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RESEARCH ARTICLE

Design and Synthesis of Novel 2, 5-Disubstituted-1, 3, 4-Thiadiazoles and Oxadiazoles as Histone Deacetylase Inhibitors

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ABSTRACT

Histone deacetylases (HDACs) have been widely recognized as promising targets for cancer treatment. Accumulated clinical studies have demonstrated that HDACs inhibitors show great potential for the treatment of cancer. SAHA (Vorinostat, trade name Zolinza®) and Trichostatin A (TSA) where approved by the FDA in 2006 for the treatment of the cutaneous manifestations of cutaneous T-cell lymphoma. As a continuity of our ongoing effort to identify novel small molecules targeting these important enzymes, we designed and synthesized two series of azole based 2, 5-Disubstituted-1, 3, 4-Thiadiazoles (5a-e) and 2, 5-Disubstituted 1, 3, 4-Oxadiazoles (5f-j) as histone deacetylase inhibitors as analogues of SAHA. Ligand-based virtual screening methods were employed to identify novel HDAC inhibitors. Docking study all compounds revealed that these compounds bound to HDAC-2 i.e. 3MAX PDB CODE with higher affinities compared to SAHA and Trichostatin A. These findings should encourage further elaboration with the azole moiety to produce more potent HDAC inhibitors with potential anti-cancer activity. They were tested for anti-cancer, anti-bacterial and anti-fungal activity. Synthesized compounds possessed good anti-bacterial and anti-fungal activity as compared to standard drug.

KEYWORDS

Anti-cancer, 2, 5-Disubstituted 1, 3, 4-Oxadiazoles, 2, 5-Disubstituted-1, 3, 4-Thiadiazoles, Histone deacetylase inhibitor, SAHA

INTRODUCTION

The change from ordinary to disease cells includes numerous etiologic pathways; it incorporates different hereditary, epigenetic and cell forms. Epigenetic is currently an up and coming field, and the most novel examination is occurring in this rising range[1]. One of the imperative epigenetic courses to carcinogenesis includes the abnormal example of histone alterations.

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It is currently surely understood that acetylation is one of the key segments in the direction of quality expression and this has fortified the investigation of histone deacetylases (HDACs) in connection to the distorted quality expression, frequently saw in malignancy[2].

Histone Deacetylase (HDAC) Enzyme

The crucial subunit of chromatin is called nucleosome, which is made out of an octamer of four center histones, i.e. H3 and H4 tetramers and two dimers H2A and H2B, encompassed by 146 base sets (bp) of DNA. Among the four histone tails, H3 and H4 are focused for different post-translational changes[3]. Translation in eukaryotic

cells is impacted by the way in which DNA is bundled[4]. The official of histones to DNA is controlled or directed by different catalysts present in the cell.

Regulation of Transcription by HAT-HDAC Enzyme

The histone acetyl transferases (HAT) add an acetyl group to the histone proteins, discharging the confined access to DNA forced by the histones. Because of this entrance to DNA, translation starts. At the point when the quality no more should be translated, compound HDAC evacuates the acetyl bunch. The expulsion of the acetyl bunch empowers histones to tie to the DNA, bringing on confined access to the DNA once more. In this way, histone can display in one of the two enemy shapes, acetylated or deacetylated. Deacetylation prompts the expulsion of an acetyl bunch from the ε-amino gatherings of the lysine side-chain of the histone atom[5].

HDAC Superfamily of Proteins

Histone deacetylase super family is grouped into four classes with general 18 individuals. Class I, containing HDACs 1–3 and 8, indicates closeness to yeast transcriptional controller Rpd3p deacetylases, while class II HDACs (4 –7, 9 and 10) are like yeast Hda1 deacetylases[6] and class IV (HDAC 11 just) is fundamentally identified with both, the class I and II HDACs. Class III HDACs show arrangement similitude to the yeast Sir2, and incorporate NAD+ - subordinate deacetylases. The class III HDACs are not delicate to the hindrance by HDAC inhibitors and their significant part in deacetylation of cell cycle proteins like p53[7] is still not clear. Class I, II, and IV are alluded to as "traditional" HDACs as they are Zn2+ subordinate chemicals, which are promptly subjected to chelation by Zn^{2+} chelating mixes, for example, hydroxamic acids. Class III individuals, requiring NAD+ for their movement, are named as 'Sirtuins'. In this way, the term "HDAC inhibitors" is normally utilized for intensifies that objective the "established" HDACs, i.e., HDACs from the classes I, II, and IV[8].

HDAC Inhibitors (HDACIs)

The HDAC inhibitors (HDACIs) are a fundamentally assorted group of operators, including both natural and synthetic mixes. The HDACIs capacity by uprooting the zinc particle and in this way rendering the charge-transfer framework broken[9]. Thorough restorative improvement endeavors have concentrated on focusing on HDAC with little particles. The HDACIs are profoundly successful in up-managing tumor silencer quality expression, decreasing tumor development and actuating modified cell demise in vitro and in disease patients in stage I and II clinical trials[10,11]. Throughout the years, various sorts of HDACIs have been produced, running from convoluted structures of bacterial or contagious starting point [trichostatinA (TSA), trapoxin] to the exceptionally basic butyrate. The HDACIs are equipped for restraining HDACs with changing productivity. Restraint of HDACs can bring about a general hyperacetylation of histones, which is trailed by the transcriptional actuation of specific qualities through unwinding of DNA adaptation. For the most part, HDACIs are known not ready to impel development capture and separation or apoptosis of tumor cells[12,13]. The majority of the hydroxamic corrosive subsidiaries share the basic attributes, which incorporate vast hydrophobic district, 5-6 carbon aliphatic chain and a dynamic useful gathering, which can collaborate with the zinc particle. It has been watched that overexpression of HDACs is related to destructive pathologies[14], and the distinctive isoforms of HDAC are communicated in a few tumor tissues with particular organic capacity[15].

Since the expanded action of HDACs is connected with carcinogenesis and tumor movement, HDAC inhibitors have been drawing in the consideration of the scientists as promising anticancer operators[16]. A few bioactive little atoms of characteristic or engineered source, have been researched as HDAC inhibitors,[17,18] and the suberoylanilide hydroxamic corrosive (SAHA) and Tricostatin A (TSA)[19] has been endorsed by the Food and Drug Administration for the treatment of cutaneous T cell lymphoma.

Other than SAHA, different inhibitors have additionally shown a helpful potential use Figure 1. The vast majority of the right now HDAC inhibitors, tried in clinical trials, are somewhat unselective, repressing it is possible that all or if nothing else a few individuals from the HDAC family. Clinical studies demonstrate that HDAC inhibitors may offer ascent to various symptoms:[20] bone marrow melancholy, looseness of the bowels, weight reduction, taste unsettling influences, electrolyte changes, confused thickening, exhaustion, and cardiovascular arrhythmias. Be that as it may, the poisonous quality profile is diverse for the different skillet HDAC inhibitors, offering ascend to various and questionable conclusions of their utilization in disease treatment. In addition, the extremely proficient antitumour impact of HDAC inhibitor ought to be credited to the synchronous hindrance of the diverse isoforms by blocking distinctive elements of malignancy cells. These undesirable impacts could be attributed to the vital part of HDACs to control distinctive proteins required in different natural procedures. Therefore, specific HDAC ligands might be liked to dish inhibitors in restorative applications[21,22].

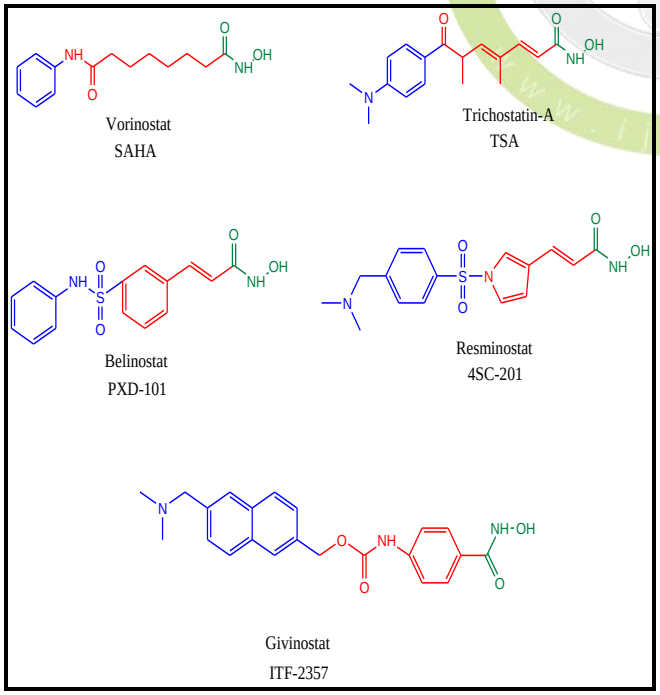


Figure 1: Structures of clinically established HDAC inhibitors. Surface recognition moiety, Linker, Zinc binding group

In this manner, the following stride in the advancement of HDAC inhibitors is to target specifically individual HDAC isoforms, with the point of meddling with basic oncogenic capacity in tumor cells and without influencing typical cells. It ought to be likewise confirmed if particular inhibitors keep up the adequacy of container inhibitors and present decreased symptoms. In this setting, as the compounds work in cells with other protein accomplices, it is hard to gadget little particles ready to specifically tie HDACs. In reality, HDACs are the reactant focus of multiprotein groups[23]. In writing, contemplates gave to create particular ligands for particular isoforms are accounted for, despite the fact that such concentrates just consider a confined number of isoforms[24]. Additionally, a large portion of the distributed works concentrated on specific ligands are just in light of natural profiles, without the auxiliary examination meant to uncover the ligand and protein components conceivably dependable of class and isoform selectivity[25]. On this premise, in the present commitment, we have followed out the auxiliary components mindful of particular authoritative in the entire scene of the restoratively pertinent HDAC isoforms.

Specifically, we have attempted to justify various trial perceptions and attempted to efficiently include new basic experiences for a focused on configuration of particular inhibitors of the diverse HDAC isoforms, centering our consideration on HDAC-2 for which few data so far are accessible in writing on expression and capacity in tumor cells, and HDAC5, missing a solid ligand inhibitory profile have not been considered in our examination. Our basic examination was performed by atomic docking computations, utilizing as ligands and class particular HDAC inhibitors reported in the writing, showing a very much characterized profile of HDACs hindrance.

Based on these characteristics, designing and synthesis of 2, 5-Disubstituted-1, 3, 4-Thiadiazoles and Oxadiazoles derivatives were undertaken. The structure of this derivative is illustrated in Figure 2.

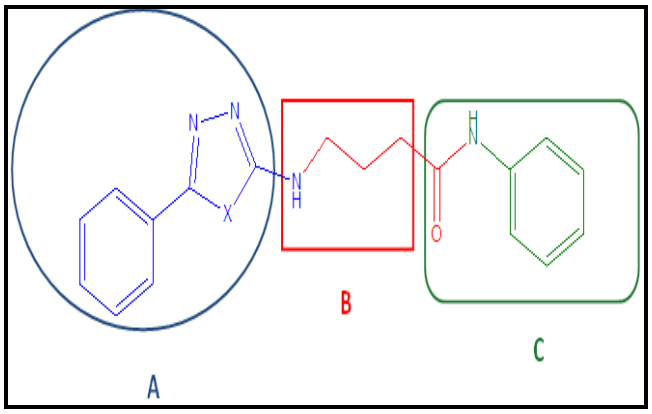


Figure 2: Structure of 2, 5-Disubstituted-1, 3, 4-Thiadiazoles and Oxadiazoles derivatives (5a-j). A = surface recognition moiety, B = Linker; C = zinc binding group

MATERIAL AND METHODS

Computational Chemistry

The first step of the project was to design the anticancer agents by using in silico method. The HDAC enzyme Inhibitors were designed using the Molecular Modeling Software "VlifeMDS" from Pune, India.

Ligand Preparation

Structures of the ligands were sketched using "build" panel on Maestro and refined for docking by the program force field, to get the low energy 3D conformers of the ligands.

Protein Preparation

The following X-Ray crystallographic structures of the HDAC enzyme were selected and downloaded from Protein Data Bank, based on the resolution, R-value, RMSD value and mode of binding of the enzyme with cocrystallized inhibitor.

1. Crystal structure of human HDAC2 complexed with N-(2-aminophenyl)benzamide: **3MAX**

The selected enzymes were processed by using protein preparation wizard and the energy of the system was minimized, using force field.

Grid Generation

"Biopredicta Tools" was used to define the docking space and then docking was performed using the Grid Docking mode.

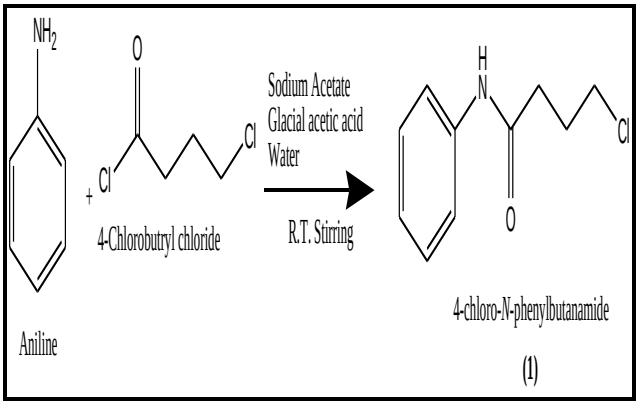
Docking

Molecular docking studies were performed using the program "VlifeMDS" to understand the interaction of ligands and the enzyme. The docking results were expressed in terms of Dock Score. Ligand binding to its receptor was evaluated based on the Dock score, hydrogen bonding (H-bond), hydrophobic bonding, charge interactions and aromatic bonding. Compounds with good Dock Score and Interactions were further synthesized and evaluated for their pharmacological activities.

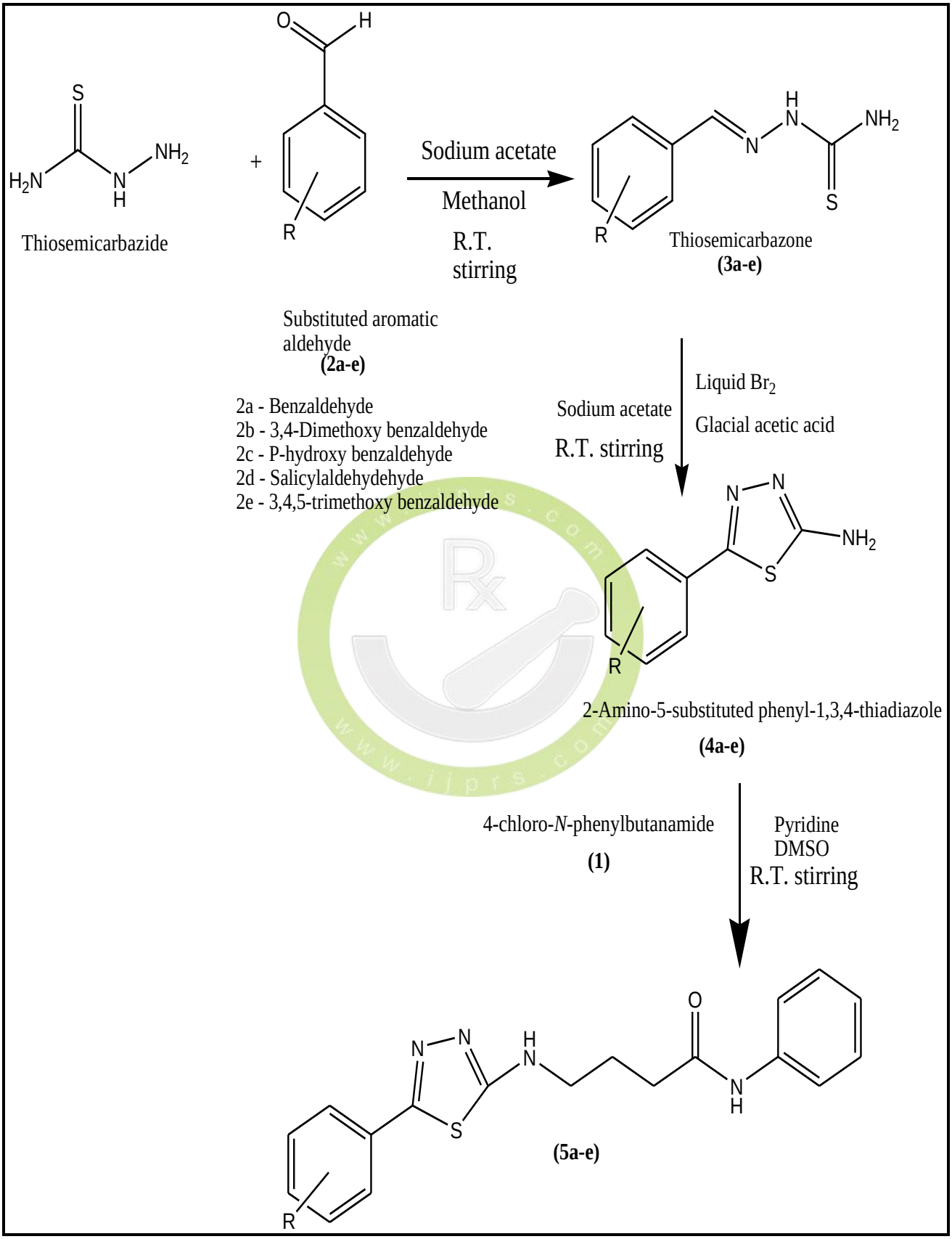
Chemistry

The general approach to synthesize compound 1 is outlined in Scheme 1. N-alkylation of aniline with 4-Chlorobutryl chloride gave 4-chloro-N-phenylbutanamide (1). The synthetic route of compounds 5a–e is outlined in Scheme 2. Thiosemicarbazide was reacted with various substituted aromatic aldehydes (2a-e) to give thiosemicarbazones (3a-e), which are further reacted with Liquid Bromine to give 2,5-Disubstituted-1,3,4-thiadiazole (4a-e).These were further reacted with compound 1 to give final compounds (5a-e).

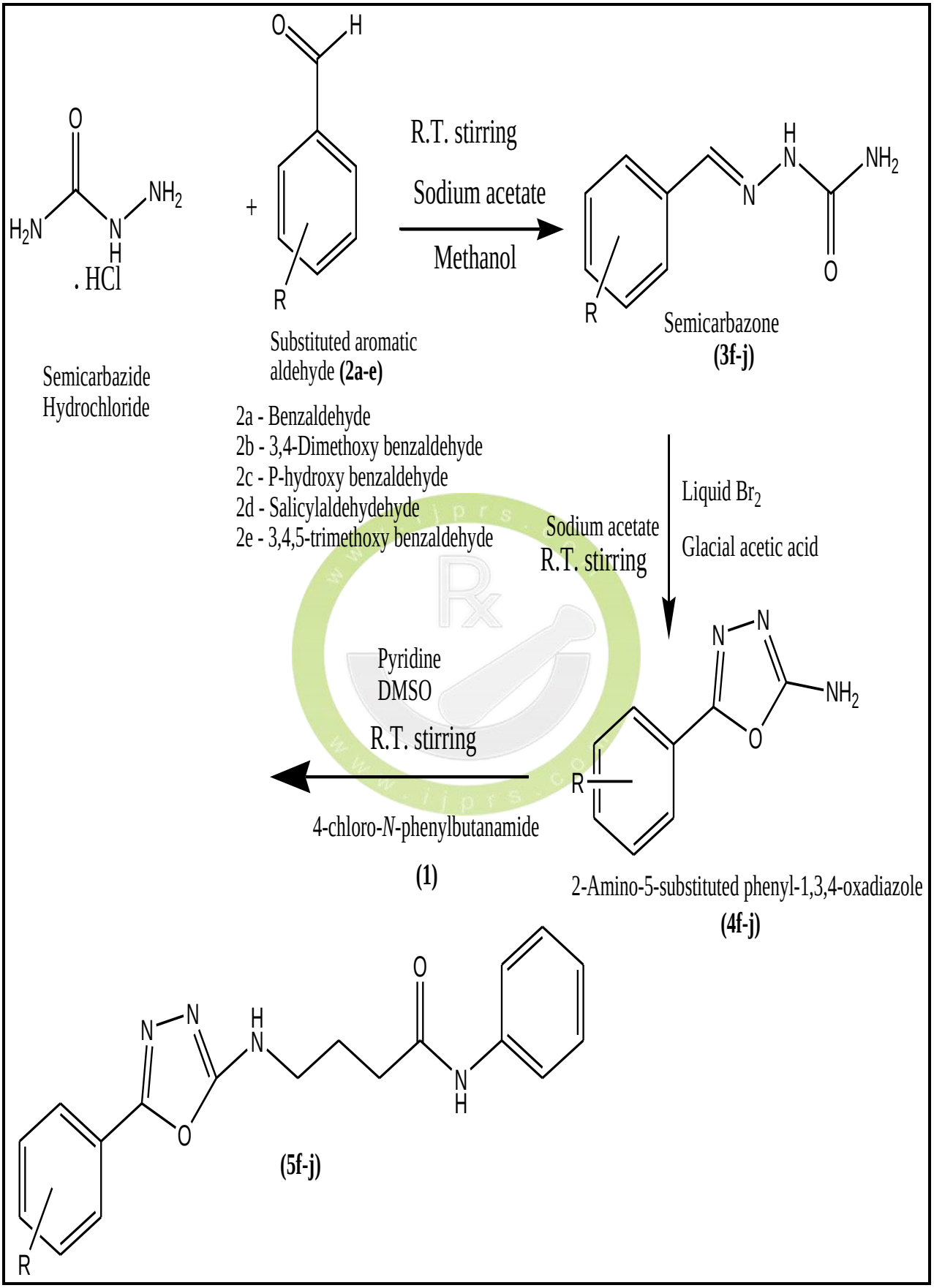
The synthetic route of compounds (5f-j) is outlined in Scheme 3. Semicarbazide hydrochloride was reacted with various substituted aromatic aldehydes (2a-e) to give semicarbazones (3f-j), which are further reacted with Liquid Bromine to give 2,5-Disubstituted-1,3,4-oxadiazole (4f-j).These were further reacted with compound 1 to give final compounds (5f-j).



Scheme 1



Scheme 2



Scheme 3

Experimental

General Experimental Procedures

Unless otherwise specified, starting materials were obtained from commercial suppliers and used without further purification.

All the melting points were determined on 'Veego' VMP-D apparatus and are uncorrected. Silica gel G plates of 3x8 cm (Sigma Aldrich) were used for TLC and spots were located by UV or in iodine chamber. The IR spectra were recorded in the 4000-400 cm-1 range using FT-IR 8400 SHIMADZU spectrometer.

1H NMR spectra were recorded on multinuclear FT NMR Spectrometer model Avance-II (Bruker) in DMSO-d6 with TMS as an internal standard and values are expressed in ppm and Liquid Chromatography Mass Spectrometry with Waters Micromass Q-Tof Micro at Sophisticated Analytical Instrumentation Facility, Punjab University Chandigarh.

Synthesis of 4-chloro-N-phenylbutanamide (1)

3.373 ml (0.037 moles, 1 equivalent) of aniline was dissolved with continuous stirring in 30 ml of solution containing glacial acetic acid and water (saturated with sodium acetate) prepared in proportion of 1:1. To this add 6.47 ml (0.037 moles, 1 equivalent) of 4-Chlorobutryl chloride was added dropwise and the mixture was stirred overnight to obtain a white precipitate. The precipitate was thoroughly washed with water to remove impurities and dried. Yield= 70-75%.

Synthesis of Thiosemicarbazones (3a-e)

0.01 mole of thiosemicarbazide and 0.02 mole of sodium acetate was mixed in 10ml water. To this substituted 0.01 mole of aromatic aldehyde **(2a-e)** was added slowly with continuous stirring. Turbid solution was formed.

To form clear solution methanol was added till clear solution is formed. It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Synthesis of 2,5-disubstituted-1,3,4-thiadiazoles (4a-e)

Mixture of 0.01 mole of thiosemicarbazone and 0.02 mole of sodium acetate was taken in round bottom flask. To this glacial acetic acid was added till all dissolves. It was stirred on magnetic stirrer for 2 minutes, then to this liquid Bromine in glacial acetic acid was added (0.7ml in 5ml). It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Synthesis of Final 2,5-disubstituted-1,3,4-thiadiazoles derivatives (5a-e)

0.01 mole of 2,5-disubstituted-1,3,4-thiadiazoles was dissolved in DMSO. To this 0.08 mole of pyridine was added and was stirred on magnetic stirrer for 30 minutes at 4˚C. To this 0.01 mole of 4-chloro-N-phenylbutanamide (1) was added slowly over a period of 2hrs. It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Synthesis of Semicarbazones (3f-j)

0.01 mole of semicarbazide hydrochloride and 0.02 mole of sodium acetate was mixed in 10ml water. To this substituted 0.01 mole of aromatic aldehyde **(2a-e)** was added slowly with continuous stirring. Turbid solution was formed. To form clear solution methanol was added till clear solution is formed. It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (4f-j)

Mixture of 0.01 mole of semicarbazone and 0.02 mole od sodium acetate was taken in round bottom flask. To this glacial acetic acid was added till all dissolves. It was stirred on magnetic stirrer for 2 minutes, then to this liquid Bromine in glacial acetic acid was added (0.7ml in 5ml).

It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Synthesis of Final 2,5-disubstituted-1,3,4-oxadiazoles derivatives (5f-j)

0.01 mole of 2,5-disubstituted-1,3,4-thiadiazoles was dissolved in DMSO. To this 0.08 mole of pyridine was added and was stirred on magnetic stirrer for 30 minutes at 4˚C. To this 0.01 mole of 4-chloro-N-phenylbutanamide (1) was added slowly over a period of 2hrs. It was stirred on magnetic stirrer and reaction was monitored using TLC. On completion of reaction it was dumped in ice cold water. Final product was filtered off. Hexane: Ethyl acetate (2:1).

Physicochemical and Spectral Characteristics of the Synthesized Compounds

2-Amino (4-N-phenylbutanamide)-5-phenyl-1,3,4-thiadiazole (5a)

$C_{18}H_{18}N_4OS$, Melting point: 235 °C, **IR Assignments:** NH-stretch= 3305 cm^{-1}, Aromatic C-H stretch=3118 cm^{-1}, C=O stretch= 1658 cm^{-1}, Aromatic C=C bending = 1523 cm^{-1}, C=N Imine stretch=1446 cm^{-1}, Aromatic C-H bending= 754,686 cm^{-1}. **NMR Assignments (DMSO, ppm)** BT: 8.421 δ (s, 1H, -NH), 6.9929-7.46 δ (m, 10H phenyl), 4.3 δ (s, 1H, -NH), 3.410 δ (q, 2H, CH_2), 2.5216 δ (t, 2H, CH_2), 1.6051 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole (5b)

$C_{20}H_{22}N_4O_3S$, Melting point: 213 °C, **IR Assignments:** NH-stretch= 3320 cm^{-1}, Aromatic C-H stretch=2966 cm^{-1}, C=O stretch= 1666 cm^{-1}, Aromatic C=C bending = 1535 cm^{-1}, C=N Imine stretch=1507 cm^{-1}, C-O-C ether stretch=1136 cm^{-1}, Aromatic C-H bending= 749 cm^{-1}. **NMR Assignments (DMSO, ppm)** DT: 8.1022 δ (s, 1H, -NH), 7.0131-7.391 δ (m, 8H phenyl), 4.2954 δ (s, 1H, -NH), 3.7621 δ (s, 6H, -O-CH_3), 2.569 δ (q, 2H, CH_2), 2.2114 δ (t, 2H, CH_2), 1.5857 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(4-hydroxyphenyl)-1,3,4-thiadiazole (5c)

$C_{18}H_{18}N_4O_2S$, Melting point: 240 °C, **IR Assignments:** NH-stretch= 3321 cm^{-1}, OH-stretch=3219 cm^{-1}, Aromatic C-H stretch=3072 cm^{-1}, C=O stretch= 1667 cm^{-1}, Aromatic C=C bending = 1598 cm^{-1}, C=N Imine stretch=1535 cm^{-1}, Aromatic C-H bending= 742 cm^{-1}. **NMR Assignments (DMSO, ppm)** PT: 10.0643 δ (s, 1H, -NH), 6.8252-8.3875 δ (m, 9H phenyl), 4.6704 δ (s, 1H, -NH), 4.0117 δ (s, 1H, -OH), 2.5730 δ (q, 2H, CH_2), 2.2114 δ (t, 2H, CH_2), 2.0626 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(2-hydroxyphenyl)-1,3,4-thiadiazole (5d)

$C_{18}H_{18}N_4O_2S$, Melting point: 224 °C, **IR Assignments:** NH-stretch= 3406 cm^{-1}, OH-stretch=3319 cm^{-1}, Aromatic C-H stretch=3051 cm^{-1}, C=O stretch= 1651 cm^{-1}, Aromatic C=C bending = 1598 cm^{-1}, C=N Imine stretch=1545 cm^{-1}, Aromatic C-H bending= 692,752 cm^{-1}. **NMR Assignments (DMSO, ppm)** ST: 9.9063 δ (s, 1H, -NH), 6.8252-8.4215 δ (m, 9H phenyl), 3.6997 δ (s, 1H, -NH), 3.5221 δ (s, 1H, -OH), 2.4432 δ (q, 2H, CH_2), 2.2649 δ (t, 2H, CH_2), 2.0426 δ (m, 2H, CH_2).

2-Amino-(4-N-phenylbutanamide)-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole (5e)

$C_{20}H_{25}N_4O_4S$, Melting point: 208 °C, **IR Assignments:** NH-stretch= 3321 cm^{-1}, Aromatic C-H stretch=2934 cm^{-1}, C=O stretch= 1665 cm^{-1}, Aromatic C=C bending = 1598 cm^{-1}, C=N Imine stretch=1527 cm^{-1}, C-O-C ether stretch=1102 cm^{-1}, Aromatic C-H bending= 675 cm^{-1}. **NMR Assignments (DMSO, ppm)** TT: 8.1022 δ (s, 1H, -NH), 7.0131-7.391 δ (m, 7H phenyl), 4.2954 δ (s, 1H, -NH), 3.7621 δ (s, 9H, -O-CH_3), 2.569 δ (q, 2H, CH_2), 2.2114 δ (t, 2H, CH_2), 1.5857 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-phenyl-1,3,4-thiadiazole (5f)

$C_{18}H_{18}N_4O_2$, Melting point: 208 °C, **IR Assignments:** NH-stretch= 3309 cm^{-1}, Aromatic C-H stretch=2974 cm^{-1}, C=O stretch= 1658 cm^{-1}, Aromatic C=C bending = 1535 cm^{-1}, C=N Imine stretch=1444 cm^{-1}, C-O-C ether stretch= 1031 cm^{-1}, Aromatic C-H bending= 736,682 cm^{-1}.

NMR Assignments (DMSO, ppm) BO: 8.421 δ (s, 1H, -NH), 6.9929-7.46 δ (m, 10H phenyl), 4.3 δ (s, 1H, -NH), 3.410 δ (q, 2H, CH_2), 2.5216 δ (t, 2H, CH_2), 1.6051 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole (5g)

$C_{20}H_{22}N_4O_4$, Melting point: 223 °C, **IR Assignments:** NH-stretch= 3396 cm^{-1}, Aromatic C-H stretch=3128 cm^{-1}, C=O stretch= 1671 cm^{-1}, Aromatic C=C bending = 1578 cm^{-1}, C=N Imine stretch=1437 cm^{-1}, C-O-C ether stretch=1026 cm^{-1}, Aromatic C-H bending= 966 cm^{-1}. **NMR Assignments (DMSO, ppm)** DO: 8.1022 δ (s, 1H, -NH), 7.0131-7.391 δ (m, 8H phenyl), 4.2954 δ (s, 1H, -NH), 3.7621 δ (s, 6H, -O-CH_3), 2.569 δ (q, 2H, CH_2), 2.2114 δ (t, 2H, CH_2), 1.5857 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(4-hydroxyphenyl)-1,3,4-oxadiazole (5h)

$C_{18}H_{18}N_4O_3$, Melting point: 242 °C, **IR Assignments:** OH-stretch=3404 cm^{-1}, NH-stretch= 3318 cm^{-1}, Aromatic C-H stretch=2971 cm^{-1}, C=O stretch= 1665 cm^{-1}, Aromatic C=C bending = 1533 cm^{-1}, C=N Imine stretch=1443 cm^{-1}, C-O-C ether stretch= 1180 cm^{-1}, Aromatic C-H bending= 742 cm^{-1}. **NMR Assignments (DMSO, ppm)** PO: 10.0643 δ (s, 1H, -NH), 6.8252-8.3875 δ (m, 9H phenyl), 4.6704 δ (s, 1H, -NH), 4.0117 δ (s, 1H, -OH), 2.5730 δ (q, 2H, CH_2), 2.2114 δ (t, 2H, CH_2), 2.0626 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(2-hydroxyphenyl)-1,3,4-oxadiazole (5i)

$C_{18}H_{18}N_4O_3$, Melting point: 248 °C, **IR Assignments:** NH-stretch= 3412 cm^{-1}, OH-stretch=3319 cm^{-1}, Aromatic C-H stretch=2968 cm^{-1}, C=O stretch= 1680 cm^{-1}, Aromatic C=C bending = 1535 cm^{-1}, C=N Imine stretch=1442 cm^{-1}, C-O-C ether stretch=1168 cm^{-1}, Aromatic C-H bending= 690 cm^{-1}. **NMR Assignments (DMSO, ppm)** SO: 9.9063 δ (s, 1H, -NH), 6.8252-8.4215 δ (m, 9H phenyl), 3.6997 δ (s, 1H, -NH), 3.5221 δ (s, 1H, -OH), 2.4432 δ (q, 2H, CH_2), 2.2649 δ (t, 2H, CH_2), 2.0426 δ (m, 2H, CH_2).

2-Amino (4-N-phenylbutanamide)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (5j)

$C_{20}H_{25}N_4O_5$, Melting point: 208 °C, **IR Assignments:** NH-stretch= 3463 cm^{-1}, Aromatic C-H stretch=2924 cm^{-1}, C=O stretch= 1665 cm^{-1}, Aromatic C=C bending = 1479 cm^{-1}, C=N Imine stretch=1385 cm^{-1}, C-O-C ether stretch=1105 cm^{-1}, Aromatic C-H bending= 921 cm^{-1}. **NMR Assignments (DMSO, ppm)** TO: 8.1022 δ (s, 1H, -NH), 7.0131-7.391 δ (m, 7H phenyl), 4.2954 δ (s, 1H, -NH), 3.7621 δ (s, 9H, -O-CH_3), 2.569 δ (q, 2H, CH_2), 2.2114 δ (t, 2H, CH_2), 1.5857 δ (m, 2H, CH_2).

Biological Activity

Compounds were studied for various biological activities like anti-cancer activity, anti-bacterial activity and anti-fungal activity. Since oxadiazoles and thiadiazoles are privileged structures whose anti-bacterial and anti-fungal activity is a proven fact. Therefore they have been tested for anti-bacterial and anti-fungal activity.

MTT Assay

The anti-proliferative activities of the 2,5-Disubstituted 1,3,4-thiadiazole and 2,5-Disubstituted 1,3,4-oxadiazole compounds were assessed by the tetrazolium-based MTT assay. Human breast carcinoma MCF-7 cell line were cultured in DMEM medium supplied with 10% FBS. Cells were seeded in 96 well plates at the density of 5000, 8000, 8000, 12,000 and 6000 cells per well, respectively. Cancer cells were treated with respective compounds for 24 h and then incubated with 100 µL of 0.5 mg/mL 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution for 4 h. The supernatant was discarded and DMSO was added to each well. Absorbance at 570 nm was measured using a SpectraMax M2 reader (Molecular Devices, Sunnyvale, CA, USA). The number of viable cells in the control group was assigned a relative value of 100%.

Anti-Bacterial and Anti-Fungal Activity

Antibacterial and antifungal activity of newly synthesized compounds 5(a-f) were determined by 'Broth Dilution Method'. Main advantage of the 'Broth Dilution Method 'for MIC

determination lies in the fact that it can readily be converted to determine the MIC as well. For antibacterial activity Gram positive strain of *Staphylococcus aureus* and Gram negative strain of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* were used. For antifungal activity *Aspergillus niger* and *Candida albicans* were used.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37^{o}C overnight. The tubes are then incubated overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation [which represents the original inoculum] is compared[26,27].

RESULTS AND DISCUSSION

Molecular Docking

Extensive literature review was done by referring various literatures available in the forms of journal, books and chemical abstracts. This formed the starting point for the proposed research work, and the data collected from literature review was used in each step of the research work.

In view of the literature search, it was therefore decided to undertake the docking of some 2,5-Disubstituted-1,3,4-thiadiazole and 2,5-Disubstituted-1,3,4-oxadiazole of potential biological activity (Anti-cancer activity).

The preliminary docking studies of 300 molecules based on literature review, were carried out using the VLife MDS software. Docking study gave the preliminary information about the binding properties of the proposed compounds with HDAC as target. The interactions were found to be on same line with the HDAC.

Docking for the Scheme I and Scheme II compounds was done using the Biopredicta module of VLife MDS software. 5 compounds were synthesized for each scheme. The conformers of these compounds showed that each conformers, of which the conformer with least energy was selected for docking purpose. The HDAC II protein 3MAX was obtained from Protein Data Bank (PDB). The 3MAX was bound to N-(2-aminophenyl)benzamide. The protein was prepared for docking by extracting the N-(2-aminophenyl)benzamide and using it as a reference. The results obtained after docking studies gave the best molecules from the proposed series of compounds which shown below:

Table 1: Dock score of all the synthesized compounds

Sr. No	Dock Score	Sr. No	Dock score
5a	-67.3471	**5f**	-66.3632
5b	-61.6924	**5g**	-65.6496
5c	-59.9561	**5h**	-63.1975
5d	-47,3869	**5i**	-60. 7881
5e	-36.5493	**5j**	-57.3972

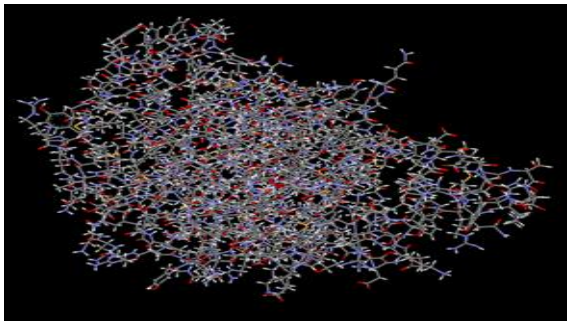


Figure 3: HDAC II protein 3MAX

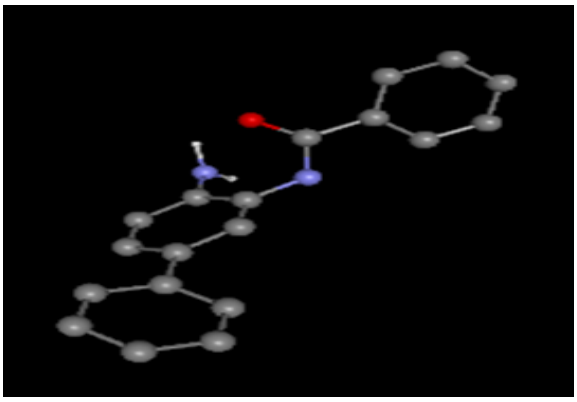


Figure 4: Reference ligand: N-(2-aminophenyl)benzamide

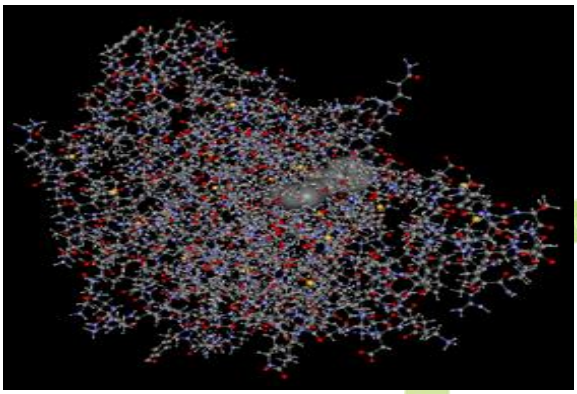


Figure 5: 3MAX with reference

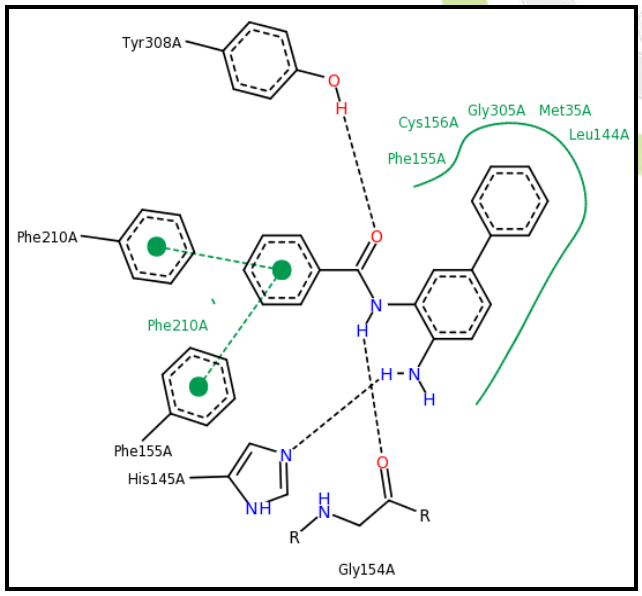


Figure 6: Interactions of the reference ligand with 3MAX

Figure 3 Indicates the HDAC II Protein 3MAX.

Figure 4 Indicates its ligand N-(2-aminophenyl)benzamide.

Figure 5 Indicates protein 3MAX complexed with co-crystalized ligand N-(2-aminophenyl)benzamide.

Figure 6 Indicates interactions of the reference ligand N-(2-aminophenyl)benzamide with protein 3MAX. (In Green it indicates Hydrophobic interactions and in Black Dash lines indicates Hydrogen bonding.)

Table 2: Interactions of all the synthesized compounds. Interactions in green are Hydrophobic interactions and in black are hydrogen bonding

Molecule	Interactions
Reference ligand	Phe155A, Phe210A, Met35A, Leu155A, Tyr308A, His145A, His144A.
SAHA	Phe210A, Met35A, Pro34A, Leu276A, Tyr308A, His145A, His144A.
Trichostatin-A	Gly154A,Phe155A,Phe210A, Met35A,Pro34A,Leu276A, Tyr308A, His145A, His144A, His183A
5a	Gly154A,Phe155A,Phe210A,Leu276A, His145,His183A
5b	Gly154A, Phe155A, Phe210A, Met35A, Pro34A, His145A, His144A.
5c	Gly154A, Phe155A, Phe210A, Leu144A, Leu276A, His145A.
5d	Gly154A,Phe155A,Phe210A,Leu276A,His145, His144A, His183A
5e	Gly154A,Phe155A,Phe210A, Met35A,Pro34A,Leu276A, Tyr308A, His145, His144
5f	Gly154A, Phe155A, Phe210A, Met35A, Pro34A, Leu276A, Tyr308A.

5g	Gly154A, Phe155A, Phe210A, Met35A, Pro34A, Leu276A, His145
5h	Gly154A, Phe155A, Phe210A, Leu276A, His146, His183A.
5i	Gly154A, Phe155A, Phe210A, Met35A, Pro34A, Leu276A, Tyr308A, His145A, His144A.
5j	Gly154A,Phe155A,Phe210A, Met35A,Pro34A, His145, His144

MTT Assay

MTT assay was performed to study its anti-cancer activity. Standard drug used was SAHA. None of the compounds were found to be in compliance with the standard drug SAHA. Its result is described in Table 3 and Figure 7.

Table 3: MTT assay results

Compound	Code	IC50 in µg/ml
5a	BT	>80
5b	DT	>80
5c	PT	>80
5d	ST	>80
5e	TT	>80
5f	BO	>80
5g	DO	>80
5h	PO	>80
5i	SO	>80
5j	TO	>80
SAHA	-	<10

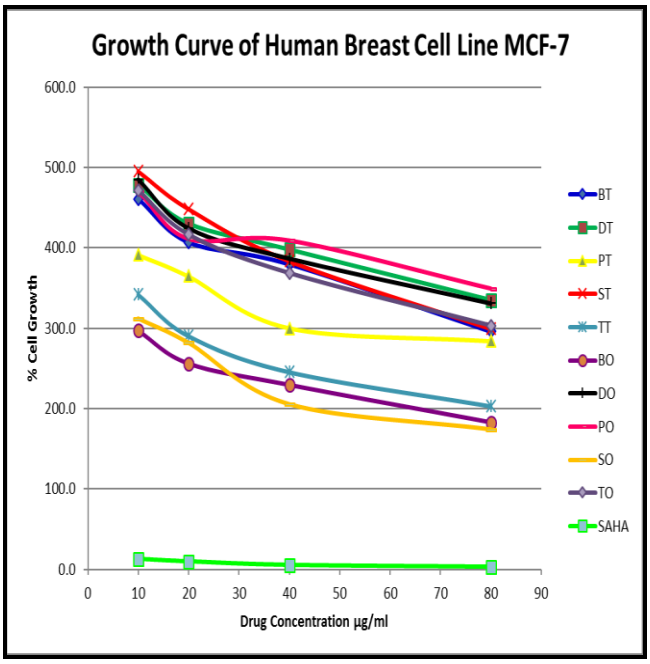


Figure 7: Graphical representation of MTT assay

Anti-bacterial and Antifungal Activity

For antibacterial activity Gram positive strain of *Staphylococcus aureus* and Gram negative strain of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were used. For antifungal activity *Aspergillus niger* and *Candida albicans* were used. Results of anti-bacterial and anti-fungal activity are mentioned in Table 4 and 5 respectively. Levofloxacin was used as the standard and has MIC values of <0.5 µg/ml against *Escherichia coli*, <0.5 µg/ml against *Staphylococcus aureus*, 64 µg/ml against *Klebsiella pneumoniae*, 32 µg/ml against *Acinetobacter baumannii* and <0.5 µg/ml against *Psedomonas aeruginosa*. The results indicated that all synthesized compounds were found to be less active as compared to the standard against Gram –ve bacteria. Compounds 5c, 5d and 5i showed some activity against gram +ve bacteria like *Staphylococcus aureus* with MIC value of 32 µg/ml. Fluconazole was used as a standard and has MIC values of 8 µg/ml against *Aspergillus niger* and 16 µg/ml against *Candida albicans* . The results indicated that all synthesized compounds were found to be less active as compared to the standard against *Aspergillus niger* and *Candida albicans* with MIC value >100 µg/ml.

Table 4: MIC value for Anti-bacterial activity

	MIC µg/ml				
Compound	**EC (*Escherichia coli*) Gram -ve**	**SA (*Staphylococcus aureus*) Gram +ve**	**KP (*Klebsiella pneumoniae*) Gram –ve**	**AB (*Acinetobacter baumannii*) Gram -ve**	**PA (*Psedomonas aeruginosa*) Gram -ve**
5a	>64	64	>64	>64	>64
5b	>64	>64	>64	>64	>64
5c	>64	32	>64	>64	>64
5d	>64	32	>64	>64	>64
5e	>64	>64	>64	>64	>64
5f	>64	>64	>64	>64	>64
5g	>64	>64	>64	>64	>64
5h	>64	>64	>64	>64	>64
5i	>64	32	>64	>64	>64
5j	>64	>64	>64	>64	>64
Levofloxacin	<0.5	<0.5	64	32	<0.5

Table 5: MIC value for Anti-fungal activity

	MIC µg/ml	
Compounds	***Aspergillus niger***	***Candida albicans***
5a	>100	>100
5b	>100	>100
5c	>100	>100
5d	>100	>100
5e	>100	>100
5f	>100	>100
5g	>100	>100
5h	>100	>100
5i	>100	>100
5j	>100	>100
Fluconazole	8	16

CONCLUSION

In this article, design, synthesis, and evaluation of newer 2,5-Disubstituted-1,3,4-thiadiazole (5a-e) and 2,5-Disubstituted-1,3,4-oxadiazole (5f-j) derivatives is reported, in an attempt to develop effective and safe anticancer agents. MTT assay was performed against Human Breast Cell Line MCF-7. They were further evaluated for anti-bacterial and anti-fungal activity. Thus, these newly synthesized compounds may serve as templates to study the 2,5-Disubstituted-1,3,4-thiadiazole and 2,5-Disubstituted-1,3,4-oxadiazole derivatives as novel anticancer agents.

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CONFLICT OF INTEREST

The authors confirm that this article's content has no conflicts of interest.

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