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Design, synthesis and *in vitro* cytotoxicity studies of novel sulfonamides

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ABSTRACT

Sulfonamides are an important class of drugs possessing antibacterial, anticarbonic anhydrase, diuretic, hypoglycemic and histone deacetylase (HDAC) inhibition activity. Inhibitors of HDAC have emerged as potential therapeutic agents against solid tumors and malignancies. Compounds from the sulfonamide class were designed by using the software Glide from Maestro 9.5 of Schrödinger, USA. Sulfonamides were synthesized from cinnamic acid as the starting material. These synthesized compounds were purified and characterized by R_f values in thin layer chromatography, melting points and IR spectroscopy. The structures were confirmed by ^1H NMR spectroscopy. The compounds were evaluated for in vitro cytotoxicity against ovarian cancer cell line (OVCAR 3), using Sulphorhodamine B (SRB) assay. The hydroxamic acid derivatives of sulfonamides showed better activity compared to their triazole derivatives. One o-phenelynediamine derivative of sulfonamide also showed significant activity.

Keywords: Sulfonamides, histone deacetylase, ovarian cancer, *in vitro* cytotoxicity

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INTRODUCTION

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths in 2008. Experts believe that cancer related deaths would further rise each year. In fact, the WHO estimates that cancer will cause 13.1 million deaths in 2030[1]. The development of molecular-targeted therapies represents an exciting new approach to cancer treatment[2]. Sulfonamides are an important class of drugs possessing antibacterial, anticarbonic anhydrase, diuretic, hypoglycemic[3] and histone deacetylase (HDAC) inhibition activities. The HDAC inhibitors trigger growth arrest, differentiation and apoptosis in tumor cells via transcriptional activation of certain genes[4]. Many HDAC inhibitors are currently undergoing clinical investigations in different types of cancer. Vorinostat or suberoylanilide hydroxamic acid (SAHA, Zolinza^{TM}) (2006) and Romedepsin (2009) are the two HDAC inhibitors approved by US-FDA[5].

There are three characteristics shared by all HDAC inhibitors, which are as follows[6]:
1) A large hydrophobic region, which binds to the hydrophobic part of the enzyme near the active site.
2)An aliphatic chain, usually consisting of 5 to 6 carbons attached to the hydrophobic region.
3) An active functional group, which is attached to the other end of the aliphatic chain, interacts with the zinc ion and the residues at the active site to disrupt the enzymatic activity of HDAC.

The connecting unit in the recognition group can be an amide, sulphonamide, ketone and ether[7].

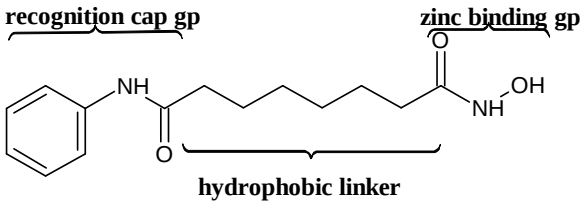


Figure 1: Vorinosat (Zolinza™)

Finn and co-workers[8] demonstrated that a series of novel sulfonamides were synthesized and evaluated for their HDAC inhibition activity. Based on these facts, it was considered worthwhile to synthesize some new sulfonamides, which are predicted to have an anticancer activity. These synthesized compounds were purified and characterized by TLC, melting points and IR spectroscopy. Their structures were confirmed by ^{1}H NMR spectroscopy. The *in vitro* cytotoxicity of the compounds was performed against ovarian cancer cell line (OVCAR 3), using Sulphorhodamine B (SRB) assay.

MATERIALS AND METHODS

2.1. Designing of compounds

Docking studies were carried out on HDAC enzyme using the software ***Glide*** from **Maestro 9.5 of Schrödinger, USA,** for the identification of potential HDAC inhibitors from the class of sulfonamides[9].

The molecular docking process was incorporated in 5 steps. They were:
1. Protein preparation and its refinement
2. Receptor grid generation
3. Validation of the protein
4. Ligand preparation
5. Ligand docking

The X –ray co-crystallized structure of HDAC enzyme (Protein Data Bank ID: 1T69) was imported from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank10. This enzyme is available as a complex with its irreversible inhibitor SAHA having a resolution of 2.91 Å. The PDB ID of HDAC enzyme (1T69) was entered in the '*Protein Preparation Wizard*' of Maestro 9.5 to download the enzyme HDAC. A number of sulfonamides were docked on the refined HDAC enzyme . The compounds showing good Glide score (G-score > -6.0) were selected for the synthesis in the laboratory.

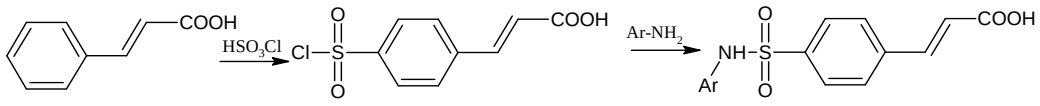
2.2 Synthesis of novel sulfonamides

All the chemicals were purchased from commercial sources. The completion of a reaction and the purity of the compounds was monitored by determining R_f values in thin layer chromatography, performed using silica gel-G pre-coated plates as the stationary phase and various mobile phases. The spots were detected by the exposure of the plate to UV- lamp at 254 nm. The melting points were determined in open glass capillaries, on Expo Hi-tech melting point apparatus. The structures of the synthesized compounds were characterized from the IR spectra using JASCO FTIR 5300 spectrophotometer. Potassium bromide pellet method was used to record the IR spectra. The structures of the compounds were confirmed by recording their ^{1}H NMR spectra on AS-1-10 spectrophotometer, using dimethyl sulphoxide (DMSO) and chloroform as solvents.

2.3 General procedure for synthesis of sulfonamides of cinnamic acid (III a-g)

Synthesis of cinnamic acid sulfonamides (Scheme 1)

[Scheme 1]



Cinnamic acid **(I)** Cinnamic acid sulphonyl chloride **(II)** 3-[4-(N-Substituted sulfamoyl)phenyl]prop-2-enoic acid **(III a-g)**

Ar = C_6H_5, C_6H_5 (2-CH_3), C_6H_5 (4-CH_3), C_6H_5 (2-OCH_3), C_6H_5 (2-Cl), $C_6H_5CH_2$, C_6H_5 (4-OCH_3)

In **Scheme 1**, cinnamic acid (**I**, 2 g, 0.0136 M) was added to chlorosulphonic acid (12.58 g, 0.108 M) in portions with stirring for 5-6 h, initially at 0-5 ^oC, and then at room temperature. The dark, viscous syrup was poured onto ice and the precipitate formed was filtered, washed with water and dried. The crude sulphonyl chloride (**II**, 1 g, 0.004 M) was dissolved in dichloromethane (DCM, 6 ml) and added to the mixture of an aromatic amine (0.0082 M) and pyridine (2 ml). The resultant mixture was stirred at 40 ^oC for 1 h to obtain sulfonamides **(III a-g)**. The solvent was recovered and the residue was dissolved in ethyl acetate. The solution was washed first with hydrochloric acid, then with water, and product was dried over anhydrous sodium sulphate. The solvent was recovered to collect the crude product **(III a-g)**, which was then purified by recrystallization.

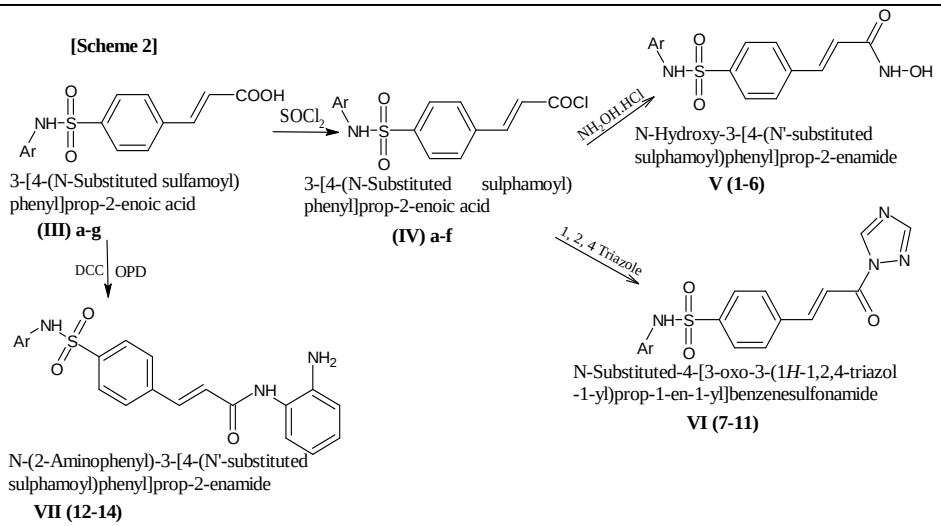
2.3.1 Synthesis of compounds [V (1-6)][11]**: (Scheme 2)**

The sulphonamide (**III a-g**, 0.0064 M) was reacted with thionyl chloride (13.06 g, 0.11 M) by refluxing for 2-3 h. Excess of thionyl chloride was distilled off. The residue was partitioned between ethyl acetate and cold water. The solvent was recovered from the ethyl acetate layer and crude acid chloride **(IV)** was used immediately for further reaction. Hydroxamic acid derivatives **[V (1-6)]** were prepared by making a suspension of hydroxylamine hydrochloride (2 g, 0.028 M) in tetrahydrofuran (THF, 14 ml) and saturated sodium bicarbonate solution (9.6 ml). The mixture was stirred for 10 min. To this mixture, acid chloride (**IV a-f**, 0.0037 M) was added by dissolving it in THF (7.6 ml) and the resultant mixture was stirred at room temperature for 1 h. The reaction mixture was partitioned between ethyl acetate and hydrochloric acid. The organic layer was washed with water. The solvent was recovered to get the crude product [**V (1-6)]**, which was then purified by recrystallization.

The IR and NMR spectral data for compounds V (1-6) is given below.

Scheme 2: Synthesis of derivatives (V, VI and VII) of sulfonamides III (a-g)

[Scheme 2]



3-[4-(N-Substituted sulfamoyl) phenyl]prop-2-enoic acid **(III) a-g**

3-[4-(N-Substituted sulphamoyl) phenyl]prop-2-enoic acid **(IV) a-f**

N-Hydroxy-3-[4-(N'-substituted sulphamoyl)phenyl]prop-2-enamide **V (1-6)**

N-(2-Aminophenyl)-3-[4-(N'-substituted sulphamoyl)phenyl]prop-2-enamide **VII (12-14)**

N-Substituted-4-[3-oxo-3-(1*H*-1,2,4-triazol -1-yl)prop-1-en-1-yl]benzenesulfonamide **VI (7-11)**

Ar = C_6H_5, 2-CH_3-C_6H_5, 4-CH_3-C_6H_5, 2-OCH_3-C_6H_5, 2-Cl-C_6H_5, C_6H_5CH

DCC = N,N'-Dicyclohexylcarbodiimide

N-Hydroxy-3-[4(phenylsulphamoyl)pheny]prop-2-enamide, V (1):
IR: 3745, O-H (str), 3383, N-H (str), 3062, C-H (str), 1693, C=O (str), 1602, N-H (ben), 1486, 1447, 1410, Ar-C=C (str), 1321, 1156, SO_2 (str). **^{1}HNMR (DMSO):** 12.5 (bs, 1H, OH), 8.1 (s, 1H, NH from NHOH), 8.0 (d, 2H, Ar-ring), 7.6 (d, 2H, Ar-ring), 7.4 (m, 5H, Ar-$NHSO_2$), 6.8 (d, 1H, CH=CH), 6.0 (d, 1H, CH=CH)

N-Hydroxy-3-{4[(2-methylphenyl)sulphamoyl]phenyl}pro-2-enamide, V (2): IR: 3417, O-H (str), 3202, N-H (str), 2921, C-H (str), 1691, C=O (str), 1628, N-H (ben), 1492, 1421 Ar-C=C (str), 1322, 1157, SO_2 (str). **^{1}HNMR (DMSO):** 10.8 (s, 1H, OH), 7.85 (d, 2H, Ar-ring), 7.7 (d, 2H, Ar-ring), 7.6 (s, 1H, NH from NHOH), 7.1 (m, 5H, Ar-$NHSO_2$), 6.95 (d, 1H, CH=CH), 6.6 (d, 1H, CH=CH), 2 (s, 3H, CH_3-aliphatic).

N-Hydroxy-3-{4[(4-methylphenyl)sulphamoyl]phenyl}pro-2-enamide, V (3): IR:
3321, O-H (str), 3203, N-H (str), 2963, C-H (str), 1688, C=O (str), 1633, N-H (ben), 1566, 1514, 1428, 1399 Ar-C=C (str), 1333, 1160, SO_2 (str). **^{1}HNMR (DMSO):** 8.25 (bs, 1H, OH), 8.1 (d, 2H, Ar-ring), 7.8 (d, 2H, Ar-ring), 7.7 (d, 1H, Ar-ring), 7.66 (s, 1H, NH from NHOH), 7.3 (m, 5H, Ar-$NHSO_2$), 6.1 (d, 1H, CH=CH), 2.5 (s, 1H, CH_3-alliphatic).

N-Hydroxy-3-{4[(2-methoxyphenyl)sulphamoyl]phenyl}pro-2-enamide, V (4): IR: 3697, O-H (str), 3211, N-H (str), 2963, C-H (str), 1695, C=O (str), 1633, N-H (ben), 1595, 1568, 1500, 1427 Ar-C=C (str), 1342, 1161, SO_2 (str). **^{1}HNMR (DMSO):** 12.5 (bs, 1H, OH), 10.6 (s, 1H, NH from NH from NHOH), 7.85 (m, 4H, Ar-ring), 7.6 (d, 1H, CH=CH), 7.1-7.2 (m, 5H, Ar- $NHSO_2$), 6.6 (d, 1H, CH=G), 3.45 (s, 1H, CH_3).

N-Hydroxy-3-{4[(2-chlorophenyl)sulphamoyl]phenyl}pro-2-enamide, V (5): IR: 3635, O-H (str), 3351, N-H (str), 2960, C-H (str), 1675, C=O (str), 1629, N-H (ben), 1590, 1529, 1481, 1440, Ar-C=C (str), 1337, 1164, SO_2 (str). **^{1}HNMR (DMSO):** 10.2 (bs, 1H, OH), 9.8 (s, 1H, NH from NHOH), 7.8 (m, 4H, Ar-ring), 7.65 (d, 1H, CH=CH), 7.45 (s, 1H, $NHSO_2$), 7.3 (m, 4H, Ar-$NHSO_2$), 7.2 (d, 1H, CH=CH).

N-Hydroxy-3-[4(benzylsulphamoyl)phenyl]pro-2-enamide, VI (6): IR: 3457, O-H (str), 3288, N-H (str), 3034, C-H (str), 1685, C=O (str), 1630, N-H (ben), 1568, 1514, 1450, 1403, Ar-C=C (str), 1322, 1158, SO_2 (str). **^{1}HNMR (DMSO):** 8.2 (bs, 1H, OH), 7.8 (m, 4H, Ar-ring), 7.65 (d, 1H, CH=CH), 7.60 (s, 1H, NH from NHOH), 7.2 (m, 5H, Ar-$NHSO_2$, $NHSO_2$), 6.9 (d, 1H, H_e).

2.3.2 Synthesis of compounds VI (7-11)[12]**: (Scheme 2)**
1, 2, 4-Triazole derivatives [**(VI) 7-11]** were prepared by dissolving acid chloride (**IV a-c, e, f**, 0.025 M), 1, 2, 4-triazole (2 g, 0.028 M) and freshly ground potassium carbonate (4 g, 0.028 M) in N, N-dimethylformamide (DMF, 0.66 ml). Reaction mixture was stirred for 12 h and then quenched with water (5 ml). Water layer was extracted thrice with ethyl acetate. Organic layers were combined, washed with brine and water and then dried over anhydrous sodium sulphate.

The IR and NMR spectral data from compounds VI (7-11) is given below:
***N*-phenyl-4-[3-oxo-3-(1*H*-1, 2, 4-triazol-1-yl)prop-1-en-1-yl]-benzene sulphonamide, VI (7):**
IR: 3211, N-H (str), 2857, C-H (str), 1693, C=O (str), 1632, N-H (ben), 1602, 1488, 1421, Ar-C=C (str), 1325, 1158, SO_2 (str). **^{1}HNMR (DMSO):** 10.4 (s, 1H, $NHSO_2$), 7.85 (d, 2H, Ar-ring), 7.75 (d, 2H, Ar-ring), 7.60 (s, 2H, CH-triazole), 7.55 (d, 1H, CH=CH), 7.25 (m, 2H, Ar-$NHSO_2$), 7.0-7.1 (m, 3H, Ar-$NHSO_2$), 6.7 (d, 1H, CH=CH).

N-(2-Methylphenyl)-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzene sulphonamide, VI (8): IR: 3202, N-H (str), 2968, C-H (str), 1692, C=O (str) 1631, N-H (ben), 1493,1423, Ar-C=C (str), 1322, 1157, SO_2 (str). **^{1}HNMR (DMSO):** 10.6 (s, 1H, $NHSO_2$), 7.9 (d, 2H, Ar-ring), 7.7 (d, 2H, Ar-ring), 7.5 (s, 2H, CH-triazole), 7.1 (m, 4H, Ar-$NHSO_2$), 6.9 (d, 1H, CH=CH), 6.7 (d, 1H, CH=CH), 2 (s, 3H, CH_3-aliphatic).

N-(4-Methylphenyl)-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzene sulphonamide, VI (9): IR: 3318, N-H (str), 2918, C-H (str), 1689, C=O (str), 1612, N-H (ben), 1592,1536, 1498,1397, Ar-C=C (str), 1326, 1159, SO_2 (str). **^{1}HNMR (DMSO):** 8.7 (s, 1H, $NHSO_2$), 8.0 (s, 2H, CH-triazole), 7.85 (d, 2H, Ar-ring), 7.7 (d, 2H, Ar-ring), 7.6 (d, 1H, CH=CH), 7.0 (m, 4H, Ar-$NHSO_2$), 6.6 (d, 1H, CH=CH), 2.5 (s, 3H, CH_3-aliphatic).

N-Benzyl-4-[3-oxo-3-(1H-1, 2, 4-triazol-1-yl)prop-1-en-1-yl]benzene sulphonamide, VI (10): IR: 3282, N-H (str), 2919, C-H (str), 1691, C=O (str) 1633, N-H (ben), 1494, 1425, Ar-C=C (str), 1320, 1158, SO_2 (str). **^{1}HNMR**

(DMSO): 8.3 (t, 1H, $NHSO_2$), 7.95 (s, 2H, CH-triazole), 7.90 (d, 2H, Ar-ring), 7.80 (d, 2H, Ar-ring), 7.65 (d, 1H, CH=CH), 7.2-7.3 (m, 5H, Ar-$NHSO_2$), 6.70 (d, 1H, CH=CH), 4.0 (d, 2H, CH_2-aliphatic).

N-(2-Chlorophenyl)-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzene sulphonamide, VI (11): IR: 3349, N-H (str), 3104, C-H (str), 1674, C=O (str), 1628, N-H (ben), 1591, 1531, 1481, 1441, Ar-C=C (str), 1336, 1165, SO_2 (str). **^{1}HNMR (DMSO):** 7.83 (s, 1H, $NHSO_2$), 7.81 (d, 2H, Ar-ring), 7.68 (d, 2H, Ar-ring), 7.60 (d, 1H, CH=CH), 7.2-7.4 (m, 4H, Ar-$NHSO_2$), 7.0 (s, 2H, CH-triazole), 6.65 (d, 1H, CH=CH).

2.3.3 Synthesis of compounds VII (12-14): (Scheme 2)
To the solution of a sulphonamide (**III a, f, g**, 0.01 M) in dichloromethane (DCM, 10 ml), was added a mixture of DCC (N', N-dicyclohexylcarbodiimide) (2 g, 0.01 M) and ortho phenylenediamine (1 g, 0.01 M). The mixture was stirred overnight, initially at 0-5 ^oC for 2 h and then at room temperature, to obtain ortho-phenylenediamine derivatives [**(VII) 12-14].** The solvent was recovered and the crude product was collected and purified by recrystallization[13].

The IR and NMR spectral data from compounds VI (12-14) is given below:

N-(2-Aminophenyl)-3-[4-(phenylsulfamoyl)phenyl]prop-2-enamide, VII (12):
IR: 3491, 3385, N-H (str) 1^o amine, 3270, N-H (str), 2932, C-H (str), 1704, C=O (str), 1603, 1535, 1496, 1449, Ar-C=C (str), 1346, 1162, SO_2 (str). **^{1}HNMR (DMSO):** 8.4 (d, 1H, NHCO) 7.7 (m, 4H, Ar-ring), 7.5 (d, 1H, CH=CH), 7.0-7.2 (m, 5H, Ar-$NHSO_2$), 6.8 (d, 1H, CH=CH), 6.3-6.5 (m, 4H, aniline ring), 4.4 (s, 2H, NH_2 from aniline).

N-(2-Aminophenyl)-3-{4-[(2-methoxyphenyl)sulfamoyl]phenyl}prop-2-enamide, VII (13): IR: 3250, 3055, N-H (str), 1^0 amine, 2992, N-H (str), 2934, C-H (str), 1704, C=O (s tr), 1649, N-H (ben), 1602, 1545, 1509,1454, Ar-C=C (str), 1333, 1158, SO_2 (str). **^{1}HNMR (DMSO):** 10.0 (s, 1H, $NHSO_2$), 8.4 (d, 1H, NHCO), 7.7 (m, 4H, Ar-ring), 7.5 (d, 1H, CH=CH), 7.0 (m, 4H, Ar-$NHSO_2$), 6.8 (m, 4H, aniline ring), 6.8 (d,1H, CH=CH), 3.5 (s, 2H, NH_2 from aniline), 3.4 (s, 3H, CH_3-aliphatic).

N-(2-Aminophenyl)-3-[4-(benzylsulfamoyl)phenyl]prop-2-enamide, VII (14): IR: 3421, 3393 N-H (str) 1^0amine, 3342, N-H (str), 2826, C-H (str), 1670, C=O (str), 1650, NH (ben), 1611, 1593, 1526,1452, Ar-C=C (str), 1317, 1159, SO_2 (str). **^{1}HNMR (DMSO):** 8.4 (d, 1H, NHCO), 8.2 (t, 1H, $NHSO_2$), 7.8 (m, 4H, Ar-ring), 7.6 (d, 1H, CH=CH), 7.2 (m, 5H, Ar-$NHSO_2$), 6.8 (d, 1H, CH=CH), 6.3-6.5 (m, 4H, aniline ring), 4.4 (s, 2H, NH_2 from aniline), 4.0 (d, 2H, CH_2-aliphatic).

2.4 Acute toxicity studies:
All the synthesized compounds were tested for their toxic effects at different dose levels (500, 1000 and 2000 mg/kg body weight) in mice. The mice were observed for 14 days after the administration of compounds as per the OECD guidelines[14].

2.5 *In vitro* cytotoxicity:
Sulpharhodamine B (SRB) is a bright pink aminoxanthine dye with two sulfonate groups. Under mild acidic condition, SRB dye binds to basic amino acid residues in trichloroacetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density [15]. The cells were placed in a 96 well micro titer plate (10^4 cells / well) for 24 h before treatment with the compound (s) to allow attachment of cells to the wall of the plate. The test compounds were dissolved in ethanol or ethyl acetate and diluted with saline to the appropriate volume. Different concentrations (0.1, 2.5, 5 and 10 µg/ml) of the compounds to be tested were added to the cell monolayer. Triplicate wells were prepared for each individual dose. The monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in an atmosphere of 5 % CO_2. After 48 h, cells were fixed with 10 % trichloroacetic acid (TCA) and washed several times with deionized water. The SRB was added to these cells and the cells were washed and dried. The plates were air dried and the bound protein stain was solubilised with 150 µl of 10 mmol/l unbuffered Tris base, tris(hyroxymethyl)aminomethane[16]. The plates were shaken vigorously for 5 min. The absorbance was measured using microplate reader at a wavelength of 540 nm.

RESULTS AND DISCUSSION

3.1 Designing of compounds

When a large number of sulfonamides were docked on an enzyme HDAC, 14 compounds resulted in the G-score more than -6.0. **Table 1** enlists the Glide score (G-score), the number of H bonds and the number of bad & ugly van der Waal (Vdw) contacts of all the fourteen designed compounds and the standard ligand SAHA.

Compounds **12, 13, 14** showed G-score -9.59, -9.54 and -9.64, respectively, which was higher than that of SAHA (-8.80).

Table 1: The Glide score (G-score), the number of hydrogen bonds (H bond), the number of bad and ugly van der Waal (vdw) contacts of all the synthesized ligands and the standard ligand SAHA

Compound No.	Docking score (G-score)	No. of H bonds	Bad vdw contacts	Ugly vdw contacts
1	-8.494	3	3	0
2	-7.974	2	2	0
3	-8.269	2	7	0
4	-6.468	2	5	0
5	-7.488	2	3	0
6	-6.450	3	8	0
7	-7.107	1	7	0
8	-6.997	1	5	0
9	-7.083	3	12	0
10	-6.595	1	5	0
11	-7.328	1	11	0
12	**-9.592**	3	8	0
13	**-9.540**	3	12	0
14	**-9.643**	3	12	0
SAHA	-8.808	3	9	0

Compounds **1** and **3** had G-score (-8.49 and -8.26, respectively), slightly lower than that of SAHA (-8.80). For five compounds **(2, 5, 7, 9, 11)**, the G-score was in the range of (-7.08 to -7.97). For compounds (**4, 6, 8, 10**), the G-score was in the range of (-6.45 to -6.99). All the designed compounds formed 1-3 hydrogen bonds with the receptor, thus, predicting good affinity towards HDAC enzyme, resulting in better efficacy. For all the designed compounds, there was an absence of ugly vdw contacts, and the bad vdw contacts were in the range of 2-12. All this information more or less matched with that of the standard ligand, SAHA.

As reported in the literature, the important amino acids involved in binding of the ligand with the HDAC enzyme, were Asp 101, His 142, His 143, Phe 152, Phe 208, Tyr 306 and Zn 378[17, 18]. Most of these compounds showed good bonding with Asp 101, His 142, His 143, Phe 152, Phe 208, Tyr 306, Zn 378, **(Table 2)**, which is required for good affinity towards the enzyme and good fitting of the compounds into the enzyme pocket. Hence, these 14 compounds were selected for the synthesis in the laboratory.

Table 2: Amino acids of the HDAC enzyme making good vdw contacts with the designed sulfonamides and SAHA

Compound No.	Amino acids involved in making good vdw contacts
1	Asp 101, His 142, Hie 180. Phe 208, Tyr 306, Zn 378
2	Gly 151, Phe 207, Phe 208, Tyr 306, Zn 378
3	His 142, His 143, Phe 152, Tyr 306, Zn 378
4	His 142, His 143, Phe 152, Phe 207, Phe 208, Tyr 306, Zn 378
5	Tyr 100, Asp 101, Gly 151, Phe 152, Phe 208, Zn 378
6	Asp 101, His 142, His 143, Phe 152, Hie 180, Zn 378
7	Asp 101, His 142, Hie 180, Phe 152, Phe 208, Zn 378
8	Tyr 100, Asp 101, His 142, His 143, Phe 208, Zn 378
9	Tyr 100, Asp 101, His 142, His 143, Phe 208, Zn 378
10	Asp 101, His 142, His 143, Phe 152, Phe 207, Phe 208, Zn 378
11	Asp 101, His 142, Hie 180, Phe 152, Zn 378
12	Asp 101, Gly 140, Trp 141, His 142, Gly 151,Phe 208, Tyr 306, Zn 378
13	Asp 101, Gly 140, Trp 141, Gly 142, Gly 151, Tyr 306, Zn 378
14	Asp 101, Gly 140, His 142, Gly 151, His 180, Tyr 306, Zn 378
SAHA	Asp 101, His 142, His143, Gly 151, Phe152, Phe 208, Tyr 306, Zn 378

3.2 Synthesis of the sulfonamides

The designed compounds were synthesized in the laboratory using schemes 1 & 2. The yield, m.p and R_f values of all 14 sulfonamides are listed in the **Table 3.**

Table 3: The characteristic parameters of the synthesized sulfonamides (Comp 1-14)

Compound no.	Name of the compound	Yield (%)	m. p. (° C)	TLC	
				R_f value	Mobile phase
1	N-Hydroxy-3-{4-[(N'-phenyl)sulphamoyl] pheny}prop-2-enamide	58	220-222	0.89	Chloroform: Methanol (2:8)
2	N-Hydroxy-3-{4-[(N'-2-methylphenyl) sulphamoyl]phenyl}pro-2-enamide	50	238-240	0.77	Hexane: Methanol (4:6)
3	N-Hydroxy-3-{4-[(N'-4-methylphenyl) sulphamoyl]phenyl}pro-2-enamide	47	210-212	0.71	Hexane: Methanol (4:6)
4	N-Hydroxy-3-{4-[(N'-2-methoxyphenyl) sulphamoyl]phenyl}pro-2-enamide	42	230-232	0.68	Hexane: Ethyl acetate (4:6)
5	N-Hydroxy-3-{4-[(N'-2-chlorophenyl) sulphamoyl]phenyl}pro-2-enamide	52	178-180	0.86	Chloroform: Methanol (4:6)
6	N-Hydroxy-3-{4-[(N'-benzyl)sulphamoyl] phenyl}pro-2-enamide	52	202-204	0.58	Chloroform: Methanol (4:6)
7	N-phenyl-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]-benzenesulfonamide	56	259-261	0.51	Hexane: Ethyl acetate (4:6)
8	N-(2-Methylphenyl)-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzenesulfonamide	57	264-266	0.76	Hexane: Ethyl acetate (4:6)
9	N-(4-Methylphenyl)-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzenesulfonamide	46	228-230	0.50	Chloroform: Methanol (4:1)
10	N-Benzyl-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzenesulfonamide	42	202-204	0.50	Hexane: Ethyl acetate (5:5)
11	N-(2-Chlorophenyl)-4-[3-oxo-3-(1H-1,2,4-triazol-1-yl)prop-1-en-1-yl]benzene sulphonamide	48	170-172	0.86	Chloroform: Methanol (2:8)
12	N-(2-Aminophenyl)-3-{4-[(N'-phenyl) sulfamoyl]phenyl}prop-2-enamide	58	85-90	0.57	Hexane: Ethyl acetate (4:6)
13	N-(2-Aminophenyl)-3-{4-[(N'-2-methoxyphenyl)sulfamoyl]phenyl}prop-2-enamide	51	182-184	0.53	Chloroform: Methanol (4:6)
14	N-(2-Aminophenyl)-3-{4-[(N'-benzyl)sulfamoyl]-phenyl}prop-2-enamide	46	158-160	0.78	Chloroform: Methanol (4:6)

3.3 Pharmacological Evaluation

3.3.1 Acute toxicity

No signs of morbidity or mortality in mice were found during the test period. No considerable decrease in the weight of mice was observed throughout the test period. Similarly, there were no changes in skin, fur, eyes and behavioral pattern.

3.3.2 *In vitro* cytotoxicity

The *in vitro* anticancer activity of all the synthesized test compounds and cisplatin was evaluated by using the SRB assay method on human ovarian cancer cell line (OVCAR-3). The results were expressed as the concentration of a compound causing 50 % inhibition of cell growth (GI_{50} value) in µMolar unit and are shown in **Table 4**.

Table 4: Anticancer activity expressed as concentration of compounds causing 50 % inhibition of cell growth (GI_{50}) for compounds 1 to 14 on OVCAR-3 cell line

Compound No.	GI_{50} (µM)	Comp. No.	GI_{50} (µM)
1	58.4	**9**	>100
2	49.4	**10**	>100
3	>100	**11**	>100
4	>100	**12**	68.0
5	>100	**13**	>100
6	85.0	**14**	>100
7	>100	**Cisplatin**	25.7
8	>100	--	--

The GI_{50} values of compounds **1, 2, 6 & 12** were less than 100 µM. Thus, their anti-proliferative action is satisfactory. All these compounds showed GI_{50} values higher than that of the standard, Cisplatin (GI_{50}, 25.7 µM).

CONCLUSION

In conclusion, the novel sulfonamides were synthesized on the basis of their docking score, number of hydrogen bonds (H-bond) and Vdw contacts with enzyme HDAC. All the synthesized compounds were purified by recrystallization. The structures of the synthesized compounds were characterized and confirmed from IR and ^{1}H NMR spectroscopy, respectively. Further, the compounds were tested for *in vitro* cyto-toxicity. Out of 14 compounds, 4 compounds (**1, 2, 6**, **12)** were found to possess satisfactory anti-proliferative activity. Thus, from the results, it can be concluded that the hydroxamic acid derivatives of sulfonamides **(Compounds 1, 2 & 6)** showed better activity, compared to triazol **(Compounds 7-11**) and OPD (**Compounds 12-14**) derivatives. One of the o-phenylenediamine derivatives of sulfonamides **(Compound 12)** also showed significant GI_{50} value (<100 µM). However, all the triazole derivatives of sulfonamides **(Compounds 7-11)** showed GI_{50} >100 µM.

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