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Design, synthesis, computational and biological evaluation of novel hydroxamic and carboxylic acid derivatives as histone decaetylase inhibitors

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One of the recent targets is histone deacetylase (HDAC) which provide a very promising new approach for anticancer drugs, which may combine clinical efficacy with relatively mild toxicological side effects. Modification of histone acetylation level, promoted by histone acetylase (HAT) and HDAC enzyme, has been recognize to play an important role in epigenetic modulation of gene expression, so HDAC inhibitors are considered a new class of anticancer agents. A new series of hydroxamic and carboxylic acid analogues based on the 1,3,4-thiadiazole scaffold has been designed and synthesized with the aim of exploring its potential as new antitumor agents. Biological results have revealed that the structural modifications proposed significantly affected inhibitory potency as well as selectivity for HDAC inhibitors. Most target compounds are significantly more active, specifically **5a**, **5b**, **5e** with IC_{50} values in the low micromolar or, the most active compounds in the series. Selected compounds have been tested on the viability of MDA-MB-231 (breast cancer cell) and K562 (chronic myelogenous leukemia cell), A549 (human lung cancer), PC3 (Prostate cancer cell lines) using MTT assay. Docking simulations suggested that the most active compounds can recognize the binding site (PDB Code 1w22 reference compound) using a similar interactions network. These results have allowed us to rationalize the observed structure–activity relationships.

Keywords: Hydroxamic acid derivatives, carboxylic acid derivatives, anticancer agents, 1,3,4-thiadiazoles,computational study

Histone acetylase and histone deacetylase two classes of enzyme work in opposing direction either by the transfer of acetyl group from acetyl Co A with the help of histoneacetylase (HATs) or removing acetyl group with histone deacetylase (HDACs) from lysine residue of histone tails[1, 2] (Figure 1). Disturbance of Histone acetylasetransferase (HAT) and Histone deacetylase (HDACs) activities has been connected with the increase of a varied range of human cancers. Histone deacetylase (HDACs) inhibitors cause o increase of the acetylated level of histones, which in turn stimulate the re-expression of silenced controlling genes in cancer cells and inverse the malignant phenotype. Outstanding to this influence, Histone deacetylase (HDACs) inhibitors have recently emerged as potential cancer therapeutic agents[3, 4].

There are four classes of HDAC on their sequence homology to *saccharomyces cerevisiae* HDACs[5, 6]. Eighteen distinct human HDACs are grouped in this four main category.The HDAC family is divided into Zn dependent (Class I and Class II) and NAD – Dependent (Class III) enzymes. The Zn-dependent enzymes have been the focus of intense research, while the Sir2 family recently implicated in acetylation and regulation of key cell cycle proteins such as p53.Till date, eleven HDAC family members in classes I and II are characterized. *i.e*. HDACs 1,2,3,8 are class 1 and HDACs 4-7,9,10 are class II, a grouping based on the sequence similarity. The most recent identified member of HDAC family is HDAC 11 comprising in Class IV. ClassI family are homologous to yeast RPD-3 (reduced potassium dependency-3). They share a compact structure. They are predominantly nuclear proteins and ubiquitously expressed in most tissues and cell lines. Class II are homologous to yeast HDA-1-protien

Figure 1 — Structural Requirement of Vorinostat

(Histone deacetylase-1). It can be subdivided into two subclasses. Class IIa (HDAC 4,5,7,9) have one catalytic domain and long amino terminal adapter domain. Class II b (HDAC 6, HDAC 10) have two catalytic domain. Class II family HDACs are mainly confined in the cytoplasm, however depending upon the phosphorylation status they are split between the cytoplasm and nucleus. Class III inhibitors are also known as sirtuins as they comprise of seven members and they share sequence homology with yeast silent information regulator-2 (Sir-2) protein. They do not contain zinc and their activity require nicotinamide adenine dinucleotide (NAD^+). Sirtuins (SIRTs) are found in three important cellular compartments: nucleus, cytoplasm and mitochondrion. Phylogenetically SIRTs are distributed into four classes (SIRT1,SIRT2 and SIRT3 belong to class1,SIRT4 to class-II, SIRT5 to Class III, and SIRT6 and SIRT7 to class IV). HDAC 11 has conserved residues in its catalytic center that are shared by both class I and Class II deacetylase and placed in Class IV[7, 8].

HDAC inhibitors of the hydroxamic class have common structural characteristics, (i) Capping group (surface recognition), that binds the protein and responsible for specificity, (ii) A straight chain alkyl, vinyl, or aryl linker that connect the ZBM and capping group and must fit in to the narrow hydrophobic group, (iii) Zinc binding moiety (ZBM) in catalytic pocket and give enzyme inhibitory activity[9].

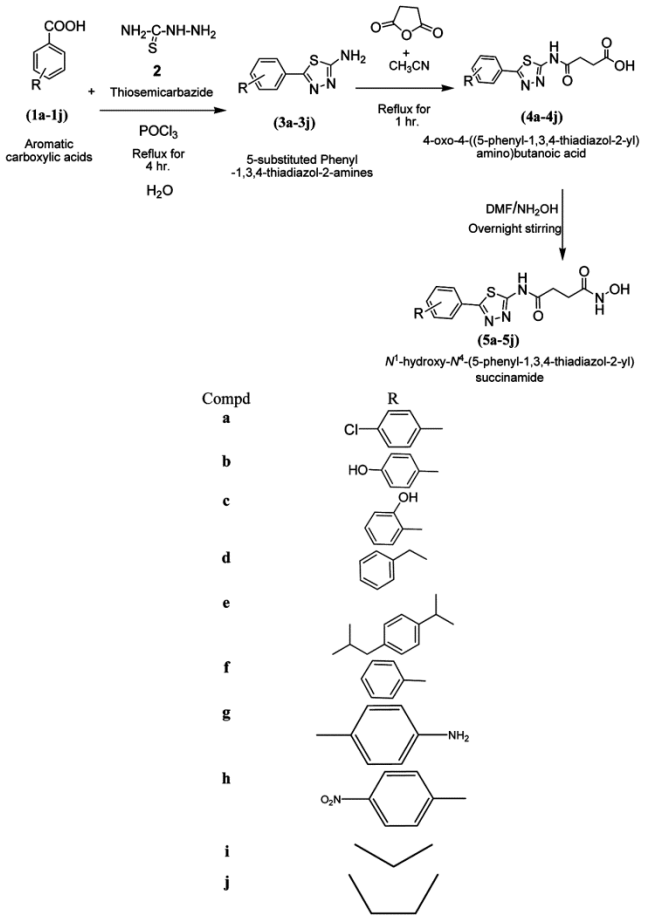
In prototypical HDAC inhibitor, the capping group is solvent exposed and interacts with the amino acids near the entrance of the active site. The metal binding group resides in the protein interior and complexes the metal ion involved in catalysis. The incorporation of substituent on the linker adjacent to the metal binding moiety has a variable influence on inhibitory activity.

HDAC enzyme remove the acetyl group from histone using a charge-relay mechanism consisting of two adjacent histidine residue and one tyrosine residue, and crucial for this charge-relay Zn^{2+} ion, which bind deep in the pocket of the enzyme.

Novel HDAC inhibitors have been generated by modification on ZBM, linker and capping group. The catalytic core comprises a Zn^{2+} ion coordinated by two histidine residues and an aspartate, two his-asp dyed and a tyrosine residue.

Results and Discussion

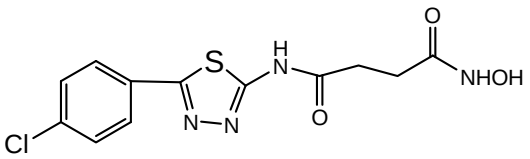
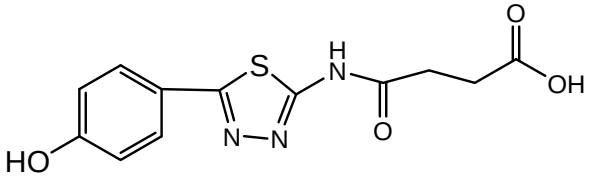
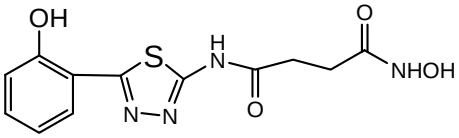
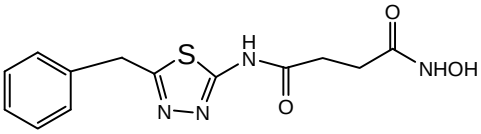
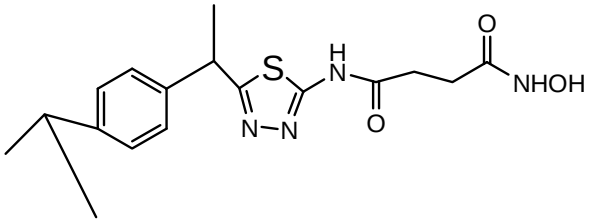
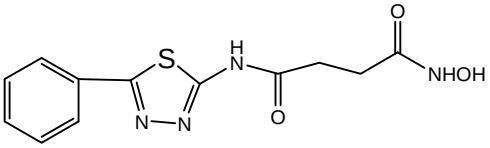
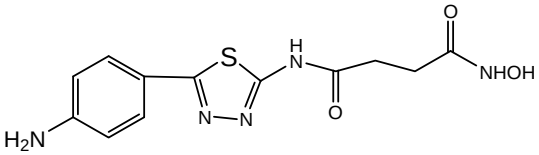
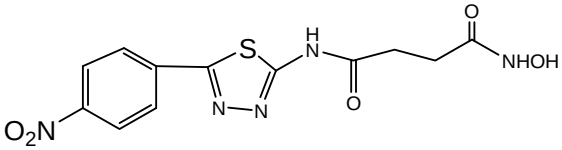
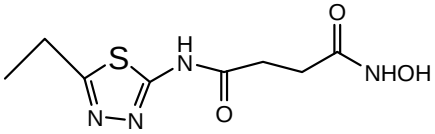
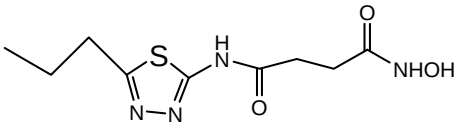
HDAC inhibition activity of the 1,3,4-thiadiazole based hydroxamates and carboxylates (Scheme I)



Scheme I

were assessed by the Color de Lys assay and the results are tabulated as IC50 values in Table I. According to the data in Table I, the substitution in 1,3,4-thiadiazole(cap group) and the terminal hydroxamate and carboxylate group (Zinc binding group) play a role in potency. For example, compounds with the hydroxamic acid substitution are found to exhibit good activity micromolar level while carboxylic acid substitution proves to have less activity. In general, the substitutions in 1,3,4-thiadiazole ring have an profound effect on the inhibitory activities against HDAC compared with the linker between zinc binding group and 1,3,4-thiadiazole ring. To further validate the utility of this set of structures at the cellular level, the effect of the exposure of selected compounds was tested on the viability of MDA-MB-231 (breast cancer cell) and K562 (chronic myelogenousleukemia cell), A549 (human lung cancer), PC3 (Prostate cancer cell lines). We evaluated compounds **5a**, **5b**, and **5e** using MTT assay. The IC50 values were summarized in Table II.

Table I — HDAC activity of substituted 1,3,4-thiadiazole hydroxamates and carboxylates derivatives

S. No	Compd	Structure	IC 50 value
1	HA01		0.19± 0.04
2	HA02		0.27 ± 0. 05
3	HA03		0.59± 0.04
4	HA04		0.42± 0.05
5	HA05		0.31± 0.004
6	HA06		1.91± 0.01
7	HA07		2.27± 0.04
8	HA08		2.86± 0.05
9	HA09		3.38± 0.04
10	HA10		3.49± 0.04

(*Contd*.)

Table I — HDAC activity of substituted 1,3,4-thiadiazole hydroxamates and carboxylates derivatives (*Contd*.)

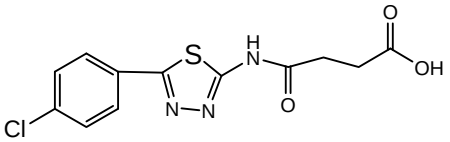
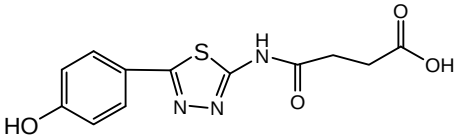
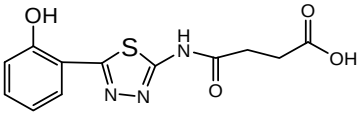
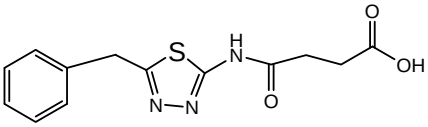
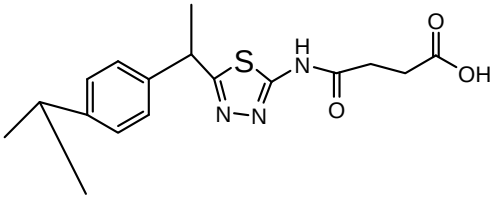
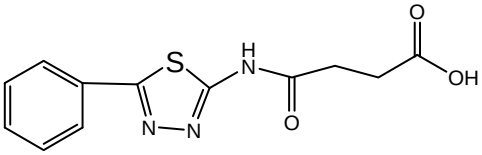
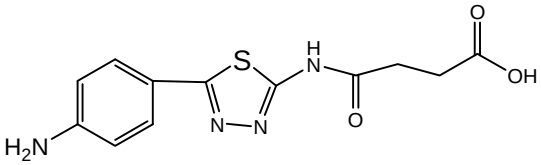
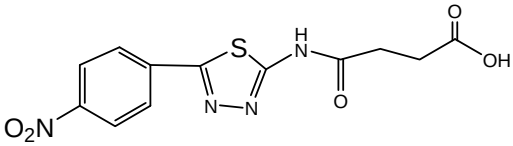
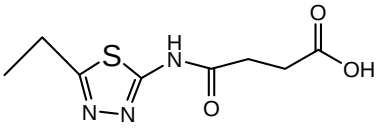
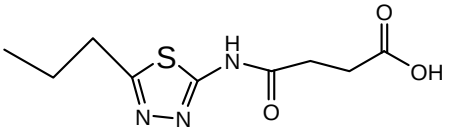
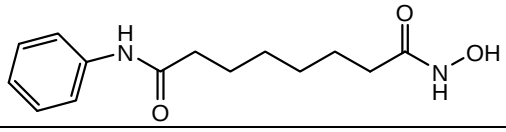
S. No	Compd	Structure	IC 50 value
11	CA01		>5
12	CA02		>5
13	CA03		>5
14	CA04		>5
15	CA05		>5
16	CA06		>5
17	CA07		>5
18	CA08		>5
19	CA09		4.39± 0.05
20	CA10		4.85± 0. 04
21	SAHA		0.15±0.02

Table II — Proliferative activities of representative compounds against various cancer cell lines

S. No	Compd	IC50 value of HDAC(μM)	MDA-MB-231 IC50 value (μM)	K562 IC50 value (μM)	A549 IC50 value (μM)	PC3 IC50 value (μM)
1	HA01	0.19± 0.04	19.43	23.59	>50	45.89
2	HA02	0.27 ± 0. 05	35.13	32.57	23.56	>50
3	HA05	0.31± 0.004	14.67	38.47	35.87	26.56
4	SAHA	0.15±0.02	1.35	1.96	4.10	5.56

Table III — Molecular docking studies of synthesized compounds

S. No.	Compd	ID	Docking Score	HBD	HBA	Mol. Wt.	CLOGP
1	**4a**	CA01	-23.2407	2	6	311.744	1.859
2	**4b**	CA02	-24.1379	3	7	293.298	0.952
3	**4c**	CA03	-35.3017	3	7	293.298	0.952
4	**4d**	CA04	-19.4404	2	6	291.326	1.229
5	**4e**	CA05	-21.5976	2	6	347.432	2.886
6	**4f**	CA06	-21.9883	2	6	277.299	1.984
7	**4g**	CA07	-21.6959	4	7	292.314	0.448
8	**4h**	CA08	-16.0321	2	9	322.297	1.089
9	**4i**	CA09	-49.5047	2	6	229.256	0.952
10	**4j**	CA10	-46.7130	2	6	243.283	0.653
11	**5a**	HA01	-81.9616	3	7	326.759	1.244
12	**5b**	HA02	-76.0022	4	8	308.313	0.337
13	**5c**	HA03	-74.1298	4	8	308.313	0.337
14	**5d**	HA04	-74.7134	3	7	306.24	0.614
15	**5e**	HA05	-75.3654	3	7	362.447	2.271
16	**5f**	HA06	-69.5264	3	7	292.314	0.579
17	**5g**	HA07	-66.3344	5	8	307.328	-0.167
18	**5h**	HA08	-62.274	3	10	337.311	0.474
19	**5i**	HA09	-74.7416	3	7	244.271	-0.418
20	**5j**	HA10	-72.0623	3	7	258.297	0.038

Among these compounds, all of them could inhibit the cell proliferation effectively activity. In molecular docking, the reference binding mode of HDAC was predicted with docking protocol (PDB Code 1w22) and a docking score of −82.29 was found. The binding interaction shows H-bond with polar amino acid (Thr306) and p-p interaction with hydrophobic residues of His142. All the test compounds were further docked and their docking scores were predicted (Table III). For test compounds a common binding mode was observed, where binding site is comprised of His 143, His 180, His 142, Phe 152, Phe 208 and Tyr 306 amino acid residues. log P values (045-1.85) for the test compounds were noted target compounds also shows mild to moderate similarity with respect to standard drug.

Materials and Methods

All the chemicals used were of laboratory grade and procured from Rankem Astron (India). and Sigma Aldrich. Melting point of synthesized compounds were performed in one end open capillary on VEEGO (VMP-PM) melting point apparatus. The purity of compound were performed by using pre-coated TLC plates and solvent systems. The TLC evaluated in UV chamber at 254 nm wave length and Iodine chamber. The FT-IR of synthesized compounds were performed on SIMADZU FT-IR 8400 by using KBr pellets. The 1H NMR (Nuclear Magnetic Resonance) and ^{13}C NMR of synthesized compounds were performed on BRUKER AVANCE-III 400 MHz FT-NMR instrument by using the DMSO/$CDCl_3$ as solvent, TMS as internal standard. The signals are quoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet and are expressed in d ppm. Mass spectra (GC-MS) of synthesized compounds were performed on SHIMADZU QP2010.

Docking

Docking predicts the binding orientation and affinity of a ligand to a target. Software used for docking is FRED 2.0,Open Eye Scientific software. It is used to dock a large collection of molecules into the active site of a target protein.It determines the

database file format from the file extension. mol. Subsequent screening is based on the crystal structure of HDAC(PDB code:1w22).Using a combination of various aspects such as, structure generation, shape alignment and flexible fitting, a ligand of interest is compared to bound ligands and its similarity to such both guides the nature of the applied algorithm and produces an accuracy estimate. Poses are analysed and selected from Discovery Studio Visualizer. Drug likeness is also calculated from the same.

***In vitro* HDAC assay**

We performed assays according to the kit instruction (BML-AK501-0001 HDAC Colorimetric acitivity assay kit). HDAC came from HeLa cell nucleus extracts, mainly including HDAC1 and HDAC2. The tested compounds and the control drug SAHA (Figure 2, Figure 3 and Figure 4) were diluted to various concentrations. On the 96-well plate, HDAC (5 lL/well) were incubated at 37ºC with 10 lL of various concentrations of samples and 25 lL of substrate. After reacting for 30 min, Color de Lys Developer (50 lL/well) was added. Then, after 15 min the ultraviolet absorption of the wells was measured on a microtiter-plate reader at 405 nm. The inhibition rates were calculated from the ultraviolet absorption readings of inhibited wells related to those of control wells. Finally, the IC50 values were determined using a regression analysis of the concentration/inhibition. Thus, this assay has two step procedure. First, COLOR DE LYS substrate comprises an acetylated lysine side chain,is incubated with the sample containing HDAC activity.Deacetylation of the substrate sensitizes the substrate so that, in second step, mixing with the COLOE DE Lys developer causes an increase in yellow color intensity, and absorption at 405nm.

MTT assay

MDA-MB-231, K562, A549 and PC3 (Prostate cancer cell lines). were respectively cultured in RPMI1640 medium containing 10% FBS at 37ºC in 5% CO2 humidified incubator. Cell proliferation was determined by the MTT (3- [4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) assay. Briefly, cells were plated in a 96-well plate at 10,000 cells per well, cultured for 4 h in complete

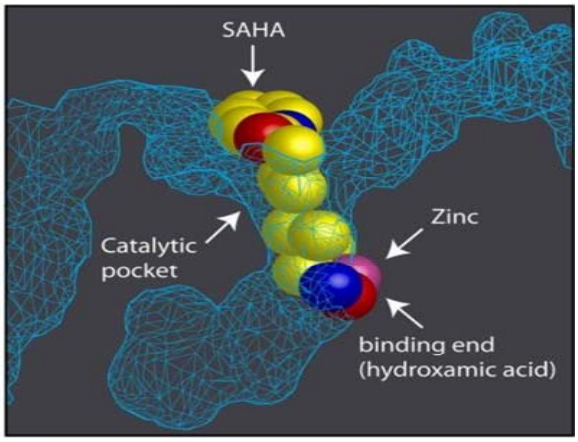


Figure 2 — The pharmacophore based on bound confirmation of SAHA

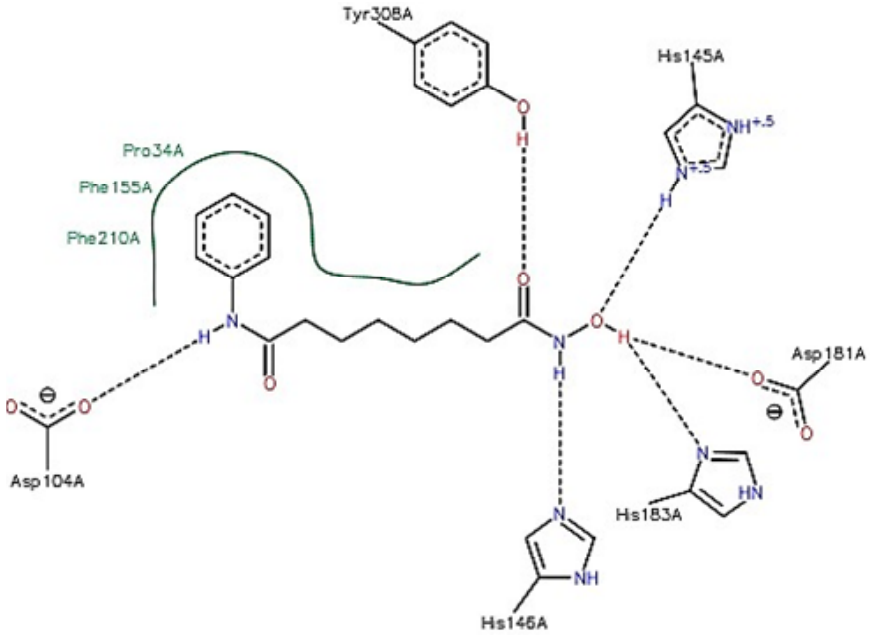


Figure 3 — Common framework for SAHA with amino-acid

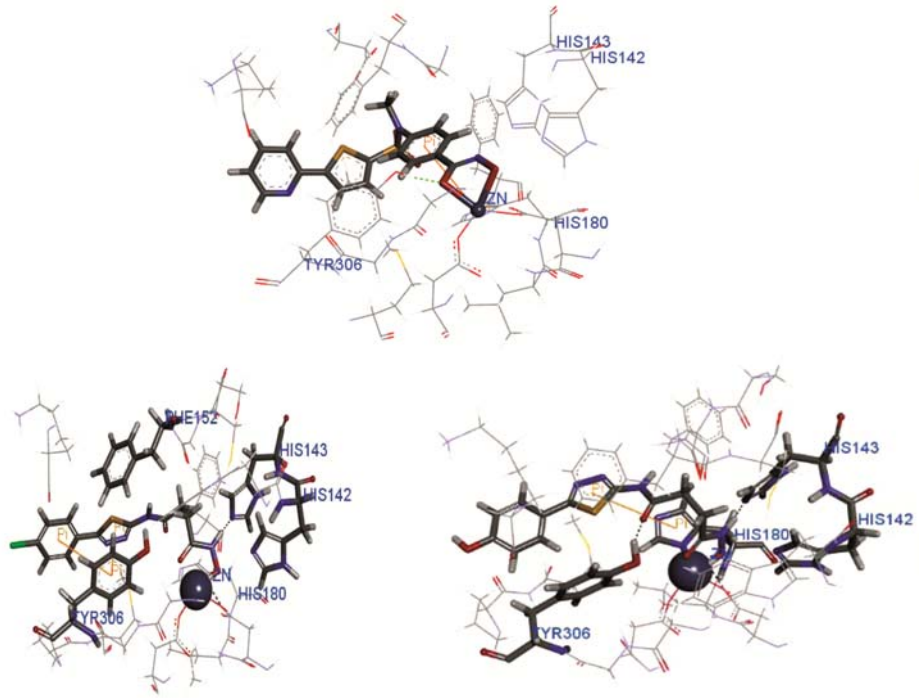


Figure 4 — Docking of the molecule with crystal structure having Pdb Code: 1w22

growth medium, then treated with 2000, 400, 80, 16, 3.2, 0.64 mg/mL of compounds for 48 h. 0.5% MTT solution was added to each well. After further incubation for 4 h, formazan formed from MTT was extracted by adding 200 lL DMSO and mixing for 15 min. Optical density was read with anmicrotiter-plate reader at 570 nm.

Experimental Section

5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amine, 3a

A strring mixture of benzoic acid (0.05 mol), N-amino-thiourea (0.05 mol) and $POCl_3$ (13 mL) was heated at 75ºC for 0.5 h. After cooling down to RT, water (10 mL) was added. The reaction mixture was refluxed for 4 hr. After cooling, the mixture was basified to *p*H 8 by the drop-wise addition of 50% NaOH solution under strring. The precipitate was filtered and recrystallized from ethanol to yield the target compound **3a** as a colorless crystal. Yield: 60%, m.p. 213-215ºC; ESI-MS *m/z*: 212; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (s, 1H, NH), 3.30-6.40 (m, 4H, Ph-C_2-C_3-C_5-C_6).

Compounds **3b-j** were synthesized following the procedure described above.

4-(5-Amino-1,3,4-thiadiazol-2-yl)phenol, 3b: Yield: 78%, m.p. 209-213ºC ESI-MS *m/z* (M+1) 195, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H, 1OH), 10.03 (s, 1H, NH), 4.70-6.31 (m, 4H, Ph-C_2-C_3-C_5-C_6).

2-(5-Amino-1,3,4-thiadiazol-2-yl)phenol, 3c: Yield: 64%, m.p. 212-215ºC ESI-MS *m/z* (M+1): 195, ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 3.70-5.31 (m, 4H, Ph-C_2-C_3-C_5-C_6).

5-Benzyl-1,3,4-thiadiazol-2-amine, 3d: Yield: 75%, m.p. 211-215ºC ESI-MS *m/z* (M-1): 190; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.01 (s, 1H, 1OH), 10.20 (s, 1H, NH), 4.70-6.90 (m, 5H, Ph-C_2-C_3- C_4-C_5-C_6).

5-(1-(4-Isopropylphenyl)ethyl)-1,3,4-thiadiazol-2-amine, 3e: Yield: 80%, m.p. 205-208ºC; ESI-MS *m/z* (M-2) 245; ^{1}H NMR (400 MHz DMSO-d_6): δ 10.39 (s, 1H, NH), 3.70-6.12 (m, 4H, Ph-C_2-C_3-C_5-C_6), 2.50-3.30 (s, 4H, CH_2-CH_2).

5-Phenyl-1,3,4-thiadiazol-2-amine, 3f: Yield: 68%, m.p. 207-209ºC ESI-MS *m/z* (M-1) 176; ^{1}H NMR (400 MHz DMSO-d_6): δ 10.39 (s, 1H, NH), 4.70-7.59 (m, 5H, Ph-C_2-C_3-C_4-C_5-C_6).

2-Amino-5-substituted-1,3,4-thiadiazole, 3g: Yield: 69%, m.p. 209-211ºC ESI-MS *m/z*: 192; ^{1}H NMR (400 MHz DMSO-d_6): δ 11.23-11.59 (d, 2H, NH), 4.70-7.52 (m, 4H, Ph-C_2-C_3-C_5-C_6).

5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-amine, 3h: Yield: 49%, m.p. 206-209ºC ESI-MS *m/z* (M-1): 221;

^{1}H NMR (400 MHz DMSO-d_6): δ 10.45 (s, 1H, NH), 4.10-6.92 (m, 4H, Ph-C_2-C_3-C_5-C_6).

5-Ethyl-1,3,4-thiadiazol-2-amine, 3i: Yield: 70%, m.p. 202-205ºC ESI-MS *m/z* (M-1) 128; ^{1}H NMR (400 MHz DMSO-d_6): δ 10.45 (s, 1H, NH), 2.50-3.32 (s, 4H, CH_2-CH_2).

5-Propyl-1,3,4-thiadiazol-2-amine, 3j: Yield: 69%, m.p. 210-213ºC ESI-MS *m/z* (M-1) 142; ^{1}H NMR (400 MHz DMSO-d_6): δ 10.10 (s, 1H, NH), 2.50-4.91 (m, 4H, CH_2-CH_2,CH_3).

4-Oxo-((5-phenyl-1,3,4-thiadiazole-2-yl)) amino) butanoic acid, 4a: Succinic anhydride (0.02mol) was added to a solution of thiadiazole in a hot acetonitrile (150 mL). The solution was heated for 1 hr under reflux. After cooling, the precipitate product was collected. Recrystallization is carried out using alcohol to give **4a** as white solid. Yield: 56%, m.p. 250-253ºC; ESI-MS *m/z* (M+1)312; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.10-12.33 (m, 2H, 2OH), 10.90 (s, 1H, NH), 6.69-7.42 (m, 4H, Ph-C_2-C_3-C_5-C_6), 2.40-2.62 (d, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.3, 176.6 (C=O), 166.03 (Thiadiazole-C_2), 153 (Thiadiazole-C_5), 134 (Ph-C_4), 129.44 (Ph-C_3-C_5), 128.85 (Ph-C_2-C_6), 30.70, 32.30 (CH_2-CH_2).

Compounds 4b-j were synthesized following the procedure described above.

4-(5-(4-Hydroxyphenyl)-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4b: Yield: 55%, m.p. 248-250ºC; ESI-MS *m/z*: (M+1) 295; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30-1.99 (m, 2H, 2OH), 10.59 (s, 1H, NH), 6.70-7.31 (m, 4H, Ph-C_2-C_3-C_5-C_6), 2.41-2.59 (d, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.30, 175.6 (C=O), 152.30 (Thiadiazole-C_5), 158.50 (Ph-C_4), 126.10 (Ph-C_1), 116.40 (Ph-C_3-C_5), 128 (Ph-C_2-C_6), 30.7- 32.30 (CH_2-CH_2).

4-(5-(2-Hydroxyphenyl)-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4c: Yield: 62%, m.p. 254-258ºC; ESI-MS *m/z* (M+1) 295; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.33-12.46 (m, 2H, 2OH), 10.093 (s, 1H, NH), 6.89-7.76 (m, 4H, Ph-C_2-C_3-C_5-C_6), 3.357 (s, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.3, 175.6 (C=O), 173.70 (Thiadiazole-C_2), 155 (Thiadiazole-C_5), 136.1 (Ph-C_4), 135.09 (Ph-C_1), 129 (Ph-C_3-C_5), 128.92 (Ph-C_2-C_6), 30.70- 32.50 (CH_2-CH_2).

4-(5-Benzyl-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4d: Yield: 65%, m.p. 238-241ºC; ESI-MS *m/z* (M-1) 291; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.90 (s, 1H, 1OH), 10.80 (s, 1H, NH), 7.06-7.16 (m, 5H, Ph-C_2-C_3-C_4-C_5-C_6), 2.30-2.50 (d, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.3, 173.7 (C=O), 161.00 (Thiadiazole-C_2), 152.80 (Thiadiazole-C_5), 136.1 (Ph-C_4), 129.15 (Ph-C_1), 128.70 (Ph-C_3-C_5), 125.80 (Ph-C_2-C_6), 30.70- 32.90 (CH_2-CH_2), 37.60 (CH_2).

4-(5-(1-(4-Isopropylphenyl)ethyl)-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4e: Yield: 76%, m.p. 238-240ºC; ESI-MS *m/z*: 277; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (s, 1H, 1OH), 11.03 (s, 1H, NH), 6.65-7.10 (m, 5H, Ph-C_2-C_3-C_4-C_5-C_6), 2.40-2.60 (m, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.3, 172.3(C=O), 163.00 (Thiadiazole-C_2), 151 (Thiadiazole-C_5), 134.1 (Ph-C_4), 126.15 (Ph-C_1), 125.70 (Ph-C_3-C_5), 123.80 (Ph-C_2-C_6), 24.00- 39.90 (m, 15H, CH_2-CH_2).

4-Oxo-4-(5-phenyl-1,3,4-thiadiazol-2-ylamino) butanoic acid, 4f: Yield: 53%, m.p. 249-253ºC; ESI-MS *m/z* (M+1) 293; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.71 (s, 1H, 1OH), 10.50 (s, 1H, NH), 7.10-7.56 (m, 5H, Ph-C_2-C_3-C_4-C_5-C_6), 2.10-2.61 (m, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.3, 175.6 (C=O), 173.70 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 129.30 (Ph-C_4), 127.09 (Ph-C_1), 133 (Ph-C_3-C_5), (Ph-C_2-C_6), 30.70- 32.50 (CH_2-CH_2).

4-(5-(4-Aminophenyl)-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4g: Yield: 59%, m.p. 238-241ºC; ESI-MS *m/z*: (M+1) 323; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.21 (s, 1H, 1OH), 10.10 (s, 1H, NH), 7.00-7.50 (m, 4H, Ph-C_2-C_3-C_5-C_6), 2.40-2.61 (d, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.30, (C=O), 152 (Thiadiazole-C_5), 136.1 (Ph-C_4), 135.09 (Ph-C_1), 128.30 (Ph-C_3-C_5), 116 (Ph-C_2-C_6), 30.70- 32.30 (CH_2-CH_2).

4-(5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4h: Yield: 42%, m.p. 242-245ºC; ESI-MS *m/z*: 322; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.10 (s, 1H, 1OH), 10.80 (s, 1H, NH), 7.15-8.23 (m, 4H, Ph-C_2-C_3-C_5-C_6), 2.31-2.61 (d, 4H, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 177.3, 170.6 (C=O), 166.03 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 148.40 (Ph-C_1), 128.31 (Ph-C_3-C_5), 116.50 (Ph-C_2-C_6), 30.70-32.30 (CH_2-CH_2).

4-(5-Ethyl-1,3,4-thiadiazol-2-ylamino)-4-oxobutanoic acid, 4i: Yield: 60%, m.p. 218-220ºC ESI-MS *m/z* (M+1): 230; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (m, 2H, 2OH), 10.10 (s, 1H, NH), 2.10-2.74 (m, CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 174.3, 170.6 (C=O), 168.03 (Thiadiazole-C_2), 150.30 (Thiadiazole-C_5), 31.70-34.30 (CH_2-CH_2), 16.70-21.30 CH_2-CH_2).

4-Oxo-4-(5-propyl-1,3,4-thiadiazol-2-ylamino) butanoic acid, 4j: Yield: 65%, m.p. 224-229ºC; ESI-MS *m/z*: 243; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (s, 1H, 1OH), 10.79 (s, 1H, NH), 1.19-4.87 (m, 11H, CH_2-CH_2). CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6):δ 175.3, 172.4 (C=O), 170.03 (Thiadiazole-C_2), 155.30 (Thiadiazole-C_5), 17.70-35.30 (m, 12C, CH_2-CH_2).

N1-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-N4-hydroxysuccinamide, 5a

To a -20ºC cooled solution of acid derivative (0.07mole) and N-methylmorpholine (0.014mole) in anhydrous N,N-dimethylformamide (15 mL) was added ClCOOBu-i (isobutyl chloroformate), and the mixture was stirred for 0.5h. The solid was filtered out and filtrate was added to freshly prepared NH_2OK in methanol (5 mL, 1.54mol/L).The resulting mixture was stirred at RT overnight, then was filtered and the residue was washed with water to give crude product. Recrystallization is carried out using ethanol to give **5a** as white solid. Yield: 52%, m.p. 207-209ºC; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 12.50 (s, 1H, OH), 11.10 (s, 1H, NH), 8.0 (s, 1H, NH), 7.42 (d, 2H, Ph-C_2-C_6), 7.33 (d, 2H, Ph-C_3-C_5), 2.48-32.451 (CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.47, 173.27 (C=O), 170.00 (Thiadiazole-C_2), 152 (Thiadiazole-C_5), 134 (Ph-C_4), 129.47 (Ph-C_1), 128.95 (Ph-C_3-C_5), 126.97 (Ph-C_2-C_6), 28.00- 31.65 (CH_2-CH_2).

Compounds **5b-j** were synthesized following the procedure described above.

N1-Hydroxy-N4-(5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)succinamide, 5b: Yield: 51%, m.p. 207-209ºC; ESI-MS *m/z* (M+1):309; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 12.30 (s, 1H, OH), 10.90 (s, 1H, NH), 8.010 (s, 1H, NH), 7.314 (d, 2H, Ph-C_2-C_6), 6.79 (d, 2H, Ph-C_3-C_5), 2.461-2.00 (m, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ 172.47, 170.27 (C=O), 163.00 (Thiadiazole-C_2), 158 (Thiadiazole-C_5), 137 (Ph-C_4), 128.77 (Ph-C_1), 128.73 (Ph-C_3-C_5), 126.97 (Ph-C_2-C_6), 27.95- 29.65 (CH_2-CH_2).

N1-Hydroxy-N4-(5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)succinamide, 5c: Yield: 56%, m.p. 207-209ºC; ESI-MS *m/z* (M-1) 307.9; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 12.10 (s, 1H, OH), 10.20 (s, 1H, NH), 8.10 (s, 1H, NH), 6.99 (d, 2H, Ph-C_2-C_6), 6.29 (d, 2H, Ph-C_3-C_5), 2.461-2.00 (m, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.00, 173.7 (C=O), 170.00 (Thiadiazole-C_2), 158 (Thiadiazole-C_5), 130 (Ph-C_4), 126.77 (Ph-C_1), 123.73 (Ph-C_3-C_5), 121.97 (Ph-C_2-C_6), 31.00- 28.00 (CH_2-CH_2).

N1-(5-Benzyl-1,3,4-thiadiazol-2-yl)-N4-hydroxysuccinamide, 5d: Yield: 61%, m.p. 207-209° C ESI-MS *m/z*: ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 13.10 (s, 1H, OH), 11.80 (s, 1H, NH), 8.010 (s, 1H, NH), 7.42 (d, 2H, Ph-C_2-C_6), 7.14 (d, 2H, Ph-C_3-C_5), 3.810 (s, 2H, CH_2), 2.481 (m, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 173.70, 170.60 (C=O), 161.00 (Thiadiazole-C_2), 152 (Thiadiazole-C_5), 136 (Ph-C_4), 129.15 (Ph-C_1), 129.90 (Ph-C_3-C_5), 128.97 (Ph-C_2-C_6), 28.00- 31.60 (CH_2-CH_2), 37.80 (CH_2).

N1-Hydroxy-N4-(5-phenyl-1,3,4-thiadiazol-2-yl) succinamide, 5e: Yield: 61%, m.p. 207-209°C; ESI-MS *m/z* (M-1) 292; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 12.63 (s, 1H, OH), 11.72 (s, 1H, NH), 8.9 (s, 1H, NH), 7.89 (d, 2H, Ph-C_2-C_6), 7.51 (d, 2H, Ph-C_3-C_5), 3.07-3.13 (m, 2H, CH_2), 2.83-2.93 (m, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 173.70 (C=O), 170.60 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 133 (Ph-C_4), 129.35 (Ph-C_1), 128.73 (Ph-C_3-C_5), 127.51 (Ph-C_2-C_6), 28.00- 31.00 (CH_2-CH_2), 34.5 CH_3.

N1-(5-(4-Aminophenyl)-1,3,4-thiadiazol-2-yl)-N4-hydroxysuccinamide, 5f: Yield: 52%, m.p. 207-209ºC; ESI-MS *m/z*: (M+1) 308; ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ 12.5 (s, 1H, OH), 10.90 (s, 1H, NH), 8.010 (s, 1H, NH), 7.23 (d, 2H, Ph-C_2-C_6), 6.51 (d, 2H, Ph-C_3-C_5), 4.00 (Ar-NH_2) 2.46-2.50 (m, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 176.00, 173.70 (C=O), 170.60 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 133 (Ph-C_4), 129.35 (Ph-C_1), 128.73 (Ph-C_3-C_5), 127.51 (Ph-C_2-C_6), 28.00- 31.00 (CH_2-CH_2).

N1-(5-(4-Aminophenyl)-1,3,4-thiadiazol-2-yl)-N4-hydroxysuccinamide, 5g: Yield: 67%, m.p. 207-209°C; ESI-MS *m/z* (M+1) 308; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 12.10 (s, 1H, OH), 8.2 (s, 1H, NH), 4.1 (s, 1H, NH_2), 6.52 (d, 2H, Ph-C_2-C_6), 7.23 (d, 2H, Ph-C_3-C_5); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.00, 173.70 (C=O), 170.00 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 148 (Ph-C_4), 128.30 (Ph-C_1), 123.30 (Ph-C_3-C_5), 116.80 (Ph-C_2-C_6), 28.00- 31.60 (CH_2-CH_2).

N1-Hydroxy-N4-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)succinamide, 5h: Yield: 60%, m.p. 204-206ºC; ESI-MS *m/z*: 337; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 12.50 (s, 1H, OH), 10.10 (s, 1H, NH), 7.56 (d, 2H, CH_2-CH_2), 7.74 (d, 2H, Ph-C_2-C_6), 2.48-2.80 (CH_2-CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.00,

173.70 (C=O), 170.00 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 148 (Ph-C_4), 128.45 (Ph-C_1), 128.40 (Ph-C_3-C_5), 121.60 (Ph-C_2-C_6), 28.00- 31.00 (CH_2-CH_2).

N1-(5-Ethyl-1,3,4-thiadiazol-2-yl)-N4-hydroxysuccinamide, 5i: Yield: 60%, m.p. 202-205ºC; ESI-MS *m/z*:244; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 10.50 (s, 1H, OH), 9.56 (s, 1H, NH), 8.0 (s, 1H, NH), 2.46-2.59 (CH_2-CH_2), 2.00 (CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.00, 173.7 (C=O), 170.00 (Thiadiazole-C_2), 152 (Thiadiazole-C_5), 31.00- 28.00 (m, 8H, CH_2-CH_2).

N1-Hydroxy-N4-(5-propyl-1,3,4-thiadiazol-2-yl) succinamide, 5j: Yield: 60%, m.p. 199-202ºC; ESI-MS *m/z*: 258.03; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 11.10 (s, 1H, OH), 10.08 (s, 1H, NH), 8.2 (s, 1H, NH), 2.48-2.59 (CH_2-CH_2), 2.00 (CH_3), 2.20 (CH_2); ^{13}C NMR (400 MHz, DMSO-d_6): δ 173.7, 170.0 (C=O), 165 (Thiadiazole-C_2), 150 (Thiadiazole-C_5), 31.00- 28.00 (m, 8H, CH_2-CH_2), 30.5 (CH_3).

Conclusions

In conclusion, we have developed various hydroxamic acid and carboxylic acid derivatives through cyclization and condensation approach through various aliphatic and aromatic substituents. The synthesis, computational studies and their evaluation for anticancer activity of new inhibitors are been carried out.The target compounds were successfully synthesized and well characterized by molecular docking studies revealed good binding interactions of target molecules. The target compounds also showed mild to moderate similarity with respect to standard drug. The pharmacokinetic and toxicity corroborate with standard compound and suggests its potential being drug-like candidates. However, further optimization might be beneficial in the future research and development of the target compounds for the refinement of the anticancer activity.

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