Journal of Pharmaceutical Research International

33(44A): 472-484, 2021; Article no.JPRI.74020
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Design, Synthesis, Molecular Docking and Biological Evaluation of Novel 2-[(2-{[5-(Pyridin-4-yl)-1, 3, 4-Oxadiazol-2-yl]Sulfanyl}Ethyl)Sulfanyl]-1,3-Benzoxazole

Anees Fathima[1], H. M. Vagdevi[2*], N. D. Jayanna[3], R. Mohammed Shafeeulla[1] and Subbaraju[4]

[1]Department of Chemistry, Sahyadri Science College, Shimoga-577203, India.
[2]Sahyadri Science College, Shimoga-577203, India.
[3]Department of Chemistry, KLE's SSMS Arts, Science & Commerce College, Atani-591304, India.
[4]Department of Microbiology, Sahyadri Science College, Shimoga577203, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i44A32640
Editor(s):
(1) Dr. Thomas F. George, University of Missouri- St. Louis, USA.
Reviewers:
(1) Saade Abdalkareem Jasim, Al-Maarif University College, Iraq.
(2) G. M. Malik, Navyug Science College, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74020

Original Research Article

Received 04 July 2021
Accepted 14 September 2021
Published 20 September 2021

ABSTRACT

In the present communication, a simple and facile method was adopted to synthesize a series of novel 2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole by fusing 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol with substituted 2-[(2-bromoethyl)sulfanyl]-1,3-benzoxazole and 2-[(2-chloroethyl)sulfanyl]-1,3-benzoxazole to obtain heterocyclic ring systems of 1,3,4 oxadiazole linked benzoxazole moiety. The synthesized compounds were characterized by the aid of LCMS, IR, ^{1}H NMR, ^{13}CNMR, and C, H, N analysis technique. All the newly synthesized compounds were assessed for antimicrobial, antioxidant and antitubercular activities against standard strains. Microbiological results showed that the compounds showcased a wide range of activities and further the results of antimicrobial, antioxidant and molecular docking studies revealed that the compounds 6c, 6d and 6e are more potent and displays excellent docking scores

**Corresponding author: E-mail: vagdevihm17@gmail.com;*

with various amino acids interaction like alkyl-alkyl, pi-alkyl and hydrogen bonding of antitubercular receptor H37R with benzoxazole moieties and displayed encouraging antitubercular results to 6c, 6d and 6e molecules. The structural activity relationship studies illuminated the obtained results.

Keywords: Benzoxazole; oxadiazole; antibacterial; antioxidant; molecular docking; antitubercular.

1. INTRODUCTION

Drug design is increasingly based on modern computational chemical techniques and also uses sophisticated knowledge of disease mechanisms and receptor properties [1]. The chemistry of heterocyclic compounds is justifiable both from the hypothetical as well as practical importance. Their study is of top priority interest. Numerous compounds inclusive of alkaloids, essential amino acids, vitamins, heamoglobin, hormones, wide variety of synthetic drugs and dyes contain heterocyclic ring frameworks. There are huge numbers of manufactured heterocyclic compounds like pyrrole, pyrrolidine, furan, thiophene, piperidine, pyridine and thiazole, having vital application and many are significant intermediates in synthesis of medicines. In the core structure, heterocycles consisting of oxygen and nitrogen atoms exhibits number of pharmacologically and biologically active compounds [2]. Amidst all the heterocyclic compounds, benzoxazole is one of the significant heterocycles showing exceptional pharmacological activities. Its aromaticity makes it relatively stable, although as a heterocycle it has reactive sites, which allows for functionalization [3]. Benzoxazole conjoined to 1,3,4 oxadiazole have been found to be of great interest also has a broad spectrum of biological activities. Literature survey showed that 1,3,4 oxadiazole has been a frontier in pharmaceutical research for synthesis of new derivatives playing a vital role in biological activities as drugs. As per the literature survey benzoxazole shows remarkable pharmacological activities like antimicrobial, antioxidant, antifungal, cytotoxic, antitubercular, Pancreatic Lipase Inhibitory, analgesic and anti-inflammatory[4-8]. Even 1,3,4 oxadiazole also shows appreciable activities as antibacterial, antifungal, nematicidal, anti-HIV, antitubercular[9-13]. Hence the combined moiety of benzoxazole and oxadiazole seem to display impeccable biological activity.

Inspired by the biological profile of benzoxazoles and oxadiazoles and their increasing importance in pharmaceutical and biological fields, in continuation of our research on biologically active heterocycles considering the scope to introduce 1,3,4-oxadiazole moiety into the benzoxazole, it is thought worthwhile to undertake the synthesis of title compounds. In view to obtain certain new chemical entities with both active pharmacophores in a single molecular frame work for the intensified biological activities.

The present work sheds light on the cost effective synthetic route for the preparation of series of novel 2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole derivatives. The synthesized conjoined moiety of benzoxazole and oxadiazole derivatives has been subjected to both dry and wet studies, which displayed significant activities against all the strains. Out of which 6c, 6d and 6e showed considerable biological screening scores.

2. METHODOLOGY

2.1 Materials and Methods

By taking the sample in a glass capillary being sealed at one end, melting points were determined in an electrically heated apparatus, which are uncorrected. Spectra like ^{1}H NMR and ^{13}C NMR were recorded on Bruker at 400MHz at Manipal Institute of Technology (MIT), Manipal, Karnataka, India, with tetramethylsilane(TMS) being an internal standard, the chemical shifts are shown in δ values (ppm). Elemental analysis (C, H and N) was determined by Perkin-Elmer 2400 Series II analyzer. By LC-MS spectroscopy, molecular weights of unknown compounds were characterized at Centralized Instrumentation Facility, Mysore University, Karnataka, India. By using a Shimadzu Fourier Transform Infrared (FT-IR Nicolet-5700) spectrometer, the FT-IR spectra of the compounds were determined. By thin layer chromatography (TLC), where silica gel coated on aluminium sheets (silica gel 60 F254), the completion of the reaction was monitored. Commercial grade solvents and reagents were used. The yield, melting point, molecular formula and molecular weight of the compounds are recorded in Table1.

2.1.1 Preparation of substituted benzoxazole-2-thiol [2]

Upon stirring KOH and methanol for nearly 10 minutes, followed by the addition of carbon

disulphide (1.1eq) at room temperature, different substituted amino phenols were added to the prior mentioned reaction mixture. The reacting substrate was refluxed for 6-8 hour on water bath. The reaction is monitored for the completion with the aid of TLC, on confirming the completion of reaction the entire mass is transferred to the crushed ice followed with the acidification by using acetic acid. The obtained solid product was separated from the mother liquor through the filtration and washed several times with water and finally recrystallized from ethyl acetate to obtain a pure product [7].White colour solid, yield (90%), M.P. 209-210℃; ^{1}H NMR (DMSO) ppm: δ7.058-7.391 (m, 3H, Ar-H), δ 2.365(s, 3H, CH_3), 13.792(s, 1H, SH).

2.1.2 Synthesis of substituted 2-[(2-bromoethyl)sulfanyl]-1,3-benzoxazole[3]

The mixture of compound 2(0.1mmol), activated K_2CO_3 (2eq) in acetonitrile was taken in a round bottom flask and stirred for 15 minutes. The reaction mixture was treated with 1, 2 dibromoethane/1,2 dichloroethane drop by drop with constant stirring for about 10 min at room temperature. This reaction mixture was heated under reflux for 5 hours and allowed to cool to room temperature and poured in to crushed ice. The obtained solid was filtered, washed with water, dried and recrystallized using ethanol (K_2CO_3 to be activated beforehand for 2-3 hours)[12-14].

Pale yellowcolor solid, yield (88%), M.P. 123^o-125℃; ^{1}H-NMR (DMSO-d_6) ppm: δ 7.046 -7.372 (m, 3H, Ar-H), δ 3.817-3.883 (t, 2H, S-CH_2), δ4.214-4.168 (t,2H, -CH_2), δ 2.447 (s, 3H, CH_3) IR (KBr) cm−1: 2923.24 (CH_3), 621.45 (C-Br), 1214.55 (N=C), 728.80(C-S), 1573.96(Ar).

2.1.3 Synthesis of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol [5]

To the solution of potassium hydroxide (2eq) in methanol (20ml) was added carbon disulphide (1.1eq) drop wise with constant stirring for about10-15 minutes followed by the addition of isoniazide (0.1mmol). The mixture was refluxed till completion of the reaction take place, which was monitored by TLC. After the completion of the reaction, the reaction mixture was poured into ice-cold water. It was filtered to remove suspended impurities and acidified with 10% acetic acid and the solid thus precipitated was filtered, washed twice with cold water and recrystallized with ethanol to give the compound as yellow crystals [12]. ^{1}HNMR (DMSO—d_6): δ 7.784-7.769 (m, 2H, Ar-H), δ 8.783-8.768 (m, 2H, Ar-H), 15.0(s, 1H, SH).

2.1.4 General procedure for the synthesis of 2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole 6(a-e)

A mixture of 2-[(2-bromoethyl)sulfanyl]-1,3-benzoxazole(3)(0.01moles) and 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol(5)(0.01moles) in 40ml dry THF was stirred in presence of triethylamine for 6 hour at room temperature. After the completion of reaction, THF was removed and ice cold water (30 ml) was added to the residue with stirring. The solid precipitated was filtered, washed with water followed by hexane, recrystallized with ethanol to give compounds 6(a-e)[6].

2.1.5 2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole 6a

IR (KBr, cm^{-1}): 1217.46 (N=C), 733.89 (C-S), 1596.82 (Ar), 3110.10 (pyridine); ^{1}H NMR (DMSO-d_6, δ ppm): 7.336-8.376 (m, 8H, Ar-H), 4.215-4.395 (t, 2H, S-CH_2), 4.640-4.786(t, 2H, S-CH_2); ^{13}C-NMR(DMSO-d_6,δppm): 168.70, 151.66, 150.56, 142.97, 138.13, 134.11, 129.36, 126.57, 125.81, 124.65, 119.23, 118.87, 117.05, 111.90, 39.37, 35.31; MS: (M+1) = 357.12.

2.1.6 4-nitro-2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole 6b

IR(KBr, cm-1): 1495.59 (NO_2), 1217.35 (N=C), 721.59 (C-S), 1595.72 (Ar), 3089.39 (pyridine); ^{1}H NMR (DMSO-d6, δ ppm): 8.65-7.52(m, 8H, Ar-H), 3.33(t, 2H,S-CH2), 3.33(t, 2H, S-CH2); ^{13}C-NMR(DMSO d6, δ ppm): 165.0,164.5,150.9,149.9,143.7,140.4,135.2,124.8,120.5, 121.4, 116.8, 36.5;MS: m/z = 401.42.

2.1.7 5-nitro-2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole 6c

IR (KBr, cm^{-1}): 1552.30 (NO_2), 1217.32 (N=C), 743.69 (C-S), 1595.72(Ar), 3088.63 (pyridine);^{1}H NMR (DMSO-d_6, δ ppm): 8.65-7.52(m, 8H, Ar-H), 3.33(t, 2H,S-CH_2), 3.33(t, 2H,S-CH_2); ^{13}C NMR(DMSO d_6, δ ppm): 165.0, 164.5, 150.9, 149.9, 143.7, 140.4, 135.2, 124.8, 120.5, 121.4, 116.8, 36.5; MS: m/z = 401.42.

2.1.8 5-methyl-2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole 6d

IR (KBr, cm^{-1}): 2919.28 (CH_3), 1218.95 (N=C), 738.21 (C-S), 1596.72 (Ar); ^1H NMR (DMSO-d_6, δ ppm): 8.399-7.350 (m, 7H, Ar-H), 4.578-4.428 (t, 2H, S-CH_2), 4.373-4.238 (t, 2H, S-CH_2), 2.548-2.246(s,3H, CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 159.60, 153.89, 142.02, 132.93, 129.62, 126.82, 125.27, 121.81, 119.18, 116.49, 106.12, 39.3, 16.8; MS: (M+1) = 371.14.

2.1.9 5-chloro-2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole 6e

IR (KBr, cm-1): 622.71 (Cl), 1217.95 (N=C), 733.51 (C-S), 1597.08(Ar),3051.04(pyridine); ^1H NMR (DMSO-d_6, δ ppm): 8.65-7.20 (m, 8H, Ar-H), 3.33 (t, 2H, S-CH_2), 3.33 (t, 2H, S-CH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 165.0, 164.5, 149.9, 148.1, 143.7, 142.9, 128.7, 122.8, 121.4, 117.7, 112.1 ; MS: m/z = 390.86.

2.2 Biological Activities

2.2.1 Antibacterial activity

The newly synthesized compounds 6(a-e) were examined for their antibacterial potency against Gram-positive bacteria, particularly *Staphylococcus aureus, Bacillus subtillus* and Gram-negative bacteria is *Pseudomonas aeruginosa* and *Klebsiella Pneumonia* by agar well diffusion technique[15]. On sterile Muller-Hinton agar plates, the 24 hours old Muller-Hinton broth cultures of test bacteria were swiped utilizing sterile cotton swab preceded by punching wells of 8mm with the assistance of sterile plug borer. To respectively labelled wells, the standard drug (Chloramphenicol 30mcg/disc), mixes of 6(a-e) compounds (200μg/mL in 10% DMSO), and controls (10% DMSO) were added. For about 30 minutes, the plates were assented to stand and in upstanding position they were incubated at 37℃ for 24 hour for recording the zone of inhibition. The antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the used standard reference. The observed inhibition zones are presented in Table 2. As a result of this, the primary screening against the bacterial strains showed good zone of inhibition as shown in Fig 2. It was noticed that the presence of electron donating and electron withdrawing groups attached to the benzoxazole ring displayed strong effect on the antibacterial activity for example the presence of nitro, methyl and chloro substituent respectively in the structure, which was responsible for the enhanced activity of the compounds of (6c, 6d and 6e) showed the potent antibacterial activity among all the tested compounds of this series.

2.2.2 Minimum inhibitory concentration (MIC)

All the compounds were screened for antibacterial activity by using cup plate method. The Minimum inhibitory concentration (MIC) of the most active synthesized compounds 6(a-e) were evaluated *in-vitro* using the serial dilution technique. The results of minimum inhibitory concentration are depicted in Table 3. The MIC study of the synthesized compounds against bacterial strains at different concentrations i.e., 100, 50, 25 and 12.5 μg/mL was evaluated. The MIC zones of inhibition for antibacterial activity of the compounds 6(a-e) are reported in Fig.3 which was evident for the inhibition of bacterial strains. The 6c, 6d and 6e compounds showed potential MIC values against bacterial strains respectively [16].

2.2.3 Antioxidant Activity (DPPH Assay)

Radical scavenging effect on DPPH free radical and also the radical scavenging capacity of compounds and the Ascorbic acid (standard) were tested. In methanol, various concentrations (25, 50, 100, 200, and 400μg/mL) of compounds and standard were prepared. 2ml of DPPH solution (0.002% in methanol) was mixed individually with 2mL of various concentrations of compounds and standard in clean and labelled test tubes which were further taken for the study. The tubes were incubated at room temperature for 30 min in dark and using UV-visible spectrophotometer, the optical density was detected at 517nm[17]. The absorbance of the DPPH control was recorded.

The scavenging activity was determined utilizing the formula:

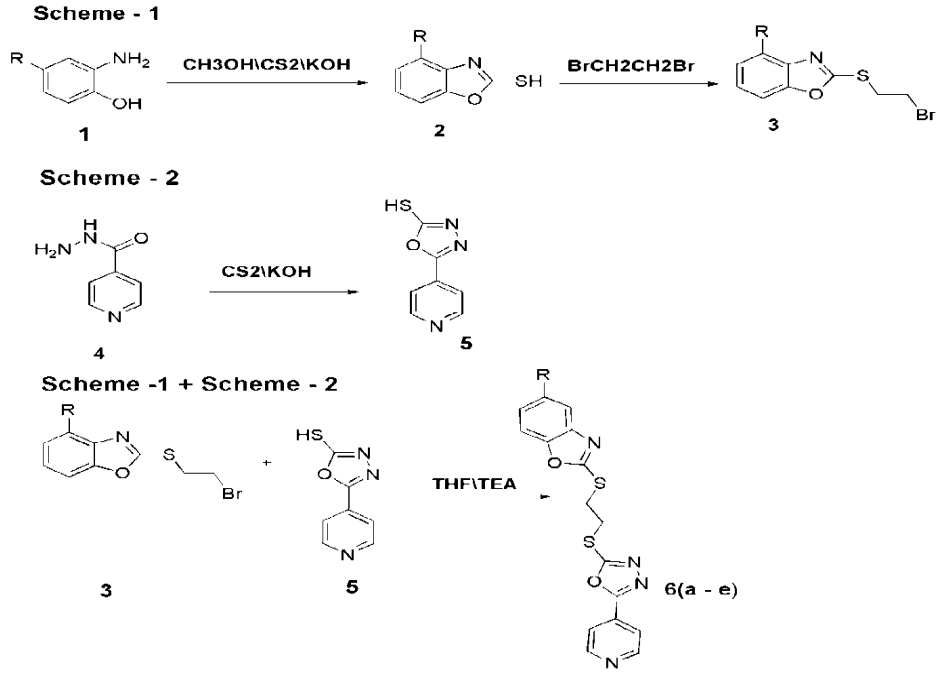
$$\text{Scavenging activity (\%)} = A\text{-}B/A\times100$$

Where A is the absorbance of DPPH and B is the absorbance of DPPH in standard combination.

The DPPH radical scavenging activity data is depicted in Table 4 and Fig 4. DPPH solution in methanol showed strong absorbance at 517 nm.

Table 1. Physical data of compounds 6(a-e)

Compound	Molecular formula	Molecular weight	M.P.(ºC)	% Yield	Found (Calculated)% C	H	N
6a	$C_{16}H_{12}N_4O_2S_2$	356.42	226	83	53.92 (53.96)	3.39 (3.41)	15.72 (15.80)
6b	$C_{16}H_{11}N_5O_4S_2$	401.42	230	76	47.87 (47.91)	2.76 (2.82)	17.45 (17.49)
6c	$C_{16}H_{11}N_5O_4S_2$	401.42	222	78	47.87 (47.92)	2.76 (2.80)	17.45 (17.50)
6d	$C_{17}H_{14}N_4O_2S_2$	370.44	218	81	55.12 (55.21)	3.81 (3.84)	15.12 (15.19)
6e	$C_{16}H_{11}ClN_4O_2S_2$	390.86	224	75	49.17 (49.23)	2.84 (2.88)	14.33 (14.36)



6a = H, 6b = 4-NO_2, 6c = 5-NO_2, 6d = CH_3, 6e= Cl

Fig. 1. Synthesis of substituted 2-[(2-{[5-(pyridin-4-yl)-1, 3, 4-oxadiazol-2 yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole derivatives

Table 2. Antibacterial activity of compounds 6(a-e)

Compound	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
6a	11±0.97	12±0.81	11±0.47	12±1.63
6b	13±0.81	12±1.63	11±0.97	11±0.81
6c	15±1.63	14±0.81	13±1.63	14±0.97
6d	17±0.47	16±0.47	14±0.81	16±0.47
6e	20±0.81	18±2.16	17±1.63	21±2.16
Standard	25±0.47	21±0.97	22±0.81	26±1.63

STD=30mcg/disc, Compound=250 μg/mL, Mean± SEM, n = 6

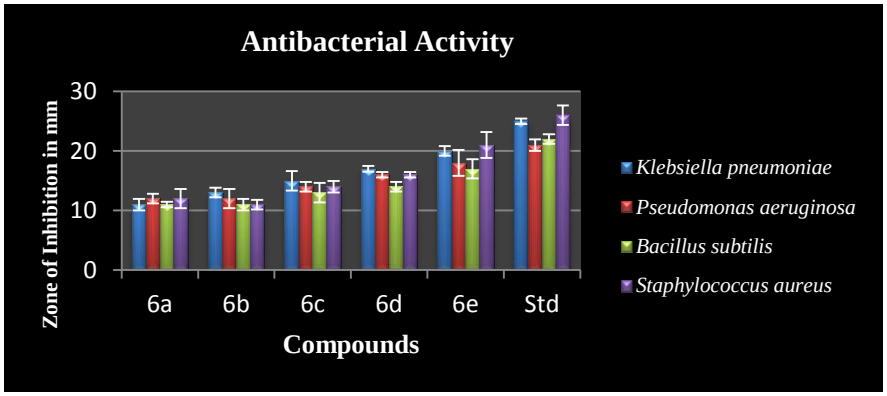


Fig. 2. Antibacterial activity of compounds 6(a-e)

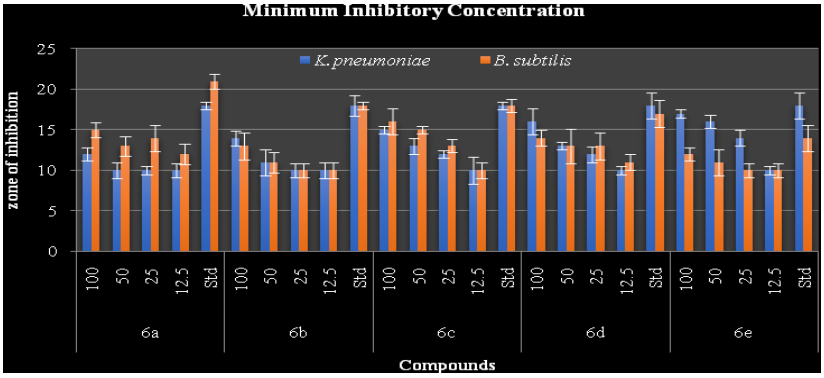


Fig. 3. Minimum inhibitory concentration compounds 6(a-e)

Table 3. Minimum inhibitory concentration of compounds 6(a-e)

Organisms	Concentration in μg/ml	Compound 6a	6b	6c	6d	6e
Klebsiellapneumoniae	100	12±0.81	14±0.94	15±0.47	16±1.63	17±0.47
	50	10±0.94	11±1.63	13±0.94	13±0.47	16±0.81
	25	10±0.47	10±0.81	12±0.47	12±0.97	14±0.97
	12.5	10±0.81	10±0.94	10±1.63	10±0.47	10±0.47
	Std	18±0.47	18±1.24	18±0.47	18±1.63	18±1.63
Bacillus subtilis	100	15±0.94	13±1.63	16±1.63	14±0.97	12±0.81
	50	13±1.24	11±1.24	15±0.47	13±2.16	11±1.63
	25	14±1.63	10±0.81	13±0.81	13±1.63	10±0.81
	12.5	12±1.24	10±0.94	10±0.97	11±0.97	10±0.81
	Std	21±0.94	18±0.47	18±0.81	17±1.63	14±1.63

Mean± SEM, n = 6

Table 4. Antioxidant activity of concentration of compounds 6(a-e)

Compound	Scavenging activity of different concentration (µg/mL) in %				
	400 µg/mL	200µg/mL	100µg/mL	50µg/mL	25µg/mL
6a	88.82±0.57	82.09±0.65	81.91±0.14	79.25±0.81	76.41±0.75
6b	89.55±1.05	88.14±0.37	87.18±0.85	85.28±0.58	85.28±0.17
6c	97.51±0.69	94.68±0.58	93.97±0.81	81.02±0.54	75.11±0.39
6d	96.09±0.38	95.92±0.57	90.95±0.70	86.17±0.70	70.74±0.52
6e	97.16±0.58	88.47±0.23	85.46±0.65	83.15±0.36	81.73±0.50
Ascorbic acid	98.89 ±1.0	95.82±0.43	94.29±0.55	88.46±0.62	84.75±0.21

Mean± SEM, n = 6.

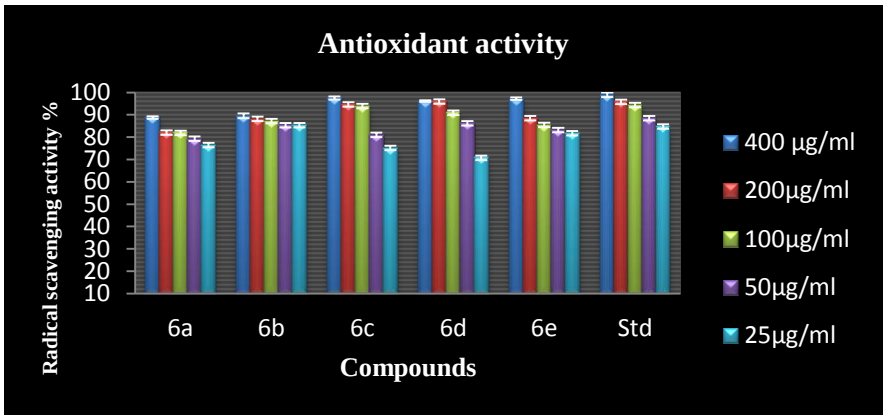


Fig. 4. Antioxidant Activity of Concentration compounds 6(a-e)

If DPPH abstracts a hydrogen radical from an external source, the absorption decreases stoichiometrically depending on the number of electrons or hydrogen atoms. The newly synthesized compounds displayed potent activity but lower when compared to standard. The compounds 6c and 6d showed potent scavenging activity almost close to the standard (ascorbic acid) and compound 6e showed better inhibition activity against free radical and rest of the synthesized compounds showed moderate activity.

2.2.4 Molecular docking studies

The molecular docking studies were eventually carried out by following the reported procedure [18, 19]. The In-silco molecular docking has been carried out on the antitubercular receptor PDB code: H37R, the crystal structure of the receptor has been obtained from the Protein Data Bank (PDB: http://www.rcsb.org/pdb). The water molecules and heteroatoms were removed before screening. The receptor structure was prepared prior to use in docking study, utilizing protein preparation module of HEX modelling package 8.0. During the protein preparation, all hetero and water molecules were removed from the crystal structure except water molecules within 5Å from the ligand. All the molecules docked at the active binding sites of the receptor, the 3D structure of each ligand with the receptor binding interactions were visualized to optimization quality by discovery studio 3.2. The in silico molecular docking scores give useful information concerning the capability of the newly synthesized compounds to bind the active sites of the receptor. Thus the obtained docking values guided us for performing wet analysis of antitubercular activity.

2.3 Antitubercular Activity

The antimycobacterial activities of compounds 6(a-e) were assessed against M. tuberculosis ATTC 2729415[20] using the micro plate Alamar Blue assay (MABA) [21]. This method is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods [22, 23]. Sterile deionized water (200µl) was added to all outer-perimeter wells of sterile 96 well plates (falcon, 3072: Becton Dickinson, Lincoln Park NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100 µl of the Middle brook 7H9 broth and a serial dilution of the compounds were made directly on plate [24]. The final drug concentrations tested

were 100-0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37° C for five days. After this time, 25 µl of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% tween 80 was added to the plate and incubated for 24 hour.

The MIC (Minimal Inhibition Concentration) was defined as the lowest drug concentration, which prevented a colour change from blue to pink. Standard Strain used: *Mycobacteria tuberculosis* (Vaccine strain, H37 RV strain): ATCC No-27294. Standard values for the Anti-Tubercular test which was performed. Pyrazinamide-3.125µg/mL, Ciprofloxacin-3.125µg/mL, Streptomycin- 6.25µg/mL.

3. RESULTS AND DISCUSSION

In the present research ultimately 5 new 2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole derivatives 6(a-e) were synthesized. For their synthesis, initially compound 2 was obtained by refluxing the appropriate amount of the substituted amino phenol in the presence of cyclising agent CS_2 and suitable amount of KOH in methanol were added. It was characterized by ^{1}H-NMR, which exhibited a multiplet at 7.058-7.391 were due to 3 aromatic protons, a singlet at 13.792 for SH proton (D2O exchangeable). For the synthesized compound 3, the compound 2 was made to react with 1,2 di-bromoethane by refluxing the reaction mixture for about 4 hours. In ^{1}H- NMR, the presence of -S–CH_2 at 4.174 and -CH_2 at 3.883 confirmed the formation of compound 3 (scheme-1). Afterwards as mentioned in the scheme-2, isoanizide was converted to 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol by reacting with CS_2 and KOH retaining the same conditions of compound 2. The formation of oxadiazolethiol was confirmed by a multiplet at 7.769-7.784 and 8.768-8.783 were due to four aromatic protons and a singlet at 15.0 for SH proton. Finally in the last step the clubbing of the compound 3 and 5 through condensation reaction yields 6(a-e) derivatives in 70-80%. Then the newly synthesized 2-[(2-{[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}ethyl)sulfanyl]-1,3-benzoxazole derivatives were evidenced by spectral data. In the IR spectra of compounds 6a, absorption bands corresponding to the -C=N and -C-S at 1217.46 and 733.89 cm^{-1} respectively. The absorption band signal for pyridine group appeared at 3110.10 cm-1, ^{1}H NMR spectrum of compound 6a showed two resonance signals at δ 4.64 and at δ 4.21 as double triplets for two protons corresponding to S-CH_2 protons. The aromatic protons of phenyl ring appeared

Table 5. Binding energies and types of binding intraction of compound 6(a–e) with receptor 4FDO

Compounds Code	H-bond	Pi-Lone pair interaction	Docking score	Pi-alkyl interaction	Alkyl- Alkyl interaction
6a	ALA20	GLY17	-238.00	ALA126	THR14
	PRO21	ARG18		LYS418	GLY15
	SER22	ALA24		ALA417	TRP16
	VAL23			LYS418	
6b	PHE379	HIS132	-244.39	LYS418	VAL121
	PHE142	THR7		LEU98	ALA126
	LEU98	THR8		TYR415	LYS418
		THR9		PHE379	
	PHE142	GLN336		PHE142	PRO21
6c	ILE131	PHE337		GLY15	SER22
	GLN336	ILE386	-259.71	TRP16	VAL23
	HIS132	TYR415			ALA24
6d	GLN336	LA417		MET34	THR14
	PHE337	TYR415	-251.40	ILE35	GLY15
	ILE386	LYS37		VAL36	TRP16
	TYR415				GLY17
6e	ALA417	LYS418		HIS160	ARG28
	TYR415	THR416	-291.59	LEU161	THR29
	LYS418	LYS418		THR162	PRO30
	TYR415	GLN336		PRO163	ASP31
(STD)			-159.50		

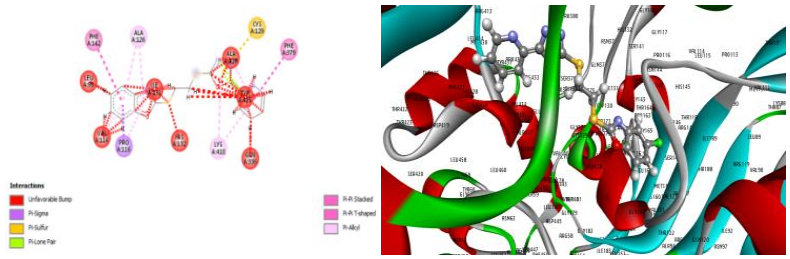


Fig. 5. 6a- 2D and 3D bonding interactions of receptor 4FDO with compound 6a

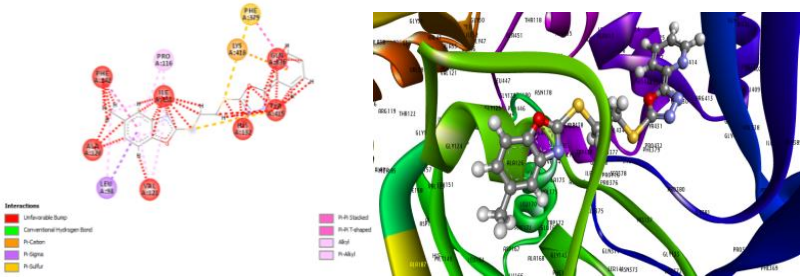


Fig. 6. 6b- 2D and 3D bonding interactions of receptor 4FDO with compound 6b

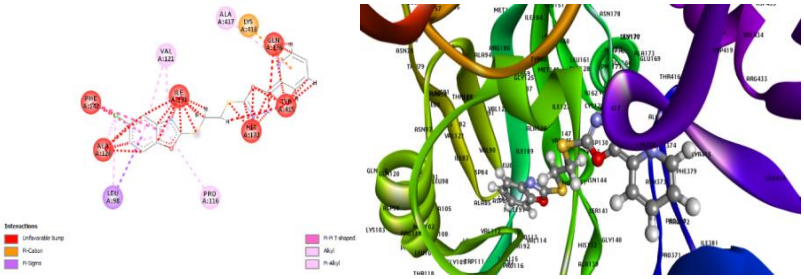


Fig. 7. 6c- 2D and 3D bonding interactions of receptor 4FDO with compound 6c

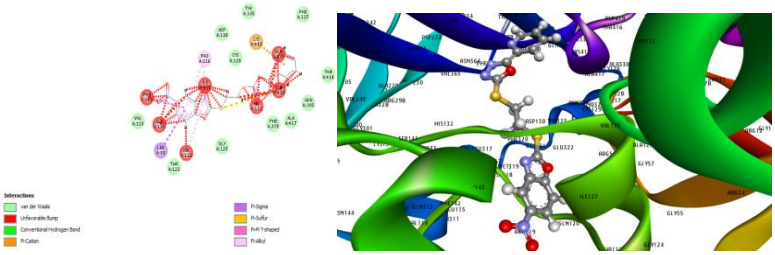


Fig. 8. 6d- 2D and 3D bonding interactions of receptor 4FDO with compound 6d

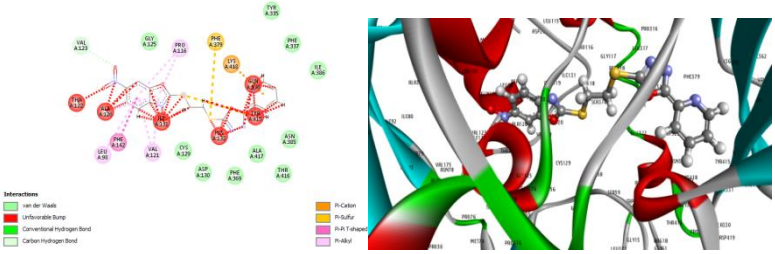


Fig. 9. 6e- 2D and 3D bonding interactions of receptor 4FDO with compound 6e

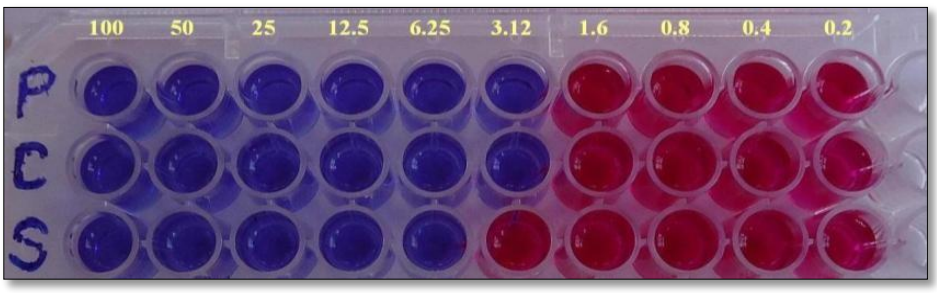


Fig. 10. Standard drug photograph of Anti tubercular activity

Table 6. Antitubercular activity of compounds 6(a-e)

Sl. No.	Compound	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
01	6a	S	R	R	R	R	R	R	R
02	6b	S	R	R	R	R	R	R	R
03	6c	S	S	R	R	R	R	R	R
04	6d	S	S	S	R	R	R	R	R
05	6e	S	S	S	R	R	R	R	R

**Note: S-Sensitive, R- Resistant*

Fig. 11. Antitubercular activity assay plate of compounds 6(a-e)

between δ 8.37-7.33 as multiplet, integrating for eight protons. Mass spectrum showed a peak at m/z 357.12 (M+1), confirming the structure of compound 6a. To summarise the IR, ^{1}HNMR ^{13}CNMR, Mass spectra and elemental analysis results are in favor with the propounded structures, further the compounds were screened for their antibacterial, antioxidant, molecular docking scores against antimycobacterial receptor (PDB ID: 4FDO) are guided for antitubercular activity.

3.1 Antibacterial and MIC

Table 2 and 3 summarizes the results of the antibacterial and Minimum Inhibitory Concentration (MIC) assays of five synthesized compounds respectively. Compared to the lead compound 6a, general improvement of antibacterial activity was observed. When the aromatic of 6a was substituted with 4-nitro amino phenol (6b), 5-nitro aminophenol (6c), 4-methyl amino phenol (6d), 4-chloro amino phenol (6e), the antibacterial activities were escalated by 8-32 folds. It appeared that substitutions in benzoxazoles provided good antibacterial activity. It was concluded that the activity was significantly effected by the presence of electron withdrawing groups (NO_2,Cl) improved the antibacterial activity against *P. aeruginosa, K. pneumonia, Bacillus subtilis S. typhi* and *Bacillus subtilis.*

Further, to quantify the lowest concentration which prevents the growth of the organism was determined by the Minimum Inhibitory Concentration (MIC) value by cup plate method. The compound 6c exhibited considerable antibacterial activity against *Bacillus subtilis* (gram +ve) but at the same concentration the compound showed lower inhibition potency against *Klebsiella pneumonia* (gram –ve) similarly compound 6d with methyl substitution on aryl group showed good inhibition in case of *Klebsiella pneumonia* and decreased potency against *Bacillus subtilis*. The compound 6e with chloro substitution on phenyl ring showed better activity against *Klebsiella pneumonia* and *Bacillus subtilis* respectively. The compounds 6a and 6b displayed moderate potency with average inhibition efficacy against the tested pathogens. The declining trend in the inhibition potency was observed in the synthesized compounds 6a and 6b with substitution –H and $–NO_2$ at 4-position. Overall, from the MIC of synthesized compounds, it was noted that the inhibition efficiency of the tested compounds against the two bacteria *Klebsiella pneumonia* (gram –ve) and *Bacillus subtilis* (gram +ve) is not the same. The compound which showed maximum inhibition against P. aeruginosa showed the least inhibition potency and vice versa.

3.2 Antioxidant

DPPH (1,1diphenylpicrylhydrazyl) radical scavenging efficacy of synthesized compounds 6(a-e) was evaluated by using Ascorbic acid as standard and the results have been shown in Fig4. It was observed that among the synthesized compounds most of the compounds showed considerable scavenging activity. Compound 6e with -Cl substitution displayed strongest radical scavenging potency followed by the compounds 6c and 6d which showed significant activity. Compounds 6a and 6b with -H and $-NO_2$ substitution on phenyl ring showed moderate activity.

3.3 Molecular Docking Studies

The molecular docking of the synthesized compounds 6(a-e) was carried out with possible biological targets for the antitubercular receptor (PDB ID: 4FDO). The binding energies of the synthesized compounds 6(a-e) with the receptors were calculated using computational docking studies with HEX 8.0 engine. These docking energy values and possible different types of binding interaction were listed in Table 5. The antitubercular docking results showed that there are possible interactions between 6c, 6d and 6e. The 6d and 6e showed a stronger interaction with 4FDO receptor than the other compounds with lowest binding energy are - 251.40 and - 291.59 kcal mol−1 respectively. The 6c, 6d and 6e have hydrophobic (Pi-alkyl), alkyl-alkyl and hydrogen bond interaction with amino acids, in the protein around the receptor molecule. As displayed in the Table 5, the best-docked orientations of 6(a-e) with receptor 4FDO are shown in Fig 5-9.

3.4 Antitubercular Activity

Synthesized set of derivatives of benzoxazole were evaluated for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis*. From the results it was evident that the compounds 6d and 6e exhibited appreciable activity against *M. tuberculosis.*

4. CONCLUSION

To be precise it was designed a series of novel target compounds by conjoining 2 pharmacophoric motifs 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol and substituted 2-[(2-bromoethyl)sulfanyl]-1,3-benzoxazole for evaluating various biological activities. The expected target compounds were prepared, structurally confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis and *in-vitro* screened for their biological activities. The data reported here in indicates that compound 6c, 6d and 6e has emerged as potentially active compounds. These molecules have shown significant results as compared to standard drug and considered as potential molecules for further developments.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

The authors are thankful to the Directorate of minorities, Bangalore, Karnataka, India, for

financial support. The authors are grateful to the Principal, Sahyadri Science College, Shivamogga for providing the necessary research facilities. We are also grateful to Sophisticated Analytical Instruments Facility, Mysore University, Karnataka India, MIT Manipal for providing 1H NMR, 13C NMR and Mass spectral facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Xiaoqian Lin, Xiu Li, and Xubo Lin. A Review on Applications of Computational Methods in Drug Screening and Design. Molecules. 2020;25:1375.
2. Katta Eswar Srikanth. Chemical Significance of 1,3-Benzoxazole Derivatives.Chemical Sceince Journal. 2020;11:5.
3. Maruthamuthu, Shameela Rajam, Christina Ruby Stella P, Bharathi Dileepan AG. Ranjith R. The chemistry and biological significance of imidazole, benzimidazole, benzoxazole, tetrazole and quinazolinone nucleus. Journal of Chemical and Pharmaceutical Research. 2016;8(5):505-526.
4. Saloni Kakkar, Sumit Tahlan1, Siong Meng Lim, Kalavathy Ramasamy, Vasudevan Mani, Syed Adnan Ali Shah, Balasubramanian Narasimhan. Benzoxazole derivatives: design, synthesis and biological evaluation. 2018;12:1-16.
5. Sudeesh K, Gururaja R. Facile Synthesis of Some Novel Derivatives of 1,3,4,-Oxadiazole Derivatives Associated with Quinolone Moiety as Cytotoxic and Antibacterial Agents. Organic ChemCurr Res. 2017;6:1-5.
6. SaloniKakkar, SumitTahlan, SiongMeng Lim, KalavathyRamasamy, Vasudevan Mani, Syed Adnan Ali, Shahand Balasubramanian Narasimhan. Benzoxazole derivatives: design, synthesis and biological evaluation. Chemistry central journal. 2018;12:92.
7. Jayanna ND, Vagdevi HM, Dharshan JC, Prashith Kekuda TR, Hanumanthappa BC, Gowdarshivannanavar BC. Synthesis and Biological Evaluation of Novel 5,7-Dichloro-1,3-benzoxazole Derivatives. Journal of Chemistry Hindawi. 2013; 1-9.
8. Ozan Gulcan, Serdar, Unlu, Erden Banoglu, Sahin MF. Synthesis of New 4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanamide derivatives and their Analgesic and Anti-Inflammatory Properties. Turk J.Chem. 2003;27:467–476.
9. Vijesh AM, Arun Isloor M, PrashanthShetty , Sundershan S , Hoong Kun Fun. New pyrazole derivatives containing 1, 2, 4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. European Journal of Medicinal Chemistry. 2013;62:410-415.
10. HemalathaGadegoni, SarangapaniManda, ShivaprasadRangu. Synthesis and Screening of Some Novel 2-[5-(Substituted phenyl)[1,3,4]oxadiazol-2-yl]-benzoxazoles as Potential Antimicrobial Agents. Journal of the Korean Chemical Society. 2013;57(2):221-226.
11. Wael A. El-Sayeda, Farag A. El-Essawyb, Omar M. Alib, Barsis S. Nasrb, Mohamed M. Abdallac, Adel A, Abdel-Rahmanb H. Anti-HIV Activity of New Substituted 1, 3, 4-Oxadiazole derivatives and their Acyclic Nucleoside Analogues. Journal of Biosciences. 2009;64(c):773-778.
12. Nadia Abdalla Khalil, AliaaMoh Kamal, Soha Hussein Emam. Design, Synthesis, and Antitumor Activity of Novel 5-Pyridyl-1, 3, 4 oxadiazole Derivatives against the Breast Cancer Cell Line MCF-7. biol. pharm. Bull. 2015;38:763-773.
13. Rakesh R, Somani, Anuj G, Agarwal, Pushkar P, Kalantri, Pratibha S, Gavarkar, Erik De Clercq. Investigation of 1, 3, 4 oxadiazole Scaffold as potentially active compounds. International journal of Drug Design and Discovery.2011;2(1):353-360.
14. Raghunath B.Toche, Ravindra A, Janaro. Synthesis,Characterization and antimicrobial evaluation of novel urea, sulfonamide and acetamide 3,4-dihydropyrazino[1,2-α]indol-1(2H)-one derivatives. Arabian journal of chemistry. 2015;12(8):1-11.
15. Meghashree KS, Latha KP. Potential antimicrobial activity of (*Amischophacelus axillaris* L.) (Commelinaceae) against certain bacterial species. Plant Archives. 2020;20:2920-2922.
16. Mounyr Balouiri N, Moulay Sadiki, Saad Koraichi Ibnsouda. Methods for in vitro evaluating antimicrobial activity: A review.

Journal of Pharmaceutical Analysis. 2016;6:71-79.

17. Mahbubur Rahman MD, Badrul Islam MD , Mohitosh Biswas, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Research Notes.2015;8:621.
18. Mohammed Shafeeulla R, Krishnamurthy G, Bhojynaik HS, Shivarudrappa HP, Yallappa Shiralgi. Spectral thermal cytotoxic and molecular docking studies of N′-2-hydroxybenzoyl; pyridine-4-carbohydrazide its complexes. BSU. J. BAS. 2017;6(1):1-13.
19. Mohammed Shafeeulla R, Krishnamurthy G, Bhojynaik HS, Manjuraj T. Synthesis, Cytotoxicity, and Molecular Docking Study of Complexes Containing Thiazole Moiety. JOTCSA. 2016;4(3):787-810.
20. Canetti J, Rist E, Grosset R. Pneumology. 1963;27:217.
21. Franzblau, Witzig SG, McLaughlin RS, Torres JC, Madico P, Hernandez G, Degnan A, Cook MT, Quenzer MB, Ferguson VK, Gilman RM, Rapid RH. Low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. J. Clin. Microbiol. 1998;36(2) :362-366.
22. Vanitha, J. D., Paramasivan, C. N. Mycobacteriology 2004;49:179.
23. Roberto Reis S, Ivan NevesJr, Sergio L. S. Lourenc¸o, Leila S. Fonseca, Maria Cristina S. Lourenc¸o. Comparison of Flow Cytometric and Alamar Blue Tests with the Proportional Method for Testing Susceptibility of Mycobacterium tuberculosis to Rifampin and Isoniazid. J. Clin. Microbiol. 2004;42(5):2247-2248.
24. Maria Lourenço CS, Marcus de Souza VN, Alessandra Pinheiro C, Marcelle de L. Ferreira, Raoni Gonçalves SB, Thais Cristina Nogueira M, Mônica Peralta A. Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC. 2007;15:181-191.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74020