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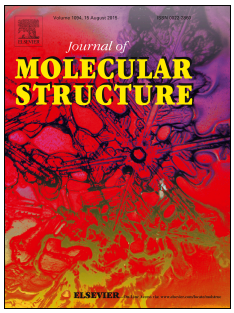

Synthesis, biological activities and docking studies of piperazine incorporated 1, 3, 4-oxadiazole derivatives

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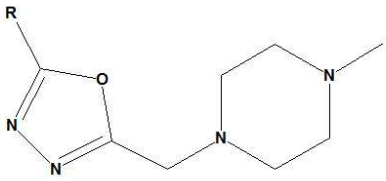
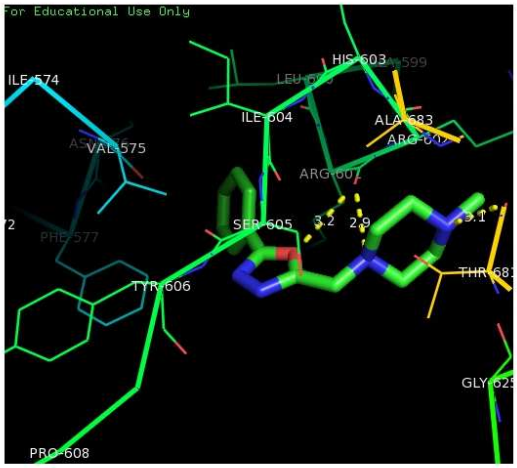
Graphical Abstract

R = C_6H_5 (**3a**),
4-ClC_6H_4 (**3b**),
4-OHC_6H_4 (**3c**),
4-$OCH_3C_6H_4$ (**3d**),
3-$NO_2C_6H_4$(**3e**)



MIC for Anti-tubular 1.0 to 50.0 µg/ml.
MIC for anti-microbial 12 to 200.0 µg/ml.
Antioxidant IC_{50} values were 62.91 µg/ml. (ascorbic acid) and 36.72 µg/ml (**3a**) .
In drug likeness model study compound **3b** possessed similar score as that of standard drug streptomycin.



Synthesis, biological activities and docking studies of piperazine incorporated 1, 3, 4-oxadiazole derivatives

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Abstract

New series of 1, 3, 4-oxadiazoles incorporating piperazine scaffolds in a single molecular framework has been reported. The structures of the synthesized derivatives were assigned by IR, NMR and mass spectral techniques. The hybrid compounds were evaluated for their antimicrobial, antitubercular and antioxidant activities. The observed MIC values of anti-tubular activities for the molecule **3a**, **3b**, **3c**, **3d** and **3e** were 6.25, 3.12, 3.12, 1.60 and 50.0 µg/ml respectively. As compared to ascorbic acid (IC_{50} = 62.91 µg/ml), molecule **3a** exhibited better antioxidant activities (IC_{50} = 36.72 µg/ml). Also, all molecules have shown significant antimicrobial activities. In addition, docking simulations were performed to study ligand-protein interactions and to determine the probable binding conformations. In drug likeness model study compound **3b** possessed maximum drug likeness model score (0.75) similar to the standard drug streptomycin. The compound **3a**, **3b** and **3c** were emerged as potential derivatives in the series and could serve as lead compound for the development of potential therapeutic agents.

Keywords: Antimicrobial; anti-tubercular; antioxidant; bioavailability; docking simulations.

1. Introduction

Antimicrobial resistant (AMR) and Tuberculosis (TB) are the global concern. The report from WHO's Global Antimicrobial Resistance Surveillance System (GLASS), 2016-17 shows that *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae* and *Salmonella species* are the most commonly reported resistant bacteria [1]. The report reveals widespread occurrence of antibiotic resistance. Emergence of antimicrobial resistance in microorganisms is a natural phenomenon and to combat antimicrobial resistance understanding of resistance mechanism, microorganism, antimicrobial drug, host, and context; parallel to new drug discovery, broad ranging, and multidisciplinary research is needed [2-5]. TB is a contagious disease caused by the bacterium *Mycobacterium tuberculosis*. Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat [6-7]. According to the WHO fact sheet on tuberculosis, In 2017, 10 million people fell ill with TB, and 1.6 million including 2, 30, 000 children died from the disease [8]. There is a critical need of design and development of novel chemotherapeutic agents to combat the emergence and increasing prevalence of resistant pathogens.

Heterocyclic compounds are key components for development of chemotherapeutic agents. Pyridine is the parent ring system of a large number of naturally occurring products and important industrial, pharmaceutical and agricultural chemicals [10]. Pyridine-based compounds have been playing a crucial role as agrochemicals or pesticides including fungicides, insecticides/ acaricides and herbicides etc. [11]. Presently there are many drugs available in the market like Pioglitazone HCl, Rosigitazone HCl, Isoniazid, Ethionamide, Prothionamide , Prothionamide, Nikethamide, etc., that have pyridine ring in their structure which is responsible for biological activities. Grombein and co-workers had presented 1-Phenylsulfinyl-3-(pyridin-3-yl) naphthalen-2-ols derivatives as potent aldosterone synthase

inhibitors [12]. Mohamed F.Abdel-Megeed and co-workers synthesized and evaluated antimicrobial activity of a series of diphenyl 1-(aryl amino)-1-(pyridin-3-yl) ethylphosphonates derivatives and found that the compounds showed high antimicrobial activity against selected bacterial and fungal strains at low concentrations (10–100 μg/mL). Also these compounds showed significant cytotoxicity anticancer activities against liver carcinoma cell line (HepG2) and human breast adenocarcinoma cell line (MCF7) [13].

Benzimidazole derivatives have been extensively studied in the field of medicinal chemistry due to their versatile and eminent biological profile. A large number of activities such as antimicrobial [14-16], anti-mycobacterial [17], analgesic [18], anticonvulsant [19] , antitumor [20] , antioxidant and anti-inflammatory [21], anti HIV activity [22], antiviral [23], antimalarial [24] and anti-hypertension activity [25] etc. had been displayed by compounds adjoining this heterocyclic system. The introduction of benzimidazole derivatives in triphenylamine-based organic dyes can improve their photovoltaic performance because of the extension of the π-conjugation structures in dye-sensitized solar cells [26]. Polybenzimidazole based nanocomposite membranes have also been reported as promising electrolyte used for high temperature proton exchange membrane fuel cells [27]. Lijun Tang and co-workers designed and synthesized benzimidazole-based fluorescent chemo sensor for Cu^{2+} and sulfide anion detection in water [28].

Oxadiazole rings are commonly employed as bioisosteric replacement for carbonyl-containing groups such as esters, amides, carbamates and hydroxamic esters and are comparatively more stable in biological media and hence they are frequently used motif in structure of drug candidates [29]. 1, 3, 4-oxadiazoles are nitrogen oxygen containing heterocyclic compounds that are known for numerous therapeutic effects and are recognized as important pharmacophore in drug discovery. 1, 3, 4-oxadiazole derivatives have occupied prominent place in medicinal chemistry and are known for their diverse range of biological

activities including antimicrobial [2-5,30,31], anti-mycobacterial [32] anti-inflammatory and analgesic [33], anticancer [34,35], anti-allergic [36],antioxidant [37] insecticidal [38], anticonvulsant [39] etc.

In the view of the facts mentioned above and in continuation to our research work, in present report, the synthesis and characterization of some novel heterocyclic compounds containing piperazine moiety has been carried out. The synthesized molecules were evaluated for their antimicrobial, anti-tubercular and antioxidant potential. Further, molecular docking studies were also carried out to understand binding interactions of ligands and selected protein targets.

2. Experimental

2.1. Chemicals and Reagents

All chemicals and reagents used in current study were of analytical grade and were used without further purification. Melting points were determined in open capillary using melting point apparatus and are uncorrected. The IR spectra of all compounds were recorded on a Perkin–Elmer FTIR spectrophotometer in KBr. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer using DMSO as a solvent and TMS as an internal standard. Chemical shifts were reported in parts per million (ppm). Mass spectra were recorded on JEOL SX 102/DA-6000 mass spectrometer data system using argon/Xenon (6 KV, 10 MA) as FAB gas. All the reactions were monitored by thin-layer chromatography (TLC) using silica gel glass plates.

2.1.1. Synthesis of Ethyl (4-methyl piperazin-1-yl) acetate (**1**)

1-methyl piperazine (0.01 mol), ethyl chloroacetate (0.02 mol), anhydrous potassium carbonate (2 g) were refluxed in 50 ml of dry acetone for 18 h. The inorganic solid was

filtered off and the filtrate was concentrated under reduced pressure. Completion of the reaction was monitored by TLC using mobile phase ethyl acetate/ hexane (6:4). Yield 80%, m.p. 88–92 ˚C, IR (KBr) cm^{-1}: 2978 (C-H str), 1720 (C=O), 1275 (C–O); ^{1}H-NMR (DMSO) □ppm: 1.15 (t, 3H, Ester- CH_3), 4.12 (q, 2H, Ester-CH_2), 1.071 (t, 4H, piperazine proton), 1.232 (s, 3H, N-CH_3 piperazine), 2.502 (dd, 4H, piperazine proton), 3.486 (s, 2H, N-CH_2); EI-MS: 186.14 (M^+); Anal calcd for $C_9H_{18}N_2O_2$: C, 58.04; H, 9.74; N, 15.04; O, 17.18; Found: C, 58.06; H, 9.72; N, 15.03; O, 17.17.

2.1.2. Synthesis of 2-(4-methyl piperazin-1-yl) acetohydrazide (**2**)

Ethyl (4-methyl piperazin-1-yl) acetate (0.01mol) and hydrazine hydrate (98%) (0.06 mol) were refluxed in 20 ml ethanol for 10 h. Completion of the reaction was monitored by TLC using mobile phase ethyl acetate/ hexane (8:4). After completion of the reaction, the mixture was cooled and kept in refrigerator for overnight. The solid so obtained was filtered, washed with cold water and recrystallized from methanol. Yield 70%; m.p.178–180 ˚C, IR (KBr) cm^{-1}: 3068.42 (-CONH), 2847.16 (C-H str.), 1687.35 (C=O) ; ^{1}H-NMR (DMSO) □ppm: 1.061 (t, 4H, piperazine proton), 1.238 (s, 3H, N-CH_3 piperazine), 2.507 (dd, 4H, piperazine proton), 3.47 (broad singlet, 2H, -NH_2), 8.167 (s,1H, CONH) ; EI-MS: 172.13 (M^+); Anal calcd for $C_7H_{16}N_4O$:C, 48.82; H, 9.36; N, 32.53; O, 9.29; Found: C, 48.80; H,9.30; N, 32.50; O, 9.31.

2.1.3. General procedure for the synthesis of compounds (**3a**-**3e**)

Equimolar amount of 2-(4-methyl piperazin-1-yl) acetohydrazide (0.005 mol) and suitable aromatic acid (0.005) were refluxed on a sand bath in the presence of cyclodehydration agent $POCl_3$ (10 ml) for 8-10 h. After completion of reaction, the mixture was cooled at room temperature and poured onto crushed ice. On basification with sodium bicarbonate (10%), a

solid mass was separated out which was filtered, dried and recrystallized from ethanol to give the target compounds **3a**-**3e**.

2.1.3.1. Synthesis of 1-methyl-4-[(5-phenyl-1, 3, 4-oxadiazol-2-yl)methyl] piperazine (**3a**). Yield 70%; m.p. 190 ˚C , IR (KBr) cm^{-1}: 3570.06 (CH arom), 2925.62 (CH aliph), 1660.13 (C=N), 1260.77 (C-O-C asymm), 1170.12 (C-N piperazine), 1105.17 (C–O–C_{symm}), 1022.94 (N–N), 925.92, 837.12 (CH); ^{1}H-NMR (DMSO) □ppm: 1.067 (t, 4H, piperazine proton), 1.236 (s, 3H, N-CH_3 piperazine), 2.502 (dd, 4H, piperazine proton), 3.427 (dd, 4H, piperazine proton), 4.27 (s, 2H, N-CH_2), 6.98-7.88 (m, 5H, Ar-H); EI-MS: 258.14 (M^+); Anal calcd for $C_{14}H_{18}N_4O$: C, 65.09; H, 7.02; N, 21.69; O, 6.19; Found: C, 65.07; H, 7.03;N, 21.66; O, 6.18.

2.1.3.2. Synthesis of 1-methyl-4-{[(5-(4-chloro phenyl)-1, 3, 4-oxadiazol-2-yl)methyl] piperazine (**3b**). Yield 50%; m.p. 220 ˚C , IR (KBr) cm^{-1}: 3580.07 (CH arom), 2921.25 (CH aliph) , 1174.33 (C-N piperazine), 1680.08 (C=N), 1282.74 (C-O-C asymm),1090.3 (C–O–Csymm), 1013.28 (N–N), 926.23, 849.54 (CH),759.15 (C-Cl); ^{1}H-NMR (DMSO) □ppm: 1.060 (t, 4H, piperazine proton), 1.235 (s, 3H, N-CH_3, piperazine), 2.507 (dd, 4H, piperazine proton), 3.437 (dd, 4H, piperazine proton), 4.28 (s, 2H, N-CH_2), 7.560-8.179 (m, 4H, aromatic); EI-MS: 292.11 (M^+); Anal calcd for $C_{14}H_{17}N_4OCl$: C, 57.44; H, 5.85; N, 19.14; O, 5.46; Found: C, 57.45; H, 5.87;N, 19.12; O, 5.47.

2.1.3.3. Synthesis of 1-methyl-4-{[(5-(4-hydroxy phenyl)-1, 3, 4-oxadiazol-2-yl)methyl] piperazine (**3c**). Yield 40%; m.p. 195 ˚C , IR (KBr) cm^{-1}: 3654.25 (OH), 3573.07 (CH arom), 2923.42 (CH aliph), 1162.20 (C-N piperazine), 1672.75 (C=N), 1272.77 (C-O-C asymm),1082.17 (C–O–C_{symm}), 1012.94 (N–N), 925.92, 837.12 (CH);^{1}H-NMR (DMSO)□

ppm: 1.056 (t, 4H, piperazine proton), 1.192 (s, 3H, N-CH_3, piperazine), 2.502 (dd, 4H, piperazine proton), 3.42 (dd, 4H, piperazine proton), 4.31 (s, 2H, N-CH_2), 6.96-7.99 (m, 4H, aromatic), 9.64 (s, 1H, Ar-OH); EI-MS: 274.14 (M^+); Anal calcd for $C_{14}H_{18}N_4O_2$: C, 61.30; H, 6.61; N, 20.42; O11.66; Found: C, 61.28; H, 6.60;N, 20.44; O, 11.67.

2.1.3.4. Synthesis of 1-{[(5-(4-methoxy phenyl)-1, 3, 4-oxadiazol-2-yl]methyl}-4-methyl piperazine (**3d**). Yield 60%; m.p. 204 ˚C , IR (KBr) cm^{-1}: 3583.06 (CH arom), 2920.82 (CH aliph), 1172.55 (C-N piperazine), 1682.75 (C=N), 1260.77 (C-O-C asymm),1105.17 (C–O–C_{symm}), 1022.94 (N–N), 925.92, 837.12 (CH);^{1}H-NMR (DMSO) □ppm: 1.058 (t, 4H, piperazine proton), 1.196 (s, 3H, N-CH_3 piperazine), 2.506 (dd, 4H, piperazine proton), 3.437 (dd, 4H, piperazine proton), 3.818 (s,3H,Ar-OCH_3), 4.29 (s, 2H, N-CH_2), 6.99-8.04 (m, 4H, aromatic); ^{13}C-NMR (DMSO) □ppm: 39.336, 39.545, 39.754, 39.962, 40.171, 55.968, 114.25, 115.285, 128.856, 162.44, 163.29, 163.94; EI-MS: 288.15 (M^+); Anal calcd for $C_{15}H_{20}N_4O_2$: C, 62.48; H, 6.99; N, 19.43; O, 11.10; Found: C, 62.49; H, 6.96; N, 19.42; O, 11.11.

2.1.3.5. Synthesis of 1-methyl-4-{[(5-(3-nitro phenyl)-1, 3, 4-oxadiazol-2-yl)methyl] piperazine (**3e**). Yield 30%; m.p. 210 ˚C , IR (KBr) cm^{-1}: 3588.06 (CH arom), 2922.84 (CH aliph), 1175.11 (C-N piperazine), 1692.05 (C=N), 1260.77 (C-O-C asymm),1125.17 (C–O–C_{symm}), 1517.98 (N=O asymm), 1317.38 (N=O symm),1064.12 (C–O–C_{symm}), 1025.12 (N–N), 940.67, 742.12 (CH); ^{1}H-NMR (DMSO)□ ppm: 1.053 (t, 4H, piperazine proton), 1.192 (s, 3H, N-CH_3 piperazine), 2.502 (dd, 4H, piperazine proton), 3.427 (dd, 4H, piperazine proton), 4.37 (s, 2H, N-CH_2), 7.56-8.82 (m, 4H, aromatic); EI-MS: 303.13 (M^+); Anal calcd for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09; O, 15.82; Found: C, 55.46; H, 5.64;N, 23.08; O, 15.80.

2.2. Prediction of Molecular Properties Descriptors

Prediction of molecular properties descriptors is significant in the discovery and development of potential therapeutics. In the present study Molinspiration program [40] was used for calculating molecular properties such as milogP (partition coefficient), TPSA (topological polar surface area), MW (molecular weight), nON (number of hydrogen bond acceptors), nOHNH (number of hydrogen bond donors), Nrotb (number of rotatable bonds), Nvio (Number of violations), Natoms (range of atoms) and Volume. The bioactivity scores of GPCR ligand, ion channel modulator, nuclear receptor ligand, kinase inhibitor, protease inhibitor and enzyme inhibitor were also calculated using molinspiration server.

2.3. Antimicrobial Activity

The synthesized compounds **3a-3e** were screened *in vitro* for their antibacterial activity against four bacterial strains i.e. *Pseudomonas aeruginosa* (MTCC 4673), *Staphylococcus aureus* (MTCC 7443), *Salmonella enterica* (MTCC 164), *Streptococcus mutans* (MTCC 497) and one fungal strain *A parasiticus* (MTCC 646) by following standard literature reported procedure of disk-diffusion method [41-45]. The cultures used in this experiment were ordered from MTCC Chandigarh (India). The media used for this purpose are nutrient agar media and potato dextrose media. 28 grams of nutrient agar and 39.0 grams of Potato dextrose agar was added to the 1 liter of double distilled water separately, it was mixed thoroughly and pH was adjusted at 7.5 ± 0.2. The solution was heated to dissolve the ingredients completely after which the media was autoclaved at 121˚C for 45 minutes at 15lbs pressure. After autoclaving, 15-20 ml of that media was poured into petridish for studying antimicrobial activities. Whattman no. 1 filter paper disks were sterilized by autoclaving at 160˚C for 1 h. Then the sterile disks were impregnated with the test compounds of different concentrations (50 ppm, 250 ppm and 500 ppm). Cultures having 10^5

CFU/mL were used against each concentration levels. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 37˚C for 24 h for bacterial strains and 48 h for fungal species. Streptomycin and ketoconazole were used as standard drug for antibacterial and anti fungal activity respectively. After incubation, the degree of inhibition was observed by formation of zones. The diameters of the zones were measured in mm.

2.4. Antitubercular Activity

The antitubercular activity of compounds **3a-3e** was assessed against *Mycobacteria tuberculosis* (Vaccine strain, H37 RV strain, ATCC-27294) using Microplate Alamar Blue Assay (MABA) [46,47]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. 200 µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. Pyrazinamide, Ciprofloxacin and Streptomycin were used as standard drugs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

2.5. Antioxidant Activity

The synthesized compounds **3a-3e** were evaluated for their antioxidant activity by using DPPH (2, 2-diphenyl-1-picrylhydrazyl), free radical scavenging method according to the reported method with slight modifications [48,49]. Briefly, 1 mg/ml solutions of compounds and ascorbic acid were prepared by dissolving them into DMSO. 25, 50, 75 and 100 µL of each was added separately to 10.0 mL amber color volumetric flasks containing 2.0 ml of 0.01mM DPPH (prepared in ethanol). The final volume was made up to 3.0 ml and after 30 minutes absorbance was checked at 517 nm by using UV-visible spectrophotometer. Pure DPPH solution (0.01mM) was used as a control and ethanol was as a blank. The decrease of in absorbance equates the DPPH radical scavenging capacity. The above process was repeated three times for ascorbic acid (positive control) and compounds. The radical scavenging ability was calculated according to the formula: Radical scavenging activity = $(A_0 - A_T / A_0) \times 100$; where, A_0 is the absorbance of pure DPPH solution (0.01mM), and A_T is the absorbance of (DPPH) and compounds. The IC50 was defined as half maximal inhibitory concentration of the compounds that inhibit DPPH radical.

2.6. Docking Simulations

The molecular docking simulations were performed to analyze drug-target interactions of the synthesized compounds using the Autodock 4.2 software [50]. The chemical structures of the compounds were drawn and their 3D structures were optimized in the ACD Labs ChemSketch 12.0 software. Docking study of antimicrobial activity was carried out against three different protein targets i.e. Isocitrate lyase (*P. aeruginosa*, PDB ID: 6g1o), Dihydrofolate reductase (*S. aureus*, PDB ID: 5isp) and Noranthrone synthase (*A. parasiticus*, PDB ID: 5kbz). Methionine aminopeptidase (*M. Tubeculosis*, PDB ID: 5yxf) and tyrosine-protein kinase (Homosapiens, PDB ID: 6il3) were selected for *in-silico* docking simulations

of antitubercular and antioxidant activity respectively of the synthesized compounds. The X-ray crystallographic structure of the target proteins were retrieved from the protein data bank [51] . Prior to the simulations all bound ligands, cofactors, and water molecules were removed from the proteins. Gasteiger charges were computed, and the Auto Dock atom types were defined using Auto Dock version 4.2. The three dimensional grid boxes were created, the spacing between grid points was 0.375 angstroms, and the grid maps representing the intact ligand in the actual docking target site were calculated with Auto Grid algorithm. Finally, Auto Dock was used to calculate the binding free energy of a given inhibitor conformation in the macromolecular structure. Lamarckian Genetic Algorithm (LGA) parameters were set to default settings, which includes 150 runs, 150 conformational possibilities, 50 populations and 2,50,0000 energy evaluations. During the docking process, a maximum of 10 conformers was considered for each compound. The results were evaluated based on the binding compatibility i.e. binding energy in kcal/mol and inhibition constant. The resultant protein-ligand complex structures were explored using Pymol program [52].

3. Results and Discussion

3.1. Chemistry

The synthesis protocol of the title compounds (**3a**-**3e**) was outlined in Scheme – 1 and Supplementary Data S1-S8. First, Ethyl (4-methyl piperazin-1-yl) acetate (**1**) was synthesized via the reaction of 1-methyl piperazine with ethyl chloro-acetate in the presence of potassium carbonate. In the next step compound (**1**) was refluxed with hydrazine hydrate in ethanol to obtain 2-(4-methyl piperazin-1-yl) aceto-hydrazide (**2**). Finally the cyclization reaction of compound (**2**) with various aromatic carboxylic acids in the presence of $POCl_3$ gave the titled compounds (**3a-3e**). The structure of the synthesized compounds was enlightened by FTIR, ^1H NMR, C^{13} NMR and mass spectral analysis.

In the IR spectra of compound (**1**), characteristic stretching vibration at 1720 cm^{-1} was observed, which confirmed the presence of carbonyl group as a part of an ester linkage. Subsequent reaction with hydrazine hydrate resulted in the formation of compound (**2**) which gave a slightly lower frequency signal at 1687.35 cm^{-1} and a new frequency mode at 3068.42 cm^{-1} supported aceto-hydrazide formation. The aceto-hydrazide when further treated with aromatic acids in $POCl_3$ underwent cyclisation yielding compounds **3a**-**3e**. The characteristic C=N band (1660–1692 cm^{-1}), C-O-C bands (1260-1282 cm^{-1} and 1105-1090 cm^{-1}) and N-N bands (1012-1517 cm^{-1}) of medium-strong intensity were identified in each IR spectra; indicating the formation of an oxadiazole ring. The ^{1}H NMR spectra of compound (**1**) gave a triplet at δ 1.15 and a quartet at δ 4.12 integrating for three and two proton respectively. Such a pattern is indicative of the fact that an ethyl group is present. A downfield signal at δ 3.486 was observed integrating for two protons confirmed the presence of a methylene group. ^{1}H NMR spectra of compound (**2**), an up field signal at δ 8.167 and a broad singlet at 3.47 accounting for one and two protons respectively , were characterized as –CONH and $–NH_2$ group. In the ^{1}H NMR spectra of the compounds **3d** a singlet at δ 4.29 and δ 3.80 integrating for two and three protons respectively were identified as methylene group and methoxy group. The other aliphatic and aromatic protons were observed at the expected regions. Further the mass spectrum of **3d** showed a (M^+) peak at m/z 288.15; (100%), which is in agreement with its molecular formula $C_{15}H_{20}N_4O_2$. ^{13}C NMR data also supported the structures of the compound **3d**.

3.2 Molecular properties descriptors

The drug likeness of the synthesized compounds (**3a**-**3e**) was estimated by calculating molecular descriptors using Molinspiration tool and results were presented in Table - 1. Lipinski's rule of five [53] predicts the molecular properties related to the pharmacokinetics

of molecules. According to this rule, the compound that have miLogP<5, molecular weight <500, the number of hydrogen bond acceptors<10 and number of hydrogen bond donors<5, possess good oral bioavailability. The compounds were in accordance with Lipinski's rule of five showing good drug likeness for drug targets with no violations. The bioactivity scores of the compound **3a**-**3e** were also predicted by calculating the activity score of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor and enzyme inhibitor by Molinspiration and were presented in Table - 2. A molecule having bioactivity score more than 0.00, it is presumed to be active, while values -0.50 to 0.00 are expected to be moderately active and if score is less than -0.50, it is considered to be inactive [40,41]. The compounds were moderately active against GPCR ligand, ion channel modulator, Kinase inhibitor, nuclear receptor ligand, protease inhibitor and Enzyme inhibitor. Compound **3c** was found to be active against kinase inhibitor with the bioactivity scores of 0.03. None of the compound was inactive against the selected drug targets. Drug likeness model scores were computed for all the compounds by molsoft software. The observed scores for the molecule **3a**, **3b**, **3c**, **3d**, **3e**, Streptomycin, Ketoconazole and Ascorbic acid were 0.37, 0.75, 0.68, 0.54, 0.21 0.75, 1.32 and 0.84 respectively. Compound **3b** possessed maximum drug likeness model score (0.75) similar to the standard drug streptomycin, followed by compound **3c** (0.68) in the series. Figure - 1 depicts graphical representation of the drug likeness model scores of compound **3b** and **3c**. The green color indicates non drug-like behavior and those fall under blue color are considered as drug-like.

Prediction of ADMET properties was done using admetSAR tool and the calculated ADMET parameters were illustrated in Table - 3. The results indicated that all the synthesized compounds **3a**-**3e** could be absorbed through intestinal tract when administrated orally. The compounds showed penetration across Blood-Brain Barrier (BBB) and also possessed Caco-2 Permeability (except compound **3e**). In terms of metabolism, compound

3a, **3b** and **3c** showed low CYP Inhibitory Promiscuity whereas compound **3d** and **3e** were found to have high CYP Inhibitory Promiscuity. The AMES toxicity test results revealed that except compound **3e**, the derivatives were found to be nontoxic which means that these compounds were non-mutagenic. The synthesized compounds **3a**-**3e** were non carcinogenic similar to the standard drugs. The computed LD_{50} doses of the synthesized compounds in rat model were found between the range 2.5899 to 2.8526 mol/Kg. Compound 3c was found to be most lethal in the series showing lowest LD_{50} dose value i.e. 2.5899 mol/Kg.

3.3. Antimicrobial Activity

The synthesized compounds (**3a-3e**) were screened for their *in-vitro* antibacterial activity against two gram negative bacterial strains i.e. *Pseudomonas aeruginosa, Salmonella enteri*ca and two gram positive bacterial strain i.e. *Staphylococcus aureus*, *Streptococcus mutans* by taking Streptomycin as standard drug while *in vitro* antifungal activity was carried out against a fungal strain *Aspergillus parasiticus* by taking Ketoconazole as standard drug at concentration 50, 250 and 500 ppm by disk diffusion method. The results of zone of inhibition measured for antibacterial and fungal activity were presented in Table - 4 and Supplementary Data S9&S10. The majority of the compounds were found to be active against selected bacterial and fungal strains. Maximum degree of inhibition was observed at 500 ppm concentration. All the molecules have shown better anti-bacterial activities. Compound **3b** has shown prominent activities against all bacterial strains. The results of antifungal activity revealed that compounds **3a** and **3b** (**3a<3b**) were found to be active against *Aspergillus parasiticus* while compound **3c, 3d** and **3e** were inactive.

The minimum inhibitory concentration (MIC) values of all compounds were evaluated and reported under Table – 3. The MIC values were ranged over 12.5 to 200 mg/l. As compared to the standards, molecules **3a** and **3b** have shown prominent antimicrobial

activities over ranged from 12.5 to 25 mg/l (Table – 3). The results of antimicrobial screening revealed that the compounds showed significant activity in comparison of standard drugs. Compounds **3a** and **3b** emerged as potent antimicrobial derivative in the series against selected bacterial and fungal strains.

3.4. Antitubercular Activity

The synthesized compounds **3a-3e** was screened for their *in vitro* antitubercular activity against *Mycobacteria tuberculosis* at concentration ranging from 0.8-100 µg/ml (Table – 5 and Supplementary Data S11) by using Micro-plate Alamar Blue assay (MABA). Pyrazinamide, Ciprofloxacin and Streptomycin were used as reference drugs. The compounds showed significant activity compared to the standard drugs. The minimum inhibitory concentration (MIC) was determined as lowest drug concentration which prevented the color change from blue to pink. The results indicated that compound **3d** having methoxy group at phenyl ring of oxadiazole ring was emerged as most effective analog with good antitubercular activity (MIC value 1.6 µg/ ml) in the series. Besides, Compound **3b** and **3c** showed MIC value 3.12 µg/ ml while compound **3a** demonstrated MIC value 6.25 µg/ ml. A high value of MIC i.e. 50 µg/ ml was observed for compound **3e**.

3.5. Antioxidant Activity

The *in vitro* antioxidant activity of the compound **3a-3e** was determined spectrophotometrically by DPPH free radical scavenging method at concentration ranging from 25 µL to 100 µL and the results were summarized in Table - 6. Ascorbic acid was used as positive control. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The compounds showed moderate scavenging activity on DPPH compared to the standard. It was observed that

antioxidant potential of the compounds increased with increase in the concentration. The compounds showed significant scavenging activity on DPPH compared to the standard. It was observed that antioxidant potential of the compounds increased with increase in the concentration. Compound **3a** appeared to be the most potent derivative in the series with IC_{50} value of 36.76 µg/ml followed by compound **3b** with IC_{50} value of 52.42 µg/ml. Compound **3a** and **3b** showed better activity compared to the standard drug ascorbic acid which showed IC_{50} value of 62.91 µg/ml

3.6. Docking Simulations

Docking is a computational simulation approach which is used to determine the affinity of binding of a small molecule to a receptor molecule to form a stable complex. To support results obtained from *in vitro* antimicrobial, antitubercular and antioxidant activities, it was thought worth-while to conduct *in sillico* docking study. AutoDock 4.2, the docking program was employed to estimate binding affinity of ligand-receptor complex in the receptor binding site. The binding energy values were calculated based on the total intermolecular energies (Kcal/mol) including hydrogen bond energy, Van Der Waals energy, desolvation energy and electrostatic energy. The receptor was kept rigid, while ligands were set flexible to rotate and explore most probable binding poses. For docking study of antibacterial activity all the synthesized derivatives **3a-3e** were docked with ATP-binding casette(ABC) transporter ComA protein (PDB ID: 3vx4). Compound **3e** showed minimum binding energy (-7.81 Kcal/mol, Ki; 1.87 µM), followed by compound **3a** (-7.51 Kcal/mol, Ki; 3.14 µM). Protein target Aflatoxin biosynthesis polyketide synthase (PDB ID: 3ils) was selected for docking study of antifungal activity. It was observed that compound **3e** showed strong affinity towards selected protein target with binding energy, - 8.31 Kcal/mol in the series. The results of docking study of antimicrobial activity were presented in Table -7. Docking studies

revealed that the synthesized compounds **3a-3e** showed good binding energy towards selected protein targets compared to the standard drugs. The derivatives **3a-3e** were also subjected to three dimensional molecular docking on crystal structure of enoyl-acyl carrier protein (EACP) reductase enzyme (PDB ID: 2nsd) and Protein-tyrosine kinase 2-β (PDB ID: 5tob) for Docking simulation of antitubercular activity and anti-oxidant activity. The results of docking of antitubercular and antioxidant activities were summarized in Table - 8. The results suggested that the derivatives **3a-3e** showed better binding energy compared to the standard drugs against the selected protein targets. Compound **3e** (-9.22 Kcal/ mol, Ki-173.83 nM), 3b (-8.44 Kcal/ mol, Ki-652.56 nM) and **3a** (-8.24 Kcal/ mol, Ki-905.77 nM) were best ligands in the series in terms of binding energy and inhibition constant against protein target 2nsd. Docking results of antioxidant activity revealed that compound **3e** (-7.49 Kcal/mol, Ki-3.23 µM), **3b** (-7.35 Kcal/mol, Ki-4.08 µM) and **3c** (-7.24 Kcal/mol, Ki-4.97 µM) showed strong affinity with protein target 5tob in the series. From the *in silico* docking simulations it is quite evident that all the synthesized compounds **3a-3e** had great potential as antimicrobial, antitubercular and antioxidant agents. Figure – 2 & 3 depicts binding mode of best conformer of compound **3a**, **3b** and **3c** against selected protein targets for docking studies.

4. Conclusion

Herein we report the synthesis of 1-methyl-4-[(5-phenyl/substituted phenyl-1, 3, 4-oxadiazol-2-yl) methyl] piperazine derivatives (**3a-3e**). The structures of the synthesized compounds were confirmed by IR, ^{1}HNMR, ^{13}C NMR and mass spectral techniques. The synthetic compounds were evaluated for their *in vitro* antimicrobial, antitubercular and antioxidant potential. The derivatives showed remarkable activity compared to the standard drugs. The derivatives were in accordance with Lipinski's rule of five and had good bioavailability. Docking simulations were performed to gain an insight into the binding modes of the

synthesized derivatives and good binding affinity was recorded against selected protein targets. A good correlation was observed between the experimental results and theoretical predictions. Among the synthesized compounds, 1-methyl-4-[(5-phenyl-1,3,4-oxadiazol-2-yl)methyl] piperazine (**3a**), 1-methyl-4-{[(5-(4-chloro phenyl)-1,3,4-oxadiazol-2-yl)methyl]piperazine (**3b**) and 1-methyl-4-{[(5-(4-hydroxy phenyl)-1,3,4-oxadiazol-2-yl)methyl]piperazine (**3c**) emerged as promising lead compound in the series and can be consider for further *in vivo* validation. Structural modification of these compounds could possibly bring more potent compounds.

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Captions of Tables, Schemes and Figures

Table - 1: Prediction of molecular properties descriptors of the titled compounds (**3a-3e**).

Table - 2: Prediction of bioactivity score of the titled compounds (**3a-3e**).

Table - 3: Prediction of ADMET profile of the title compounds **(3a-3e).**

Table - 4: Antimicrobial activities of the synthesized compounds (**3a-3e**) with Minimum inhibitory concentration MIC mg/l (MBC/MFC) values.

Table - 5: Anti-tubercular activity of the synthesized compounds (**3a-3e**).

Table - 6: Antioxidant activity of the synthesized compounds (**3a-3e**).

Table - 7: Docking simulations for antimicrobial activity of the synthesized compounds (**3a-3e**).

Table - 8: Docking simulations for antitubercular and antioxidant activity of the synthesized compounds (**3a-3e**).

Scheme - 1: Synthetic route of compounds from **1** to **3** including **3a** to **3e**.

Figure - 1: Graph showing drug-likeness model score of compound 3b (**a**) and 3c (**b**) respectively.

Figure - 2: Binding modes of compound **3a** with protein targets 3vx4 (**a**), 3ils (**b**), 2nsd (**c**) and 5tob (**d**) respectively visualized using Pymol software.

Figure - 3: Binding modes of compound **3b** with protein targets 3vx4 (**a**), 3ils (**b**), 2nsd (**c**) and 5tob (**d**) respectively visualized using Pymol software.

Table - 1: Prediction of molecular properties descriptors of the titled compounds (**3a**-**3e**).

Comp.	miLogP	TPSA	MW (mol/g)	n-ON	n-OH or n-NH	N_{rotb}	N_{vio}	Volume	N_{atom}
3a	1.06	45.40	258.32	5	0	3	0	244.00	19
3b	1.73	45.40	292.77	5	0	3	0	257.54	20
3c	0.58	65.63	274.32	6	1	3	0	252.02	20
3d	1.11	54.63	288.35	6	0	4	0	269.55	21
3e	0.99	91.22	303.32	8	0	4	0	267.34	22
X	-5.35	336.45	581.58	19	16	9	3	497.25	40
Y	3.77	69.08	531.44	8	0	7	1	452.47	36
Z	-1.40	107.22	176.12	6	4	2	0	139.71	12

Here, X = Streptomycin, Y = Ketoconazole, Z = Ascorbic acid, logarithm of compound partition coefficient between n-octanol and water (miLogP), topological polar surface area (TPSA), percentage of absorption (% ABS), molecular weight (MW), number of hydrogen bond acceptors (n-ON), number of hydrogen bond donors (nOH/NH), number of rotatable bonds (Nrotb), Number of violations (Nvio).

Table - 2: Prediction of bioactivity score of the titled compounds (**3a**-**3e**).

Comp.	GPCR	ICM	KI	NRL	PI	EI
3a	-0.20	-0.25	-0.07	-0.73	-0.29	-0.16
3b	-0.15	-0.25	-0.05	-0.67	-0.29	-0.18
3c	-0.09	-0.19	0.03	-0.47	-0.22	-0.07
3d	-0.16	-0.32	-0.03	-0.59	-0.25	-0.19
3e	-0.25	-0.30	-0.10	-0.64	-0.31	-0.24
X	0.09	-0.16	-0.17	-0.18	0.65	0.38
Y	0.26	-0.14	-0.17	-0.32	-0.21	0.15
Z	-0.53	-0.24	-1.09	-1.01	-0.81	0.20

Here, X = Streptomycin, Y = Ketoconazole, Z = Ascorbic acid, Ligand (GPCR, GPCR), Ion channel modulator (ICM), Kinase inhibitor (KI), Nuclear receptor ligand (NRL), Protease inhibitor (PI), Enzyme inhibitor (EI).

Table - 3: Prediction of ADMET profile of the title compounds (**3a-3e**)

Compd.	(BBB)	(HIA)	CaP	CIP	AT	C	LD_{50} (mol/Kg)
3a	BBB^+	HIA^+	$Caco^{2+}$	Low	No	No	2.8526
3b	BBB^+	HIA^+	$Caco^{2+}$	Low	No	No	2.8073
3c	BBB^+	HIA^+	$Caco^{2+}$	Low	No	No	2.5899
3d	BBB^+	HIA^+	$Caco^{2+}$	High	No	No	2.6038
3e	BBB^+	HIA^+	$Caco^{2-}$	High	Toxic	No	2.6791
X	BBB^-	HIA^-	$Caco^{2-}$	Low	Toxic	No	1.8409
Y	BBB^+	HIA^+	$Caco^{2+}$	High	No	No	3.4739
Z	BBB^+	HIA^+	$Caco^{2-}$	Low	No	No	1.3059

Here, X = Streptomycin, Y = Ketoconazole, Z = Ascorbic acid, Blood-Brain Barrier (BBB), Human Intestinal Absorption (HIA), Caco-2 Permeability (CaP), CYP Inhibitory Promiscuity(CIP), AMES toxicity (AT), Carcinogenicity (C), Rat Acute Toxicity LD_{50} mol/Kg (LD_{50}).

Table - 4: Antimicrobial activities of the synthesized compounds (**3a**-**3e**) with Minimum inhibitory concentration MIC mg/l (MBC/MFC) values.

Strain	**Conc.**	Inhibition zone (mm)[a]						
	(mg/l)	**3a**	**3b**	**3c**	**3d**	**3e**	**X**	**Y**
P. aeruginosa (MTCC 4673)[b]	50	7	8	6	7	7	11	NA
	250	9	15	6	8	13	15	NA
	500	15	19	15	15	17	34	NA
S. aureus (MTCC 7443)[b]	50	6	9	9	6	6	8	NA
	250	7	15	11	8	8	16	NA
	500	10	25	17	10	10	28	NA
S. enterica (MTCC 164)[b]	50	7	7	7	ND	ND	8	NA
	250	8	13	9	7	7	11	NA
	500	10	18	20	13	10	30	NA
S. mutans (MTCC 497)[b]	50	8	8	7	8	7	9	NA
	250	25	17	7	10	13	12	NA
	500	27	23	13	15	17	26	NA
A. parasiticus (MTCC 646)[f]	50	7	11	ND	ND	ND	NA	10
	250	9	13	6	ND	ND	NA	15
	500	13	18	8	7	ND	NA	28

	Minimum inhibitory concentration MIC (MBC/MFC) mg/l						
Strain	**3a**	**3b**	**3c**	**3d**	**3e**	**X**	**Y**
P. aeruginosa (MTCC 4673)[b]	25 (>100)	25 (100)	50 (>200)	100 (>200)	12.5 (50)	12.5	NA
S. aureus (MTCC 7443)[b]	50 (>100)	50 (100)	50 (>100)	100 (>200)	25 (50)	25	NA
S. enterica (MTCC 164)[b]	----	----	----	----	25 (50)	25	NA
S. mutans (MTCC 497)[b]	25 (100)	25 (>100)	50 (>100)	50 (>200)	25 (50)	25	NA
A. parasiticus (MTCC 646)[f]	50 (200)	25 (100)	----	----	----	NA	12.5

Here X (standard) = Streptomycin, Y (standard) = Streptomycin, a = mean of triplicates, b = bacterial strain, f = fungal strain, ND = not detected, NA = not applicable.

Table - 5: Anti-tubercular activity of the synthesized compounds (**3a**-**3e**).

Compd.	**Concentration (µg/ml)**								**MIC µg/ml**
	100	50	25	12.5	6.25	3.12	1.6	0.8	
3a	S	S	S	S	S	R	R	R	6.25
3b	S	S	S	S	S	S	R	R	3.12
3c	S	S	S	S	S	S	R	R	3.12
3d	S	S	S	S	S	S	S	R	1.60
3e	S	S	R	R	R	R	R	R	50.0
K	S	S	S	S	S	S	R	R	3.12
L	S	S	S	S	S	S	R	R	3.12
M	S	S	S	S	S	R	R	R	6.25

Here, S – Sensitive R- Resistant, K = Pyrazinamide, L = Ciprofloxacin, M = Streptomycin.

Table - 6: Antioxidant activity of the synthesized compounds (**3a**-**3e**).

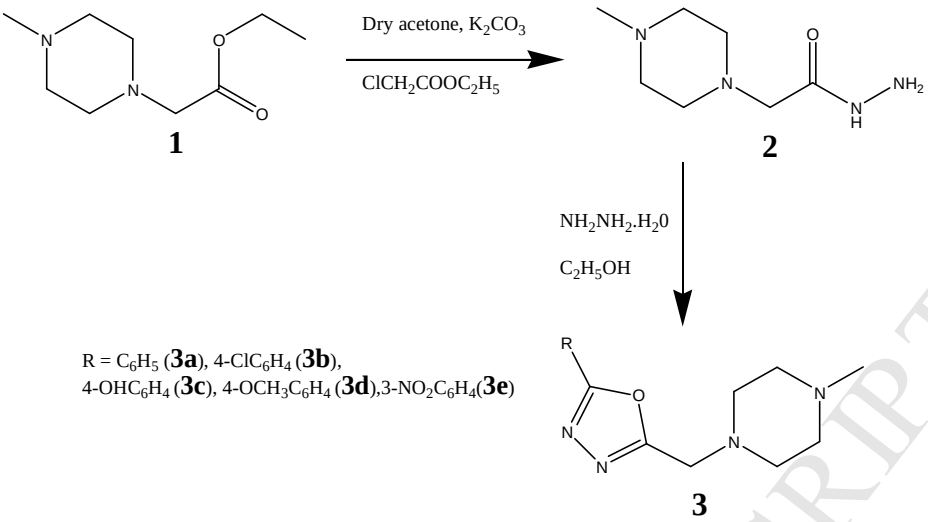
Compd.	**% inhibition at different concentrations (µg/ml)**				**IC_{50} (µg/ml)**
	25	50	75	100	
3a	46.43	54.48	59.08	65.51	36.76
3b	42.29	49.88	55.86	62.98	52.42
3c	21.14	34.94	52.87	64.59	73.65
3d	15.17	29.88	51.26	61.83	78.31
3e	28.04	37.70	48.27	60.22	77.61
Ascorbic Acid	32.87	40.68	55.40	70.34	62.91

Table - 7: Docking simulations for antimicrobial activity of the synthesized compounds (**3a**-**3e**).

Compd.	**PDB ID: 6g1o**		**PDB ID: 5isp**		**PDB ID: 5kbz**	
	Binding Energy (Kcal/mol)	Ki Inhibition Constant (µM)	Binding Energy (Kcal/mol)	Ki Inhibition Constant (nM)	Binding Energy (Kcal/mol)	Ki Inhibition Constant (nM)
4a	-7.51	3.14	-7.93	1.53	-7.51	3.14
4b	-7.24	4.95	-7.93	1.54	-7.24	4.95
4c	-7.28	4.62	-7.75	2.09	-7.28	4.62
4d	-6.62	14.05	-7.12	6.01	-6.62	14.05
4e	-7.81	1.87	-8.31	804.97nM	-7.81	1.87
Streptomycin	-2.58	12.78mM	-	-	-2.58	12.78mM
Ketoconazole	-	-	-8.31	807.99 nM	-	-

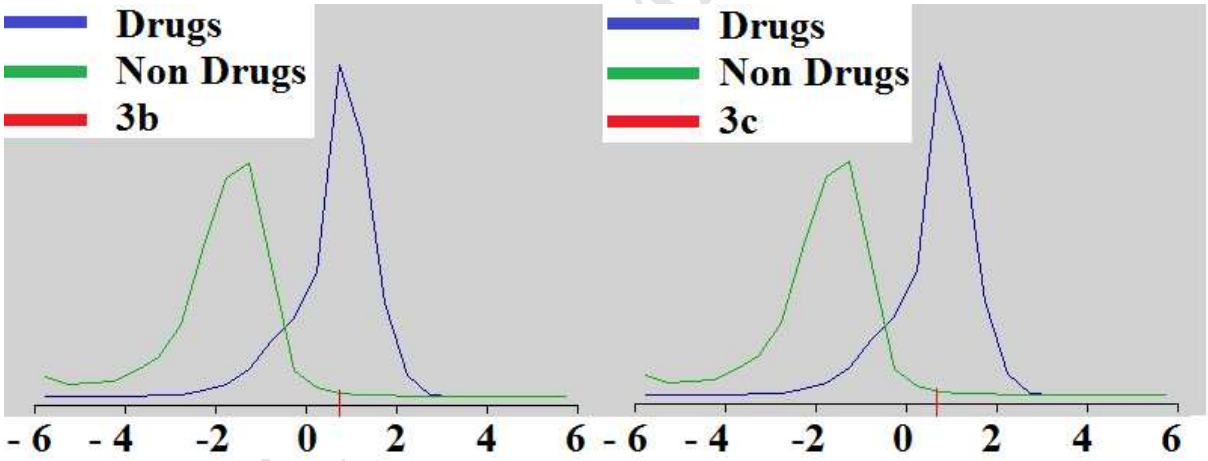
Table - 8: Docking simulations for anti-tubercular and antioxidant activity of the synthesized compounds (**3a**-**3e**).

Compd.	**PDB ID: 5yxf**		**PDB ID: 6il3**	
	Binding Energy (Kcal/mol)	Ki Inhibition Constant (nM)	Binding Energy (Kcal/mol)	Ki Inhibition Constant (nM)
3a	-8.24	905.77	-7.2	5.29
3b	-8.44	652.56	-7.35	4.08
3c	-8.09	1.17 µM	-7.24	4.97
3d	-7.8	1.93 µM	-6.97	7.84
3e	-9.22	173.83	-7.49	3.23
Streptomycin	-2.34	19.28 mM	-	-
Ascorbic acid	-	-	-5.16	165.58



Scheme - 1: Synthetic route of compounds from **1** to **3** including **3a** to **3e**.

Figure - 1: Graph showing drug-likeness model score of compound 3b (**a**) and 3c (**b**) respectively.



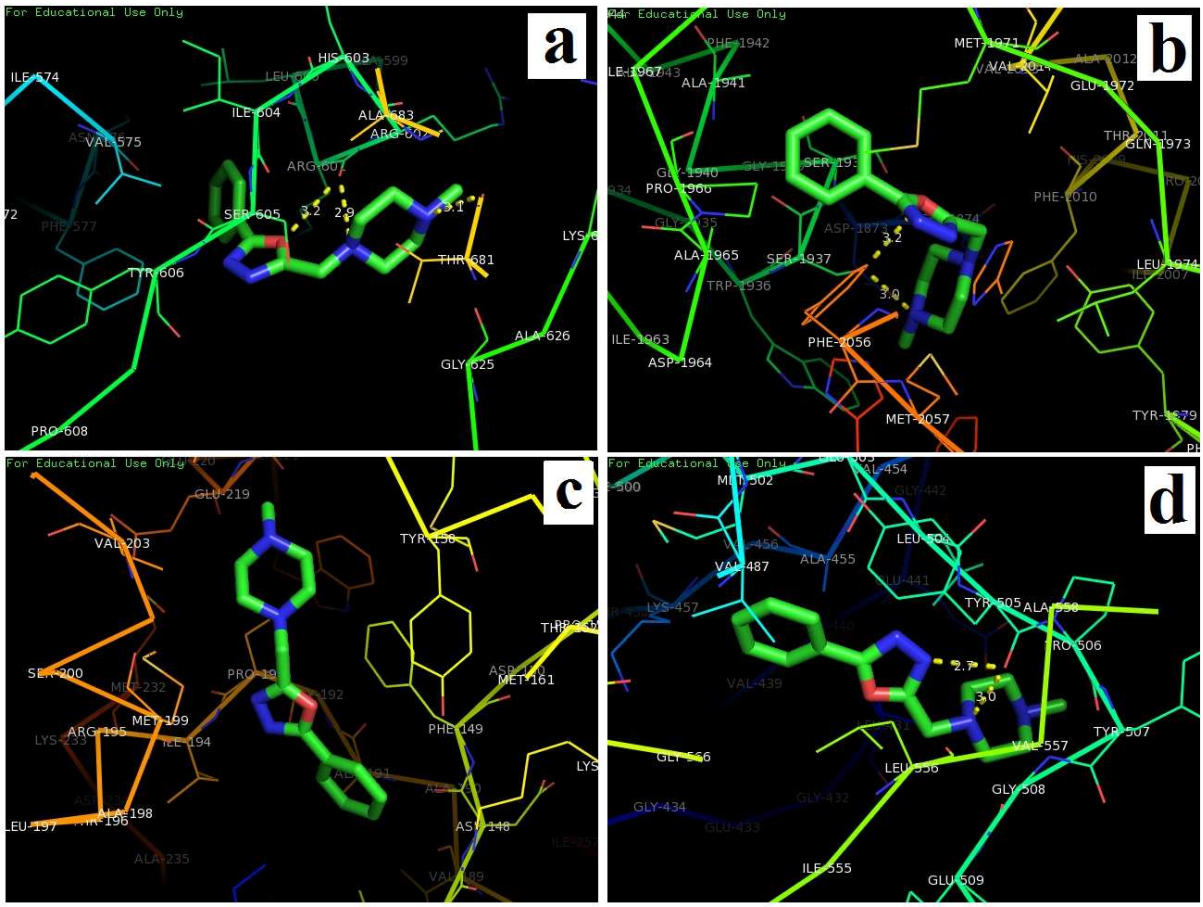


Figure - 2: Binding modes of compound **3a** with protein targets 3vx4 (**a**), 3ils (**b**), 2nsd (**c**) and 5tob (**d**) respectively visualized using Pymol software.

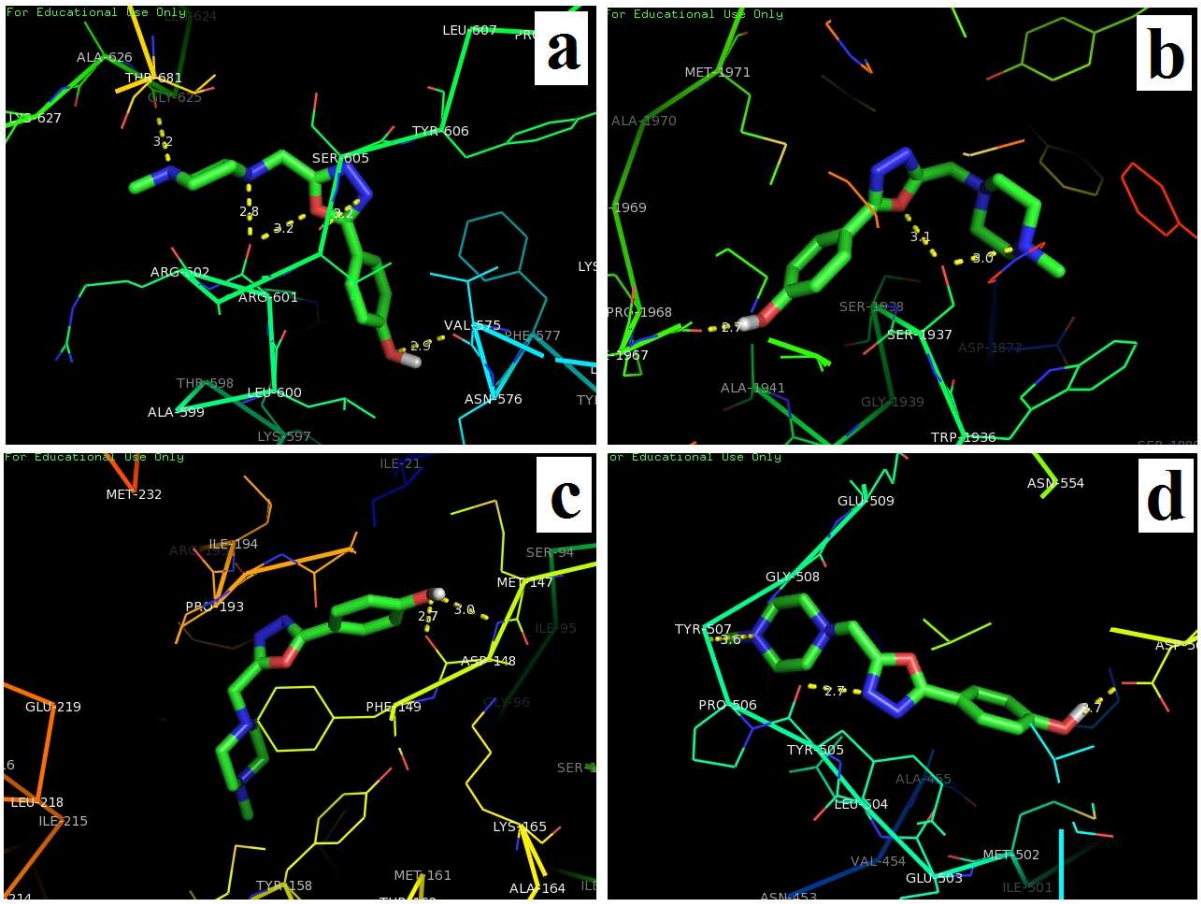


Figure - 3: Binding modes of compound **3b** with protein targets 3vx4 (**a**), 3ils (**b**), 2nsd (**c**) and 5tob (**d**) respectively visualized using Pymol software.

Highlights

- New series of 1, 3, 4-oxadiazoles incorporating piperazine scaffolds in a single molecular framework has been reported.
- The observed MIC values of anti-tubular activities for the molecule **3a**, **3b**, **3c**, **3d** and **3e** were 6.25, 3.12, 3.12, 1.60 and 50.0 μg/ml respectively.
- As compared to ascorbic acid (IC_{50} = 62.91 μg/ml), molecule **3a** exhibited better antioxidant activities (IC_{50} = 36.72 μg/ml).
- All the molecules have shown prominent antimicrobial activities.
- In addition, docking simulations were performed to study ligand-protein interactions and to determine the probable binding conformations.
- In drug likeness model study compound **3b** possessed maximum drug likeness model score (0.75) similar to the standard drug streptomycin.