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DESIGN, SYNTHESIS, DOCKING STUDIES AND BIOLOGICAL EVALUATION OF NOVEL HYDROXAMIC AND CARBOXYLIC ACID DERIVATIVES AS HISTONE DEACETYLASE ENZYME INHIBITORS

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Keywords:

Hydroxamic acid derivatives, Carboxylic acid derivatives, Anticancer agents, 1,3,4-thiadiazoles, *in-silico* docking studies

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ABSTRACT: A new series of hydroxamic (5a-5j) and carboxylic acid analogs (4a-4j) based on the 1, 3, 4-thiadiazole scaffold was designed and synthesized to explore its potential as new antitumor agents. The chemical structures of the compounds were confirmed based on TLC, IR, ^{1}H NMR, and 13C NMR analysis. Molecular docking studies showed that the target compounds correctly dock into the binding pocket of histone deacetylase inhibitor (PDB Code 1w22 reference compound), while their bioavailability / drug-likeness was predicted to be acceptable. The predicted physicochemical were compared with those of a reference compound. Biological results revealed that the structural modifications proposed significantly affected inhibitory potency as well as selectivity for HDAC inhibitors. Most target compounds were 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 were 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.

INTRODUCTION: Histone acetylase and histone deacetylase are two classes of enzyme work in a divergent direction either by the transfer of acetyl group from acetyl Co A with the help of histone acetylase (HATs) or eliminating acetyl group with histone deacetylase (HDACs) from lysine residue of histone tails [1]. Disruption of Histone acetylase transferase (HAT) and Histone deacetylase (HDACs) activities has been associated with the rise of a variety of human cancers [2].

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Histone deacetylase (HDACs) inhibitors cause an increase of the acetylated level of histones, which in turn arouse the re-expression of silenced controlling genes in cancer cells and reverse the malignant phenotype. Due to this influence, Histone deacetylase (HDACs) inhibitors have recently developed as potential cancer therapeutic agents [3].

There are four classes of HDAC on their sequence homology to *Saccharomyces cerevisiae* HDACs [4]. Eighteen distinct human HDACs are grouped in these four key categories. 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 connected 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 utmost recent identified member of the HDAC family is HDAC 11 comprising in Class IV [5].

HDAC inhibitors of the hydroxamic class have common structural characteristics, 1. Capping group (surface recognition), that binds the protein and responsible for specificity, 2. A straight-chain alkyl, vinyl, or aryl linker that connect the ZBM and capping group and must fit into the narrow hydrophobic group, 3. Zinc binding moiety (ZBM) in the catalytic pocket and give enzyme inhibitory activity [6, 7].

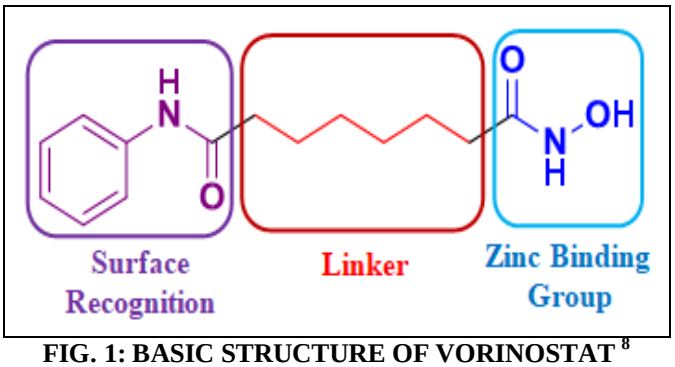


FIG. 1: BASIC STRUCTURE OF VORINOSTAT [8]

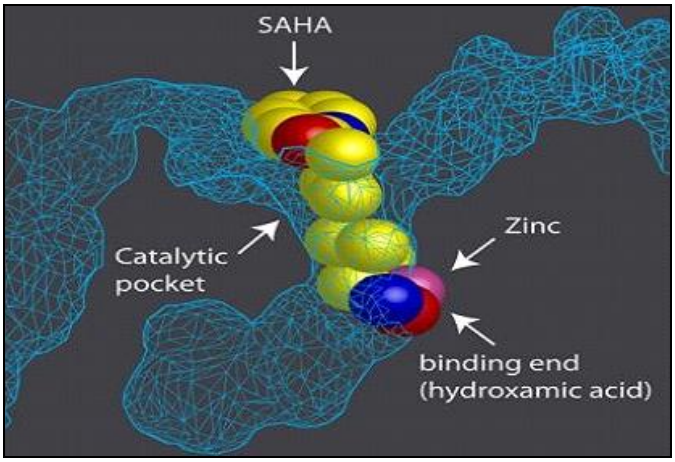


FIG. 2: THE PHARMACOPHORE BASED ON BOUND CONFIRMATION OF SAHA [6]

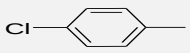
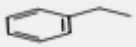
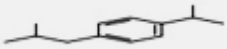
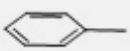
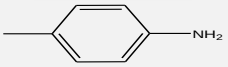
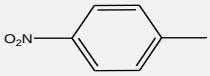
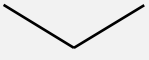
Here we design new molecules by structural modifications in surface recognition and zinc-binding site **Scheme 1** and evaluated as anticancer agents acting as histone deacetylase inhibitors. The main structural features explored included changes in the relative position of the substituent group of zinc-binding site. In Molecular docking studies, the physicochemical similarity of the target compounds was assessed by calculating physicochemical properties, and molecular modeling was performed by using an online software program (FRED 2.0). The target compounds (4a-j and 5a-j) were evaluated for anticancer activity using the MTT assay.

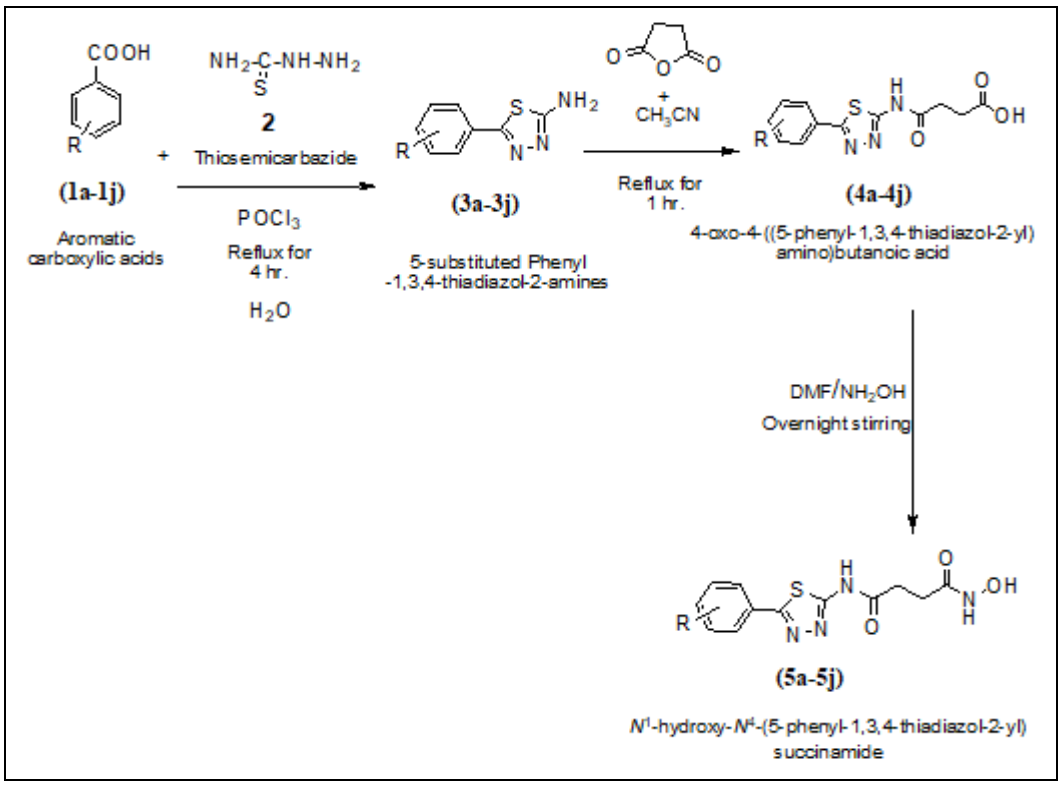
MATERIALS AND METHOD:

Experimental: [8] All the chemicals used were of laboratory grade and procured from Rankem, Astron (India) and Sigma Aldrich. The melting point of synthesized compounds was performed in one end open capillary on VEEGO (VMP-PM) melting point apparatus. The purity of the compound was performed by using pre-coated TLC plates and solvent systems. The TLC evaluated in UV chamber at 254 nm wavelength and Iodine chamber. The FT-IR of synthesized compounds were performed on SHIMADZU FT-IR 8400 by using KBr pellets.

The ^{1}HNMR (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 a solvent, TMS as an internal standard. The signals are quoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; broad singlet and are expressed in d ppm. Mass spectra (GC-MS) of synthesized compounds were performed on SHIMADZU QP2010.

TABLE 1: SUBSTITUENTS OF COMPOUNDS FOR 4a-j AND 5a-j [8]

Compound	R
a	
b	
c	
d	
e	
f	
g	
h	
i	
j	



SCHEME 1: SYNTHETIC SCHEME FOR THE PREPARATION OF TARGET COMPOUNDS

Synthesis of Compounds: [8]

Synthesis of 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (3a): A stirring mixture of benzoic acid (0.05 mol), N-amino-thiourea (0.05 mol) and $POCl_3$ (13 ml) was heated at 75 ºC for 0.5 h. After cooling down to room temperature, water (10 ml) was added. The reaction mixture was refluxed for 4 h. After cooling, the mixture was basified to pH 8 by the dropwise addition of 50% NaOH solution under stirring.

The precipitate was filtered and recrystallized from ethanol to yield 8.14g of the target compound 3a as a colorless crystal. Yield: 60%, mp: 213-215 ºC, ESI-MS m/z: 212, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.30 (s, 1H, NH), 3.30-6.40 (m, 4H, Ph-C_2-C_3-C_5-C_6).

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

4-(5-amino-1,3,4-thiadiazol-2-yl) phenol (3b): Yield: 78%, mp: 209-213 ºC ESI-MS m/z (M+1) 195, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 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%, mp: 212-215 ºC ESI-MS m/z (M+1): 195, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 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%, mp: 211-215 ºC ESI-MS m/z (M-1): 190,^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 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%, mp: 205-208 ºC, ESI-MS m/z (M-2) 245; ^{1}H NMR (400 MHz DMSO-d_6) δ ppm: 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%, mp: 207-209 ºC ESI-MS m/z (M-1) 176; ^{1}H NMR (400 MHz DMSO-d_6) δ ppm: 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%, mp: 209-211 ºC ESI-MS m/z: 192; ^{1}H NMR (400 MHz DMSO-d_6) δ ppm: 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%, mp: 206-209 ºC ESI-MS m/z (M-1): 221; ^{1}H NMR (400 MHz DMSO-d_6) δ ppm: 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%, mp: 202-205 ºC ESI-MS m/z (M-1): 128; ^{1}H NMR (400 MHz DMSO-*d_6*) δ ppm: 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%, mp: 210-213 ºC ESI-MS m/z (M-1) 142; ^{1}H NMR (400 MHz DMSO-*d_6*) δ ppm: 10.10 (s, 1H, NH), 2.50-4.91 (m, 4H, CH_2-CH_2,CH_3).

General Procedure for the synthesis of 4a-4j:

4- oxo- ((5-phenyl-1,3,4-thiadiazole-2-yl)) amino) butanoic acid (4a): Succinic anhydride (0.02 mol) was added to a solution of thiadiazole in hot acetonitrile (150 ml). The solution was heated for 1 h under reflux. After cooling, the precipitated product was collected. Recrystallization is carried out using alcohol to give 0.16 gm as white solid. Yield: 56%, mp: 250-253 ºC, ESI-MS m/z (M+1) 312; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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 ppm: 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-4j were synthesized following the procedure described above.

4- (5- (4- hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl amino)-4-oxobutanoic acid (4b): Yield: 55%, mp: 248-250 ºC, ESI-MS m/z: (M+1) 295; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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 ppm:

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%, mp: 254-258 ºC, ESI-MS m/z (M+1) 295; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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 ppm: 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%, mp: 238-241 ºC, ESI-MS m/z (M-1): 291; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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 ppm: 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%, mp: 238-240 ºC, ESI-MS m/z: 277; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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 ppm: 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%, mp: 249-253 ºC, ESI-MS m/z (M+1) 293; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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 ppm: 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- yl amino)-4-oxobutanoic acid (4g): Yield: 59%, mp: 238-241 ºC, ESI-MS m/z: (M+1) 323; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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-*d6*) δ ppm: 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%, mp: 242-245 ºC, ESI-MS m/z: 322; ^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm: 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-*d6*) δ ppm: 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- oxo-butanoic acid (4i): Yield: 60%, mp: 218-220 ºC ESI-MS m/z (M+1): 230; ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 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-*d6*) δ ppm: 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%, mp: 224-229 ºC, ESI-MS m/z: 243; ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 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-*d6*) δ ppm: 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).

General Procedure for the Synthesis of 5a-5j:

N1-(5-(4-chlorophenyl)-1, 3, 4-thiadiazol- 2- yl)-N4-hydroxy succinimide (5a): To a -20 ºC cooled solution of acid derivative (0.07mole) and N-methyl morpholine (0.014mole) in anhydrous N,N-dimethylformamide (15 ml) was added ClCOOBu-i (isobutyl chloroformate), and the mixture was stirred for 0.5 h. The solid was filtered out, and the filtrate was added to freshly prepared NH_2OK in methanol (5 ml, 1.54 mol/L). The resulting mixture was stirred at room temperature overnight, then was filtered and the residue was washed with water to give the crude product. Recrystallization is carried out using ethanol to give 0.33 gm as white solid. Yield: 52%, mp: 207-209 ºC, ESI-MS m/z: ^{1}H NMR (400 MHz, $CDCl_3$-*d6*) δ ppm: 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-*d6*) δ ppm: 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-5j were synthesized following the procedure described above.

N1-hydroxy-N4-(5- (4- hydroxyphenyl)- 1, 3, 4-thiadiazol-2-yl)succinamide (5b): Yield: 51%, mp: 207-209 ºC, ESI-MS m/z (M+1): 309; ^{1}H NMR (400 MHz, $CDCl_3$-*d6*) δ ppm: 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-*d6*) δ ppm: 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%, mp: 207-209 ºC, ESI-MS m/z (M-1) 307.9; ^{1}H NMR (400 MHz, $CDCl_3$-*d6*) δ ppm: 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-*d6*) δ ppm: 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-hydroxy-succinamide (5d): Yield: 61%, mp: 207-209 ºC ESI-MS m/z: ^{1}H NMR (400 MHz, $CDCl_3$-*d6*) δ ppm: 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-*d6*) δ ppm: 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%, mp: 207-209 ºC, ESI-MS m/z (M-1) 292, ^{1}H NMR (400 MHz, $CDCl_3$-*d6*) δ ppm: 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-*d6*) δ ppm: 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%, mp: 207-209 ºC, ESI-MS m/z: (M+1) 308, ^{1}H NMR (400 MHz, $CDCl_3$-*d6*) δ ppm: 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 ppm: 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%, mp: 207-209 ºC, ESI-MS m/z (M+1) 308; ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ ppm: 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 ppm: 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%, mp: 204-206 ºC, ESI-MS m/z: 337; ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ ppm: 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 ppm: 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- 1.00 (CH_2-CH_2).

N1-(5-ethyl-1, 3, 4-thiadiazol- 2- yl)-N4-hydroxy-succinamide (5i): Yield: 60%, mp: 202-205 ºC, ESI-MS m/z: 244; ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ ppm: 12.87 (s, 1H, OH), 11.04 (s, 1H, NH), 8.00 (s, 1H, NH), 2.461-2.590 (m, 2H, CH_2), 2.00-2.460 (m, 2H, CH_2). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 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), 28.00- 31.60 (CH_2-CH_2), 24.10 (CH_2), 14.30(CH_3).

N1-hydroxy-N4-(5-propyl-1, 3, 4-thiadiazol-2-yl) succinamide (5j): Yield: 60%, mp: 199-202 ºC, ESI-MS m/z: 258; ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ ppm: 12.55 (s, 1H, OH), 11.51 (s, 1H, NH), 8.00 (s, 1H, NH), 2.550-2.481 (m, 2H, CH_2), 1.83-2.00 (m, 2H, CH_2). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 173.70, 170.60 (C=O), 168.50 (Thiadiazole-C_2), 152.30 (Thiadiazole-C_5), 28.09-31.06 (CH_2-CH_2), 23.09 (CH_2), 13.70(CH_3).

Molecular Docking Studies: 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 analyzed and selected from Discovery Studio Visualizer. Drug likeness is also calculated from the same **Table 2**.

Pharmacological Evaluation:

***In-vitro* HDAC Assay:** We performed assays according to the kit instruction (BML-AK501-0001 HDAC Colorimetric activity assay kit). HDAC came from HeLa cell nucleus extracts, mainly including HDAC1 and HDAC2. The tested compounds and the control drug SAHA were diluted to various concentrations. On the 96-well plate, HDAC (5 µl /well) were incubated at 37 ºC with 10 µL of various concentrations of samples and 25 µl of the substrate. After reacting for 30 min, Colored Lys Developer (50 µl /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 IC_{50} values were determined using a regression analysis of the concentration/inhibition.

MTT Assay: MDA-MB-231 (Breast Cancer Cell) K562 (Chronic Myelogenous Leukemia Cell), A549 (Human Lung Cancer) and PC3 (Prostate Cancer Cell Lines.) were respectively cultured in DMEM with high glucose. The cell line was cultured in DMEM medium, which was supplemented with 10% heat-inactivated fetal calf serum (FBS) and 1% Antibiotic - Antimycotic 100 X solution. The cells were seeded at a density of approximately 5×10^3cells/well in a 96-well flat-bottom microplate and maintained at 37 ºC in 95% humidity and 5% CO_2 for overnight. Different concentration (1000, 500, 250, 125, 62.5 µg/mL) of samples was treated. The cells were incubated for another 24 h.

The cells in well were washed twice with phosphate buffer solution, and 20 µL of the MTT staining solution (5 mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37 ºC. After 4 h, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using microplate reader, and the IC_{50} of the samples can be calculated using GraphPad version 5.1.

Formula:

Surviving cells (%) = Mean OD of test compound / Mean OD of Negative control ×100

Inhibiting cells (%) = 100 - Surviving cells

RESULT AND DISCUSSION: The two series of novel 1,3,4-thiadiazole based hydroxamates (5a-j) and carboxylates (4a-j) drugs were designed and synthesized to obtain drugs with improved biological activities. Our synthetic route to target compounds is outlined in **Scheme 1**. Novel HDAC inhibitors have been generated by modification on Zinc binding molecule, 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. They were characterized by analytical and spectroscopic methods. HDAC inhibition activity was assessed by the Color de Lys assay, and the results are tabulated as IC_{50} values in **Table 4**.

According to the data in **Table 3**, 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 the 1,3,4-thiadiazole ring have a 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 IC_{50} values were summarized in **Table 5**.

Among these compounds, all of them could inhibit 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 in **Table 2** and **3**. For test compounds, a common binding mode was observed, where the binding site is comprised of His143, His180, His142, Phe 152, Phe208 and Tyr306 amino acid residues. log P values (.045-1.85) for the test compounds were noted; target compounds also show mild to moderate similarity with respect to standard drug.

TABLE 2: MOLECULAR DOCKING STUDIES OF SYNTHESIZED COMPOUNDS ALONG WITH VARIOUS BINDING INTERACTIONS

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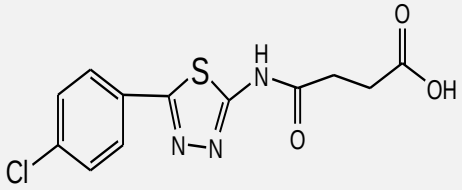
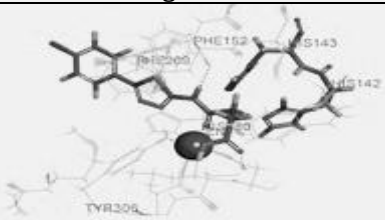
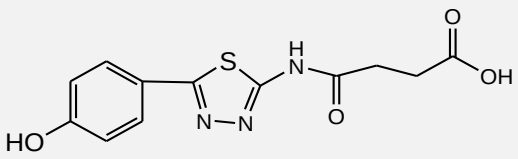
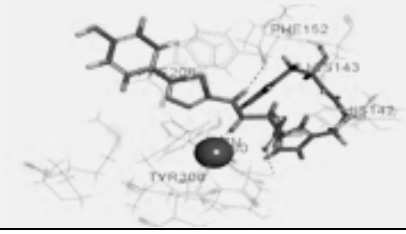
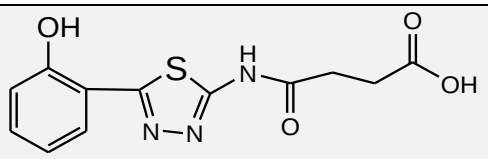
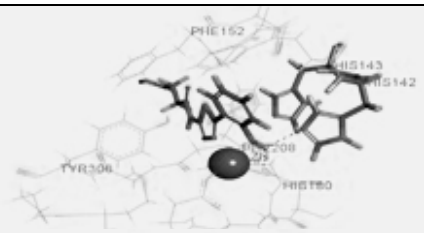
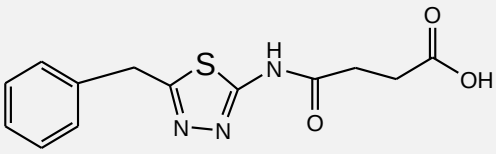
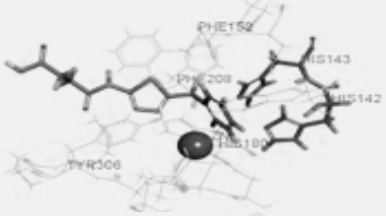
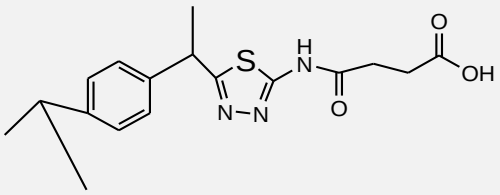
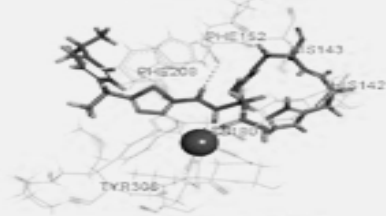
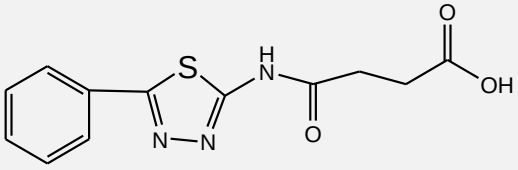
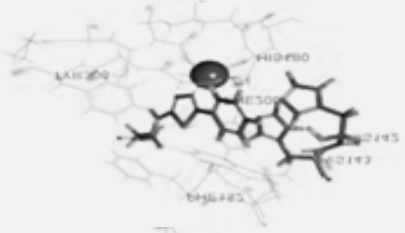
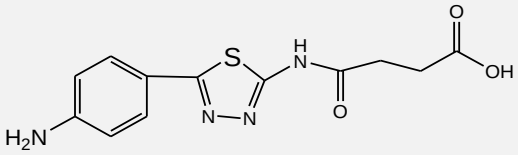
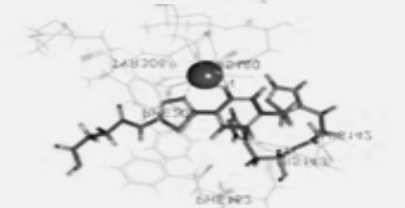
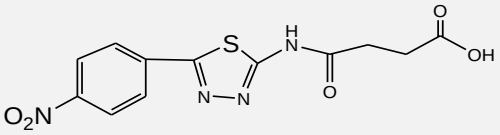
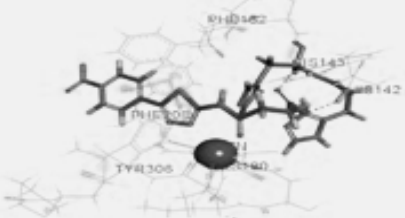
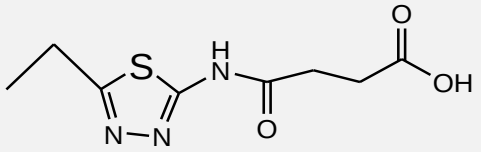
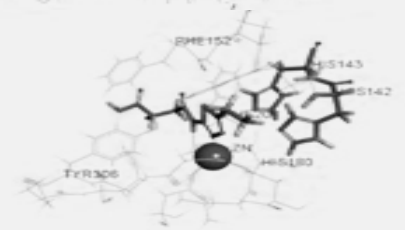
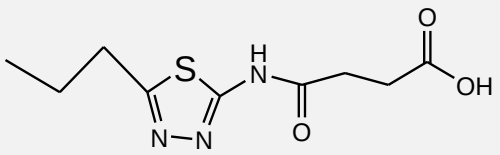
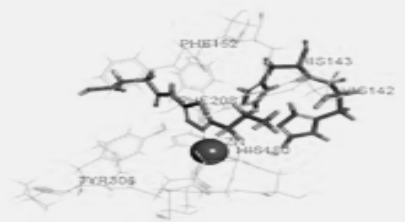
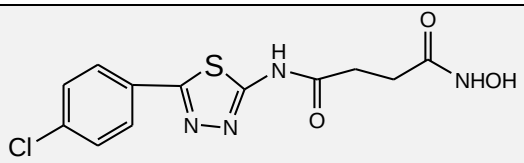
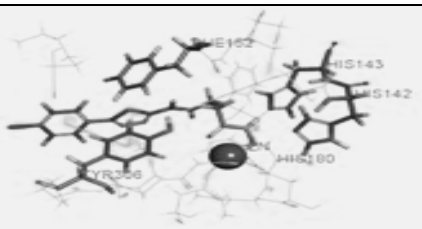
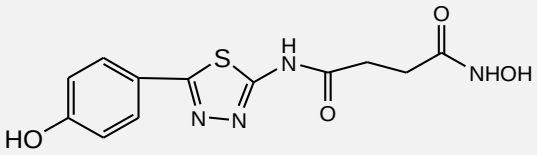
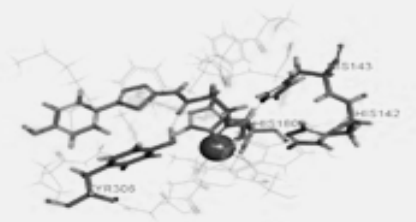
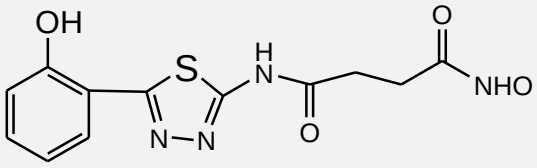
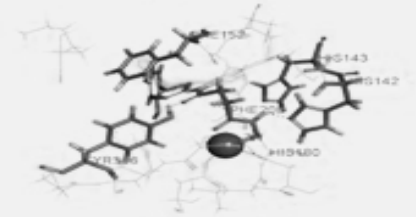
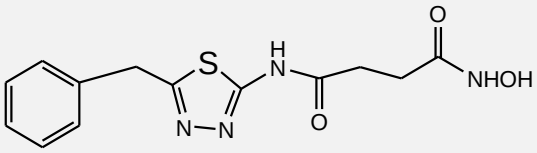
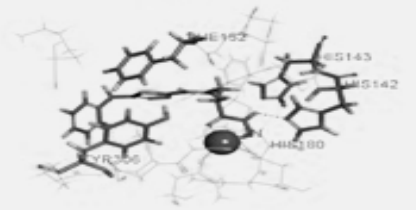
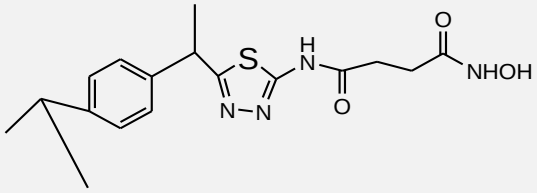
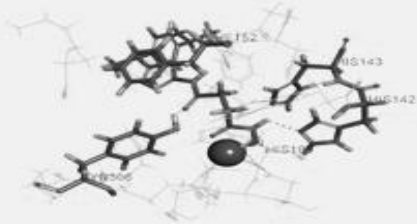
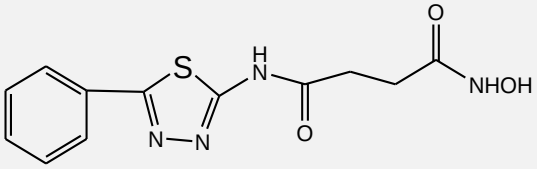
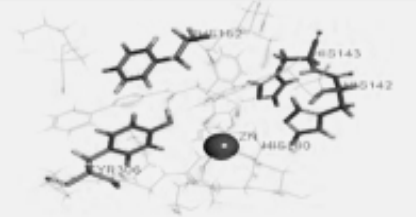
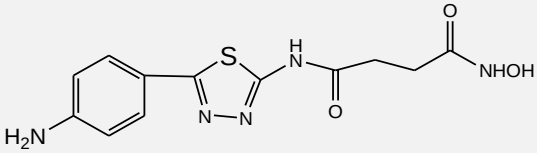
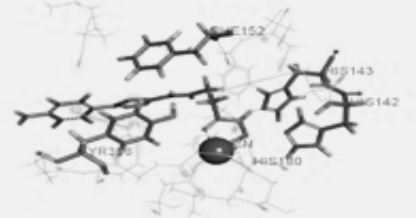
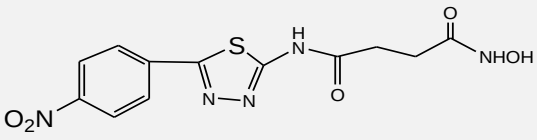
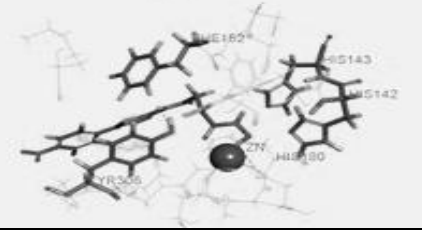
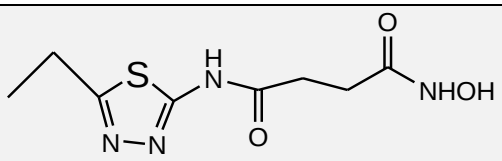
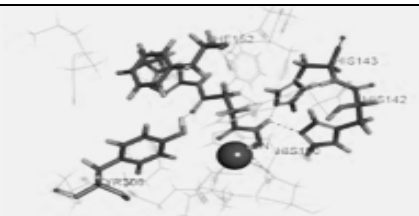
1	CA01		
2	CA02		

3	CA03		
4	CA04		
5	CA05		
6	CA06		
7	CA07		
8	CA08		
9	CA09		
10	CA10		

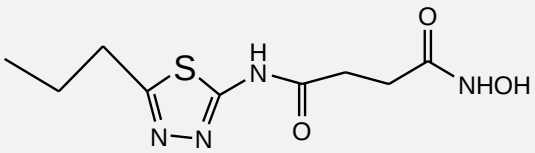
11	HA01		
12	HA02		
13	HA03		
14	HA04		
15	HA05		
16	HA06		
17	HA07		
18	HA08		

19 HA09





20 HA10



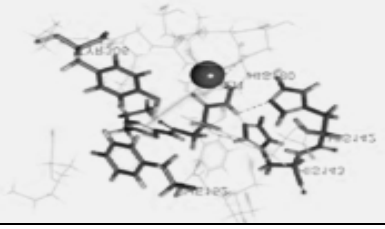
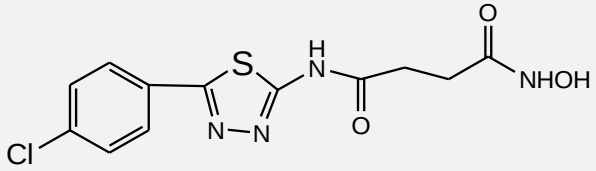
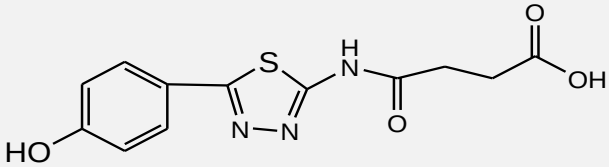
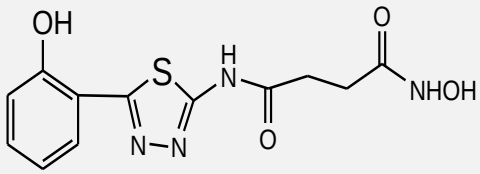
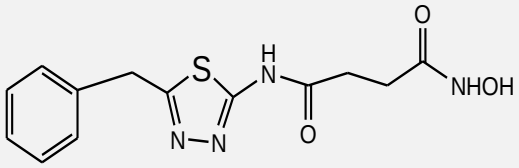
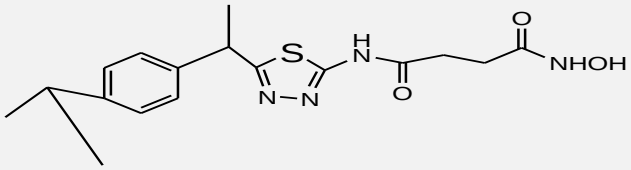
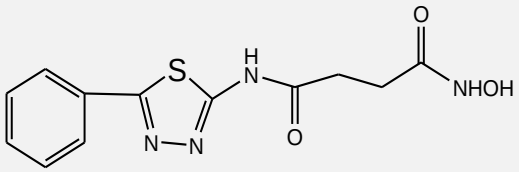
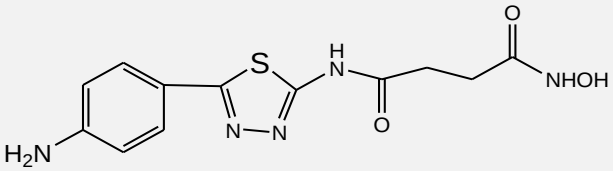
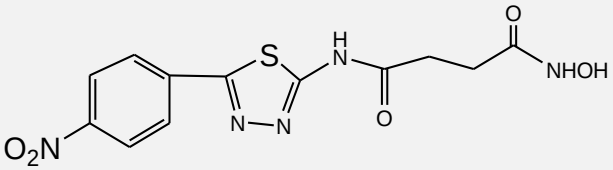
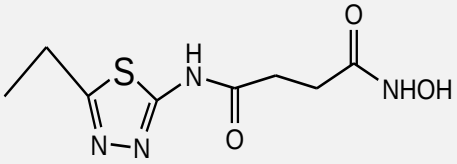
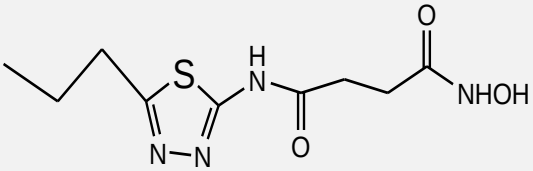
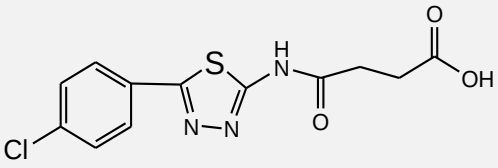
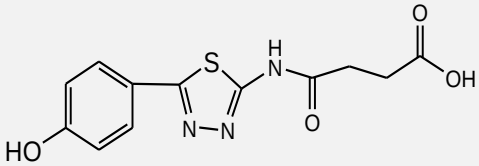
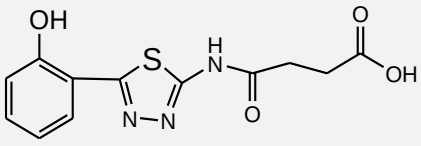
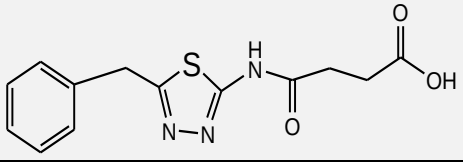


TABLE 3: MOLECULAR DOCKING STUDIES OF SYNTHESIZED COMPOUNDS

S. no.	Comp	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

TABLE 4: HDAC ACTIVITY OF SUBSTITUTED 1,3,4-THIADIAZOLE HYDROXAMATES AND CARBOXYLATES DERIVATIVES

S. no.	Compound ID	Structure	IC_{50} value (µM)
1	HA01		0.20± 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
11	CA01		>5
12	CA02		>5
13	CA03		>5
14	CA04		>5

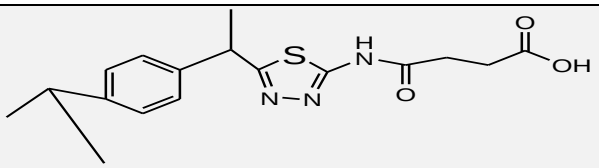
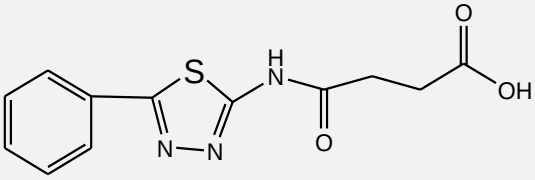
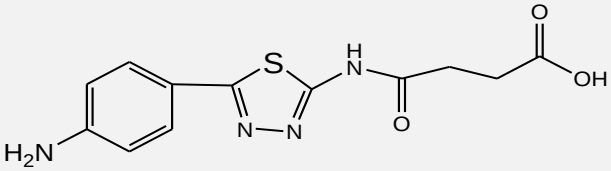
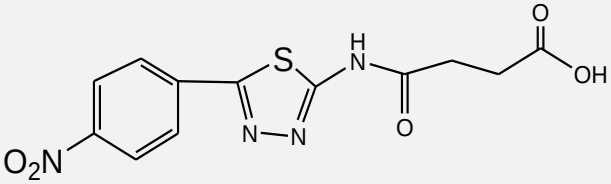
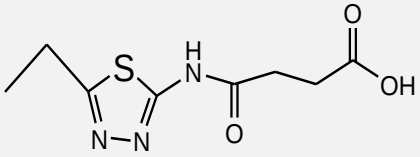
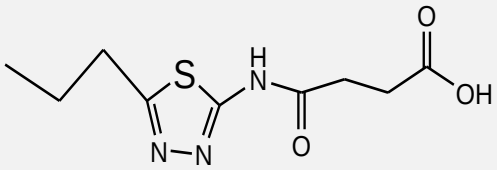
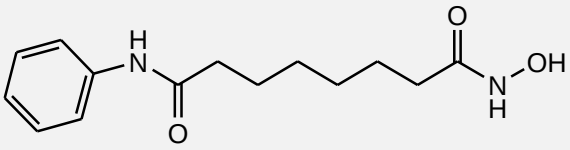
15	CA05		>5
16	CA06		>5
17	CA07		>5
18	CA08		>5
19	CA09		4.39± 0.05
20	CA10		4.85± 0 .04
21	SAHA		0.18±0.02

TABLE 5: PROLIFERATIVE ACTIVITIES OF REPRESENTATIVE COMPOUNDS AGAINST VARIOUS CANCER CELL LINES

S. no.	Compound ID	IC_{50} value of HDAC (µM)	MDA-MB-231 IC_{50} value (µM)	K562 IC_{50} value (µM)	A549 IC_{50} value (µM)	PC3 IC_{50} value (µM)
1	HA01	0.20 ± 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	0.18 ± 0.02	1.35	1.96	4.10	5.56

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 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 concerning the standard drug. The pharmacokinetics 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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CONFLICTS OF INTEREST: Nil

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