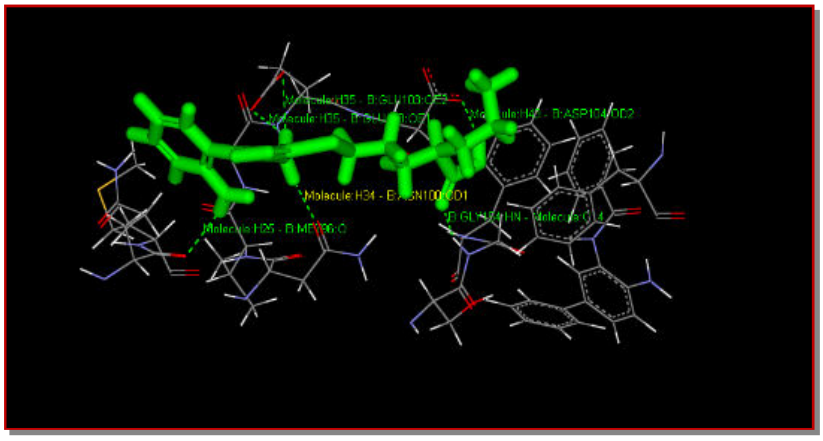

Figure 4: Docking of ligand VA8 with 3 maxB at site 6

could be inferred from Log P value. When its value is increasing, the drug will be more hydrophobic. When the drug is more hydrophobic, then the drug will be able to circulate longer in our body, because it wouldn't be easy to secrete it. The Table VI shows, that the Log P values of the P2, P4-P8, V2-V8, VA1, VA3,VA4 and VA6 modified ligands are larger than the SAHA standard ligand. It shows that the modified ligands are more hydrophobic than SAHA. Normally, drugs, which interact with enzyme inside human body, have Log P value between 2 and 5 (Copeland et al., 2005). The drug likeness value of standard and modified ligand shows the fragment content of the drugs. If the drug likeness values are increasing, than it has the same fragment content with existing drugs. From Table VI, it is shown that the drug likeness values of most ligands are larger than the SAHA standard ligand. This result tells us, that the modified ligand has the most fragments content of

Table VI

Drug likeness and scores of SAHA standard and modified ligands based on Osiris property explorer

Compounds	cLogP	Solubility	Mol Wt	Drug likeness	Drug score
SAHA	2.28	-3.33	264	-8.87	0.26
P1	1.05	-4.9	259	-7.8	0.22
P2	2.49	-7.31	353	-0.11	0.21
P3	0.8	-5.16	277	-0.25	0.3
P4	4.27	-7.75	411	-6.98	0.07
P5	4.42	-9.13	411	-8.03	0.11
P6	5.32	-6.85	399	-20.7	0.11
P7	3.69	-9.21	426	-11.89	0.07
P8	4.12	-8.83	427	-11.84	0.11
P9	1.59	-8.07	366	-7.8	0.14
P10	1.97	-8.23	408	-7.64	0.13
P11	2.01	-7.12	350	-7.81	0.15
P12	2.34	-7.39	392	-6.03	0.14
V1	1.14	-1.81	131	-11.48	0.17
V2	2.49	-7.31	353	-0.11	0.21
V3	1.82	-1.85	173	-2	0.29
V4	4.03	-8.02	429	0.52	0.11
V5	4.17	-9.39	429	-0.64	0.15
V6	2.86	-7.58	242	-3.12	0.1
V7	3.45	-9.47	444	-4.5	0.07
V8	3.87	-9.1	445	-4.45	0.11
V9	1.34	-8.34	384	-0.41	0.19
V10	1.72	-8.5	426	-0.27	0.19
V11	1.77	-7.38	368	-0.42	0.2
V12	2.1	-7.65	410	1.28	0.24
VA1	3.15	-2.24	158	-6.27	0.36
VA2	1.67	-5.97	192	-2.15	0.34
VA3	3.36	-8.06	268	-2.76	0.25
VA4	6.06	-8.63	332	-21.9	0.15
VA5	2.63	-8.13	283	-6.57	0.09
VA6	3.06	-7.76	284	-6.53	0.24
VA7	0.53	-7	223	-2.45	0.18
VA8	0.91	-7.16	265	-2.2	0.18
VA9	0.95	-6.05	207	-2.41	0.33
VA10	1.28	-6.31	249	-0.46	0.41

drugs. The drug score values are the combination of drug likeness, Log P, solubility, molecular weight, and toxicity risk within one useful practical value. It could be used for evaluating the potential of the drug candidate (Lindemann et al., 2004). When the drug score is better, then the compound has a better chance to be a drug candidate. Table VI shows that only modified P3 and V3 ligands have better drug score than SAHA standard ligand.

The toxicity of molecules is predicted using Osiris Property Explorer, and Lazar. All of them have different parameters for determining the toxicity of compounds. The prediction using Osiris Property Explorer was shown in colour codes. The result of toxicity analysis of SAHA standard ligand, first, and second modified ligands is shown in Table VII. Green colour shows the low toxicity tendency, yellow shows the mediocre tendency and red shows high tendency. Lazar is a software package with functionality of detecting mutagenic or carcinogenic properties based on the functional group similarity with mutagenic or carcinogenic ones. Lazar verified the mutagenicity of compounds by conducting assay test with *Salmonella typhimurium*. The carcinogenicity of compounds was verified by animal testing, with rat, and mouse (Table

Table VII

Toxicity of SAHA standard and modified ligand based on Osiris property explorer

Compounds	Mutagenic	Tumorigenic	Irritant	Reproductive effect
SAHA	Red	Green	Green	Green
P1	Red	Green	Green	Green
P2	Red	Green	Green	Green
P3	Red	Green	Green	Green
P4	Red	Red	Green	Green
P5	Red	Green	Green	Green
P6	Red	Green	Green	Green
P7	Red	Red	Green	Green
P8	Red	Green	Green	Green
P9	Red	Green	Green	Green
P10	Red	Green	Green	Green
P11	Red	Green	Green	Green
P12	Red	Green	Green	Green
V1	Red	Green	Red	Green
V2	Red	Green	Red	Green
V3	Red	Green	Green	Green
V4	Red	Red	Green	Green
V5	Red	Green	Green	Green
V6	Red	Yellow	Green	Yellow
V7	Red	Red	Green	Green
V8	Red	Green	Green	Green
V9	Red	Green	Green	Green
V10	Red	Green	Green	Green
V11	Red	Green	Green	Green
V12	Red	Green	Green	Green
VA1	Green	Green	Green	Yellow
VA2	Green	Green	Green	Green
VA3	Green	Green	Green	Green
VA4	Green	Green	Green	Green
VA5	Red	Red	Green	Green
VA6	Green	Green	Green	Green
VA7	Red	Green	Green	Green
VA8	Red	Green	Green	Green
VA9	Green	Green	Green	Green
VA10	Green	Green	Green	Green

VIII). The best ligands for HDAC2 *Homo sapiens* could be determined based on drug scan and docking analysis (Alonso et al., 2006).

The docking result of SAHA standard, first, and second modified ligands toward HDAC2 shows that those ligands have same type of interaction toward HDAC2. The analysis of ΔG binding and Score show that modified ligand have smaller ΔG binding than SAHA standard ligand. It could be inferred modified ligand has better binding affinity than SAHA standard ligand. Every modified ligand has good pharmacological properties, and it could be inferred by its accordance with Lipinski's Rule, hydrophobicity based on log P value, and good drug likeness and drug score. However, the best ligands according to the binding energy and drug scan analysis are P2, P6 and V6 ligands, in this end; our SAR study has proven that P2, P6 and V6 inhibitors are the best inhibitor as alternatives of SAHA.

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Table VIII

Toxicity analysis result by using lazar

Name of the compound	Mutagenicity		Carcinogencity		
	DBS Mutagenicity	Salmonella typhimurium (Kazius/Bursi)	Mouse	Rat	Multi cell call
P1	No	No	No	No	No
P2	No	No	No	No	No
P3	No	No	No	No	No
P4	No	Yes	No	No	No
P5	No	Yes	No	No	No
P6	No	No	No	No	No
P7	No	No	No	Yes	No
P8	Yes	No	No	No	No
P9	No	No	No	No	No
P10	No	No	No	No	No
P11	No	Yes	No	No	No
P12	No	Yes	No	No	No
V1	No	No	No	No	No
V2	No	No	Yes	No	No
V3	No	No	Yes	Yes	No
V4	Yes	No	Yes	No	No
V5	No	No	Yes	No	No
V6	No	No	No	No	No
V7	Yes	Yes	Yes	Yes	Yes
V8	Yes	No	No	No	Yes
V9	No	No	No	No	Yes
V10	Yes	Yes	No	No	Yes
V11	Yes	Yes	Yes	-	Yes
V12	Yes	Yes	Yes	-	No
VA1	No	No	No	No	No
VA2	No	No	Yes	No	No
VA3	No	Yes	Yes	No	No
VA4	No	No	No	No	No
VA5	Yes	Yes	Yes	Yes	No
VA6	No	Yes	No	No	No
VA7	No	No	No	No	No
VA8	No	No	No	No	No
VA9	Yes	Yes	Yes	No	No
VA10	Yes	Yes	Yes	No	No

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