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Design, Synthesis and *In Vitro* Biological Evaluation of Isoform Selective Histone Deacetylase Inhibitors

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Abstract

Histone deacetylase (HDAC) inhibitors are used as anticancer agents. Isoform selective HDAC inhibitors are more effective with fewer side effects for treatment of certain cancer types. Here five benzamide derivatives are designed and developed as HDAC8 inhibitor. All developed HDAC inhibitors were tested for enzyme inhibition assay as well as tested for antiproliferative activity using cell lines COLO 320DM, HCT-116 and COLO 205. Antiproliferative activity for cell line COLO 320DM, HCT-116 and COLO 205 was found in range12.21µM - 27.25 µM,12.36µM - 20.53µM and 12.21µM - 25.36 µM respectively. The synthesized compounds showed HDAC8 inhibitory activity in range 12.11 µM – 21.34µM.Docking studies were carried out for synthesized compounds using V-Life MDS software. Synthesized compounds were characterized by instrumental techniques such as Infrared spectroscopy, Nuclear magnetic resonance and elemental analysis.

Keywords: Histone Deacetylase (HDAC), HDAC Inhibitors, antiproliferative, IR, NMR.

Introduction

Histone proteins deacetylation, is controlled by the histone deacetylase (HDAC) enzyme which is a zinc dependent metalloenzyme and the acetylation of histone proteins by a Histone acetyl transferase (HAT) enzyme. Histone protein N-terminal lysine residue's deacetylation is HDAC enzyme catalysed. The positive charge on the N-terminal of histone proteins increased through the deacetylation. The interaction of negatively charged DNA and positively charged histone proteins results into binding of Histone and DNA which is compact one. This compact binding of DNA with histone proteinslimits access of transcription factors which leads to transcriptional gene silencing. The HAT causes acetylation ofN-terminal lysine residue

histone protein. Due to acetylation lysine get neutralized and do not show binding interaction with DNA. This results into slack DNA from histone proteins and gene-transcription activation due to the more relaxed chromatin state. Histone acetylation also linked with additional genome functions like chromatin assembly, recombination and repair of DNA[2].

Materials and Methods

Molecular Docking Studies[1-8]

Trichostatin A complexes in human HDAC8 having PDB code 5fcw was downloaded from the Protein Data Bank (RCSB PDB) and was used for validation of the docking protocol. From protein structure water molecules were deleted. The zinc metal kept as it is and hydrogens were added equivalent to pH 6.8. The selected HDAC8 protein was prepared using force field of VLifeMDS®4.6 to minimize energy. The proposed structures (inhibitors) were first energy-minimized then using Chem3D Pro 12.0 and ChemBio Draw Ultra 12.0 (CambridgeSoft) converted to pdb files. The further constraints were permitted to change during the docking: (a) the ligand's dihedral angles (b) the ring geometries of ligand (flipping ring corners) (c) the OH and NH_2 groups dihedral angles and (d) H-bonds mappings between the enzyme and ligand. All these allowed parameters were randomized at start of docking run. Best fit 5 molecules were selected for synthesis. Before docking SciFinder of proposed molecules was done from University of Mumbai department of organic chemistry to find existence of molecules or research done related to selected molecules. There was no any exact match for proposed molecules were found as per SciFinder search.

Synthesis of proposed molecules[9-11]

Chemicals: 3-phenoxybenzoic acid, 2-phenoxybenzoic acid, diphenyl acetic acid, biphenyl carboxylic acid, napthaiene-1-carboxylic acid, 1,2 phenylenediamine, Boc2O, TFA, DCM, EtOAc, sodium bicarbonate.

Procedure: The reaction mixture of acid (3-phenoxybenzoic acid/2-phenoxybenzoic acid/diphenyl acetic acid/biphenyl carboxylic acid/napthaiene-1-carboxylic acid) (0.15 mM), hydroxybenzotriazole (0.15 mM) and EDC (0.6 mM) in DMF (6 ml) to this triethyl amine (0.45 mM) was added. To the above reaction mixture pretreated 1,2 phenylenediamine (0.9mM) was added. This final reaction mixture's stirring was carried out and then diluted using sodium bicarbonate (5% w/v, 20 ml). The extraction of reaction mixture was carried out using EtOAc, resulting extracts was washed by first water followed by saturated NaCl and lastly dried to get product an amide. The resulting product

was mixed with TFA and DCM 1:1 solution for removal of Boc2O.

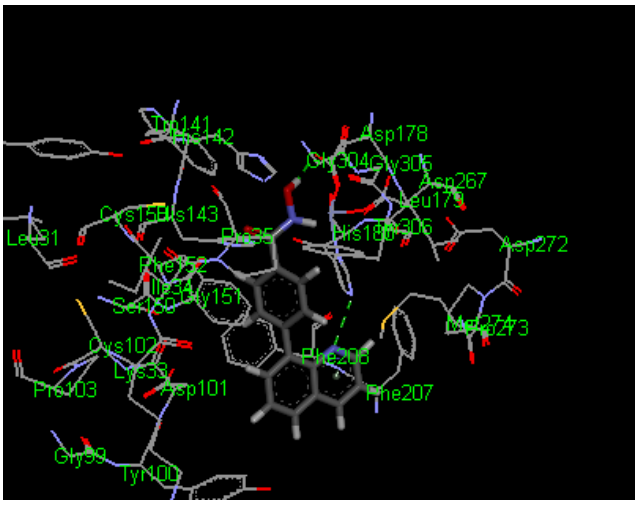
In vitro HDAC8 inhibition activity[12]

As selected target for study is HDAC8 enzyme the activity of synthesized compounds for inhibition of HDAC8 enzyme need to be study. For this purpose HDAC inhibitory assay can be carried out. The enzyme inhibition assay for synthesized compounds gives precision about the inhibitory effect of synthesized compound on target enzyme. In this assay method inhibitor is allowed to interact with enzyme in presence of substrate then after certain incubation period quantity of product formed evaluated. Less quantity of product formed more the efficacy of inhibitor and vice versa.

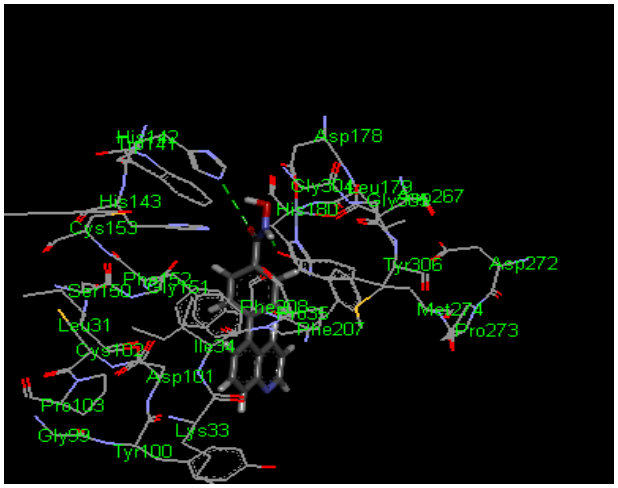
In vitro antiproliferative activity[13-19]

In vitro antiproliferative activity was studied using MTT assay method. For study of antiproliferative activity study colon cancer cell lines HCT 116, COLO 320 DM and COLO 205 were used. The assay based on cell viability study. The resulting readings were analyzed using GraphPad prism 8 for ANOVA and for IC_{50}.

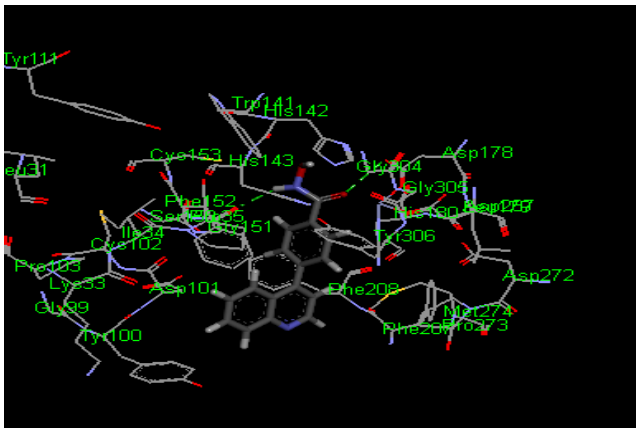
Results and Discussion



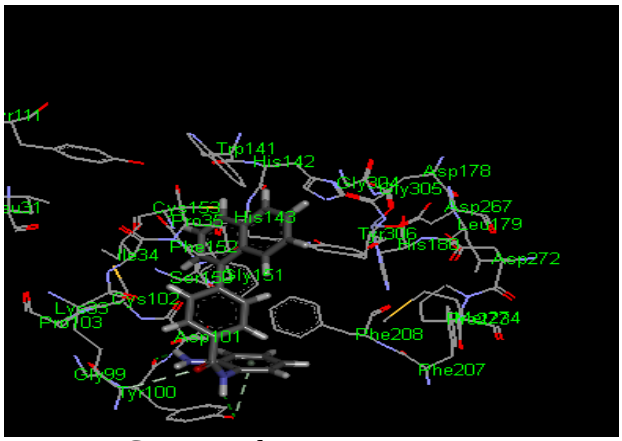
B. Compound -2



A. Compound -1



C. Compound -3



D. Compound -5

Figure No.1: Docking interactions between 5fcw HDAC protein with A. Compound 1; B. Compound 2; C. Compound 3 and D. Compound 5

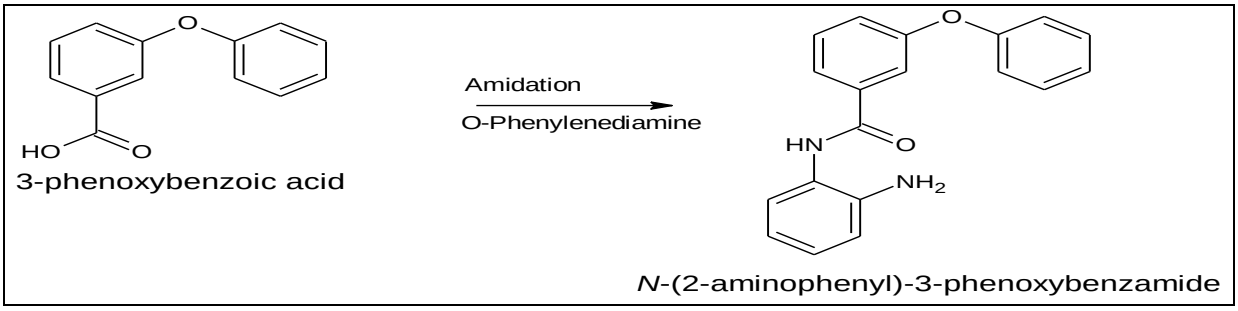


Figure No.2: Synthesis of N-(2-aminophenyl)-3-phenoxybenzamide (Compound 1)

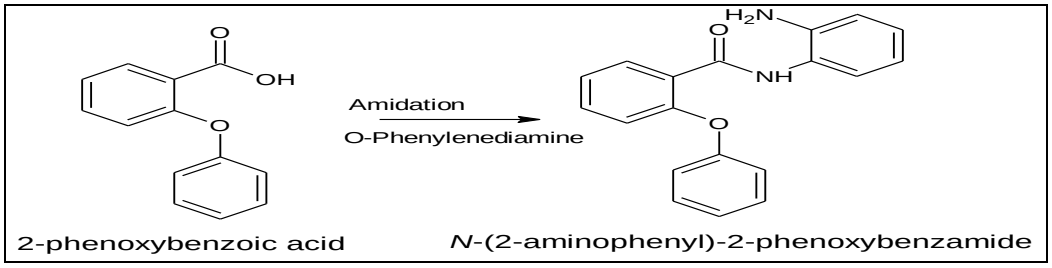


Figure No. 3: Synthesis of N-(2-aminophenyl)-2-phenoxybenzamide(Compound 2)

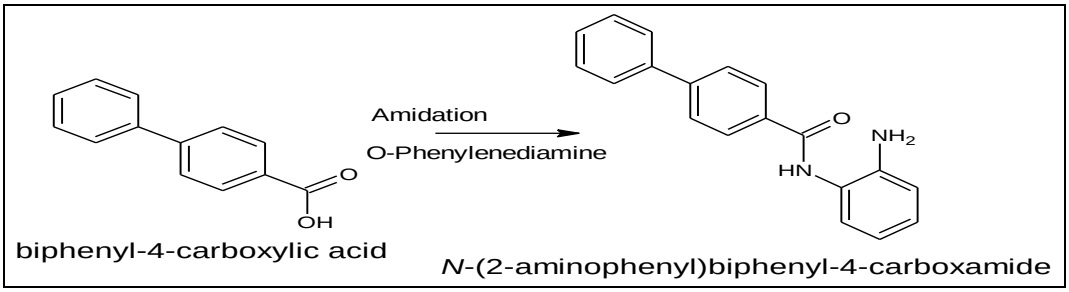


Figure No. 4: Synthesis of N-(2-aminophenyl) biphenyl-4-carboxamide (Compound 3)

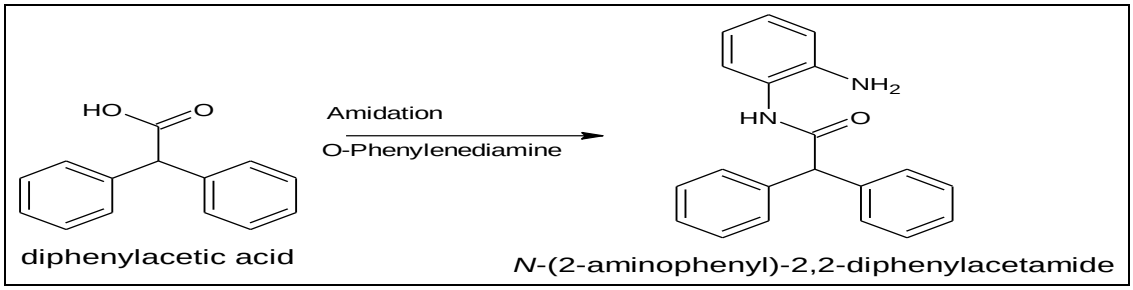


Figure No. 5: Synthesis of N-(2-aminophenyl)-2,2-diphenylacetamide (Compound 4)

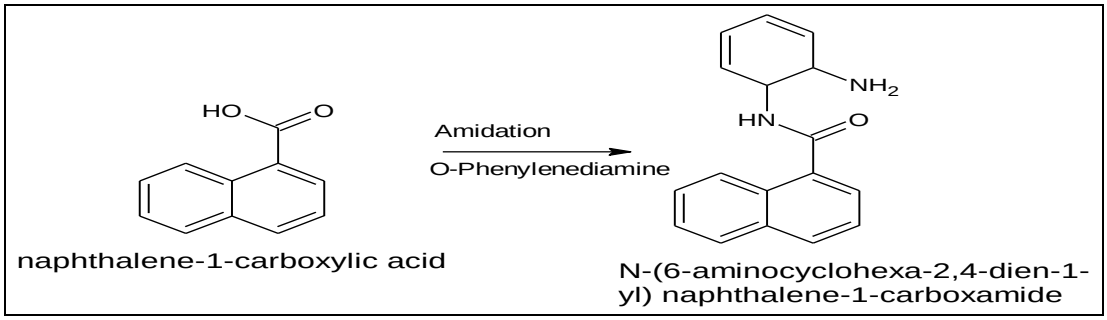


Figure No. 6: synthesis of N-(6-aminocyclohexa-2,4-dien-1-yl) naphthalene-1-carboxamide (Compound 5)

Table No.1: Showing % Yield and Melting Points of Synthesized compounds

Compound Code	% yield	M. P
1	84.43	250
2	78.47	227
3	86.22	263
4	76.23	273
5	81.34	284

Table No. 2: Predicted and detected HDAC8 inhibitory activity

Compound Code	50% HDAC8 activity inhibition (µM)	Predicted IC_{50} (µM)
1	12.11	12.45
2	21.31	21.23
3	18.23	17.94
4	17.87	18.14
5	14.45	13.96

Table No. 3: Detected antiproliferative activity of synthesized compounds using cell lines.

Sr. No	Cell Line / Compounds	HCT-116 IC_{50} (in µM)	COLO 205 IC_{50} (in µM)	COLO320 DM IC_{50} (in µM)
1.	1	23.47	13.16	21.09
2.	2	12.75	17.4	17.31
3.	3	25.36	12.36	12.21
4.	4	23.54	20.53	14.36
5.	5	17.42	16.19	27.25

Table No. 4: Binding energy and PLP score for compounds

Sr. No.	Compound Code	Energy of Molecule	Energy of optimized Complex	Δ Binding Energy	PLP Score
1	1	76.436	-468.342	-544.778	-71.122
2	2	97.435	-386.452	-483.887	-64.886
3	3	64.785	-376.325	-441.11	-69.74
4	4	58.834	-463.324	-522.158	-60.613
5	5	86.675	-385.453	-472.128	-64.345

Instrumental analysis results

1. **N-(2-aminophenyl)-3-phenoxy benzamide: IR interpretation-** N(1425 cm^{-1}), NH(1590 cm^{-1}, 3300 cm^{-1}),C=O(1660 cm^{-1}), Ar-H(3050 cm^{-1}), C-O(1100 cm^{-1}); **NMR interpretation:** 6.7 (2H), 7.7 (2H), 7.2(2H), 7.6(1H), 7(1H), 7.3(2H), 7.4(3H), 4.5(2H), 8.9(1H); **Mass:** $[M+H]^+$: 306.128454 Da; Calculated: C (74.98%), H (5.30%), N **(**9.20%), **O (**10.51%); Found: C (75.00%), H (5.32%), N (9.13%), O (10.55%)
2. **N-(2-aminophenyl)-2-phenoxy benzamide: IR interpretation:**C-N(1425 cm^{-1}), NH(1590 cm^{-1}, 3300 cm^{-1}),C=O(1660 cm^{-1}), Ar-H(3050 cm^{-1}), C-O(1150 cm^{-1})**NMR interpretation:** 7.3(2H), 7.6 (3H), 7.2 (2H), 7.9 (2H), 6.8 (2H), 7.7 (2H), 4.5(2H), 8.8 (2H)**Mass:** [M+H]+:

306.128454 Da, Calculated: C (74.98%), H (5.30%), N(9.20%), O (10.51%); Found: C (74.96%), H (5.31%), N (9.24%), O (10.49%)

3. **N-(2-aminophenyl) biphenyl-4-carboxamide: IR interpretation:** C-N(1425 cm^{-1}), NH(1590 cm^{-1}, 3300 cm^{-1}),C=O(1660 cm^{-1}), Ar-H(3050 cm^{-1})**NMR interpretation:** 7.4 (6H), 7.8 (3H), 6.7(2H), 7.7(2H), 4.5(2H), 8.7(1H). **Mass:** $[M+H]^+$: 289.13354 Da, Calculated: C (79.14%), H (5.59%), N(9.72%), O (5.55%); Found: C (79.19%), H (5.51%), N (9.77%), O (5.53%)
4. **N-(2-aminophenyl)-2,2-diphenyl acetamide: IR interpretation:** C-N(1425 cm^{-1}), NH(1590 cm^{-1}, 3300 cm^{-1}),C=O(1660 cm^{-1}), Ar-C-H(3050 cm^{-1}), Ar-C(2900 cm^{-1})**NMR interpretation:** 7.3(4H), 7.8(6H), 6.8 (2H), 7.6(2H), 4.4(1H), 8.9 (2H), 2.6 (1H)**Mass:** $[M+H]^+$: 303.14919 Da, Calculated: C (79.44%), H (6.00%), N(9.26%), O (6.29%); Found: C (79.44%), H (6.01%), N (9.28%), O (6.26%)
5. **N-(6-aminocyclohexa-2,4-dien-1-yl) naphthalene-1-carboxamide: IR interpretation:** C-N(1425 cm^{-1}), NH(1590 cm^{-1}, 3300 cm^{-1}),C=O(1660 cm^{-1}), Ar-C-H(3050 cm^{-1})**NMR interpretation:** 7.4 (4H), 7.6 (3H), 6.7(2H), 7.8(2H), 4.5(1H), 8.8(2H).**Mass:** $[M+H]^+$: 266.13354 Da, Calculated: C (77.25%), H (6.10%), N(10.60%), O (6.05%); Found: C (77.27%), H (6.07%), N (10.61%), O (6.05%)

Discussion

Five compounds were synthesized as HDAC8 inhibitor. Prior to start of docking studies availability of molecules were checked using SciFinder. Compound 1 showed good enzyme inhibition activity. Synthesized compounds in addition tested for antiproliferative activity using cell lines HCT 116, COLO 205 and COLO 320DM.

Conclusion

The synthesized molecules are effective as HDAC inhibitor. The synthesized compounds are characterized by instrumental methods. As these molecules are showing HDAC8 inhibitory effect their inhibitory activity should be studied for other HDAC isoforms.

Acknowledgements

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