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Research Paper

Synthesis and antimicrobial activity of some methyl (4-(benzo[*d*]oxazol-2-yl)phenyl) carbamodithioate amine derivatives

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ABSTRACT: *In the present study, a new series of methyl (4-(benzo[d]oxazol-2-yl)phenyl) carbamodithioate amine derived from methyl (4-(benzo[d]oxazol-2-yl)phenyl) carbamodithioate (4TO1-TO6) have been synthesized by reacting the thio methyl group with different amines in presence of ethenol. The structural assessment of the compounds (TO 1- TO 6) was made on the basis of spectral data. The synthesized compounds were screened for their in vitro growth inhibiting activity against different strains of bacteria and fungi viz., Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, S. aureus, Candida albicans and Cryptococcus neoformans were compared with standard agents such as Norfloxacin (10µg/ml) and Amphotericin B (10µg/ml) using broth dilution method. Compounds exhibit moderate to high antibacterial and antifungal activity.*

Keywords*: Synthesis, Benzoxazole, Thiourea, Antimicrobial Activity, Antibacterial*

I. INTRODUCTION

The chemistry of thioureas and their derivatives has attracted lot of attention due to their interesting physicochemical properties. The synthetic ease of thioureas, and use of inexpensive chemicals and reagents in synthesis and their wide range of pharmaceutical application has made them potential moiety for designing of new compounds. The modification that may be done on either nitrogen atoms of thiourea enhances the physical and chemical properties and their biological activities. Benzoxazoles possess most remarkable and a wide range of biological activities[1]. The 2-substituted benzoxazoles have been shown to exhibit antimicrobial[2-3], fungicidal [4], analgesic[5], insecticidal, antiviral [6], anticonvulsant [7] and anticancer [8] activities and serve as topoisomerase I poisons. In the last few years, it has been reported that 2, 5-disubstituted benzoxazoles, benzimidazoles, thiocarbazides and thiocarbamides and oxazoles have potent antimicrobial activities against some Gram-positive, Gram-negative bacteria and the yeast Candida albicans, providing a wide variety of in-vitro antimicrobial effects, especially indicating significant activity against the enterobacter Pseudomonas aeruginosa. These examples highlight the level of interest in new synthetic approaches to benzoxazole derivatives and have prompted researches around the globe to synthesize and explore the wide applicability of this important pharmacophoric scaffold.

Thiocarbamide, thiourea derivatives have biological activity such as antibacterial [9,10] , antimicrobial [11] antioxidant [12] anti HIV [13,14] anti malarial [15], and anticancer [16]. Substituted thiourea are useful catalysts for organic synthesis, the phenomenon is called thioureaorgano catalysis[17].

II. MATERIALS AND METHODS

The identification and purity of the products was checked by TLC with different combination and strength of mobile phases, i.e. hexane: ethyl acetate (2:8) or methanol: chloroform (1:9) using iodine vapours and UV light as detecting agents. Melting points were measured in open capillaries in a liquid paraffin bath and are uncorrected. IR Spectra were recorded on a SHIMADZU FTIR 8400 Spectrophotometer using potassium bromide pellets. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer/ Data System using Argon/Xenon (6kV, 10 m A) as the FAB gas. The NMR spectra were recorded on Bruker DRX-300 spectrometer and Elemental analysis was performed on Elemental Vario EL III analyzer. All the chemicals used were of synthetic grade and were procured from S.D. Fine Chem. Ltd and Merck, Mumbai, India.

2.1 Synthesis of benzoxazole derivatives of thiourea

2.1.1 Synthesis of 4-Benzoxazol-2-yl-phenylamine:

Equimolar quantities of o-aminophenol (0.002mol, 218 mg) and p-amino benzoic acid (0.002 mol, 274 mg) were mixed with polyphosphoric acid (10 ml) in a FBF and a stirrable paste was prepared and refluxed on dimmer-stat. The reaction mixture was heated slowly to 200°C. Heating was continued for 4 hours at 200°C (± 5°C). At the end of the reaction, the resulting solution was cooled to 100°C and then poured on crushed ice with constant stirring. The product was extracted using ethyl acetate and then washed with dilute solution of 10% sodium bicarbonate, then with brine and citric acid solution. Ethyl acetate portion was concentrated and the residue was decolourized and purified by passing through a silica gel column to get the 4-Benzoxazol-2-yl-phenylamine which was recrystallized from ethyl alcohol. The characterization data, yield and melting point of the product was determined and is summarized below.

Yield: 87.38%; Melting range: 160-164°C; IR (KBr, cm^{-1}): 3193, 2921(Aromatic C-H $_{str}$),1606, 1498, 1454 (Aromatic C=C $_{str}$), 746 (Meta substituted Benzene), 1290 (Asymmetric C-O-C $_{str}$), 1054 (5-Membered C-O $_{str}$), 1311(Tertiary aromatic amine C-N $_{str}$) 3471, 3299 (Primary amine); MS (FAB) m/z: 210 (M^+), 211 (M^++1).

1.1.2 Synthesis of dithiocarbamate derivative

To a solution of 0.15 moles of KOH in ethanol and 0.15 moles of 4-Benzoxazol-2-yl-phenylamine was added 0.15 moles of CS_2. The mixture was diluted with ethanol and stirred at room temperature for 12-16 h. the mixture was then neutralized with conc. HCl and the resulting precipitate was filtered off, washed with water and recrystallized form ethanol.

1.1.3 Synthesis of methyl (4-(benzo[d]oxazol-2-yl)phenyl) carbamodithioate

In a 100 mL round bottom flask, 0.0275 moles of the product of previous step, 0.038 moles of sodium carbonate and 0.042 moles of dimethyl sulfate were placed. The mixture was heated substantially until the temperature reached 75°C and the mixture started to liquefy. Heating was continued for 30 min at this temperature and then the temperature was slowly increased to 85-87°C. At this temperature the mixture started to thicken; 30 mL of water was added slowly to the mixture maintaining the temperature at 85-87°C and continued heating for another 1.5 h. On completion of the reaction as monitored by TLC, 250 mL of hot water was added to the mixture with stirring. The mixture was allowed to cool and the solid obtained was filtered at pump and washed with water to obtain the product.

1.1.4 Synthesis of thiourea derivative of benzoxazole (TO 1- TO 6)

To 0.1 methyl (4-(benzo[d]oxazol-2-yl)phenyl) carbamodithioate was added 0.15 mole of appropriate amine in ethanol and the mixture was refluxed for 2-3 h with continuous stirring. The product obtained was filtered, washed with ethanol, dried and characterized.

2.1.4.1 3-(4-(benzo[d]oxazol-2-yl) phenyl)-1-methyl-1-phenylthiourea, TO 1:

Yield: 71.56%; Melting Range: 280-286°C; IR (KBr, cm^{-1}): 2935 (Aromatic C-H $_{str}$), 1575, 1496, (Aromatic C=C $_{str}$), 1610 (HC=N $_{str}$) 1247 (Asymmetric C-O-C $_{str}$), 1134 (5-Membered C-O $_{str}$), 1228.50 (C=S $_{str}$); MS (FAB) m/z **:** 359.11 (M^+), 359, 36.0; 1H-NMR(δ ppm): 7.7, 7.5 (CH Benzoxazole) , 6.9, 6. (CH aromatic); Anal. Calcd for $C_{21}H_{17}N_3OS$ C, 70.17; H, 4.77; N, 11.69; O, 4.45; S, 8.92; found: C, 69.56; H, 4.64; N, 10.92; O, 4.11; S, 7.29;

2.1.4.2 1-(4-(benzo[d]oxazol-2-yl) phenyl)-3-p-tolylthiourea, TO2 :

Yield: 83%; Melting Range: 296-300°C; IR (KBr, cm^{-1}): 2836 (Aromatic C-H $_{str}$), 3611 (N-H $_{str}$), 1699.57 (HC=N $_{str}$), 1587, 1488,1454 Aromatic C=C $_{str}$), 1410 (Asymmetric C-O-C $_{str}$), 1051 (5-Membered C-O $_{str}$), 1258 (C=S_{st}) ; MS (FAB) m/z **:** 315 (M^++1); 1H-NMR (δ ppm): 7.7, 7.5 (CH Benzoxazole) , 6.9, 6.3 (CH aromatic), 8.8 (1H CH), 7.1, 7.5, 7.6(4H benzylidine), 2.39 (CH_3). Anal. Calcd for $C_{21}H_{17}N_3OS$ was C, 70.17; H, 4.77; N, 11.69; O, 4.45; S, 8.92; found C, 69.17; H, 4.47; N, 11.42; O, 4.24; S, 8.86;

2.1.4.3 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-benzylthiourea, TO3:

Yield: 65.25%; Melting Range: 286-298°C; IR (KBr, cm^{-1}): 3089 (Aromatic C-H $_{str}$), 1614 (HC=N $_{str}$), 1610 (Aromatic C-C_{str}), 3089 (C-H aromatic) 1051 (5-Membered C-O $_{str}$), 1325 (Tertiary aromatic amine C-N $_{st}$), 1132 (C-O) 1224 (C=S) ; MS (FAB) m/z **:** 360.1 (M^++1); 1H-NMR (δ ppm): 7.2, 7.9 (4H Benzoxazole) , 6.67 (4H aromatic), 4.01 (C-NH aromatic). Anal. Calcd for $C_{21}H_{17}N_3OS$ was C, 70.17; H, 4.77; N, 11.69; O, 4.45; S, 8.92; found C, 70.07; H, 4.68; N, 11.65; O, 4.31; S, 8.76;

2.1.4.4 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea, TO4 :

Yield: 73.4 %; Melting Range: 276-284°C; IR (KBr, cm^{-1}): 2978 (Aromatic C-H $_{str}$), 1379 (C-N $_{str}$), 3222 (N-H $_{str}$), 1649 (C=N $_{str}$),1610 (Aromatic C-C $_{str}$), 3085 (C-H aromatic) 1578 (N=O $_{str}$), 862 (C-NO_2 $_{str}$), 1054 (5-Membered C-O $_{str}$),1134 (C-O) 1224 (C=S) ; MS (FAB) m/z **:** 390.1 (M^++1); 1H-NMR (δ ppm): 7.56, 7.76 (4H Benzoxazole) , 6.9 (CH Naphthalene) 6.63 (CH Benzene) 4.01 (C-NH aromatic); Anal. Calcd for $C_{20}H_{14}N_4O_3S$ C, 61.53; H, 3.61; N, 14.35; O, 12.29; S, 8.21; found C, 61.43; H, 3.45; N, 14.21; O, 12.16; S, 8.09;

2.1.4.5 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(naphthalene-1-yl)thiourea, TO5

Yield: 67.4 %; Melting Range: 278-288°C; IR (KBr, cm^{-1}): 2978 (Aromatic C-H $_{str}$), 1666 (HC=N $_{str}$), 1612 (Aromatic C-C $_{str}$), 1054 (5-Membered C-O $_{str}$), 1320 (Tertiary aromatic amine C-N $_{st}$) 1141 (C-O) 1228 (C=S) ; MS (FAB) m/z **:** 396 (M^++1); 1H-NMR (δ ppm): 8.07, 7.74 (4H Benzoxazole) , 6.9 (CH Naphthalene) 6.66 (CH Benzene) 4.01 (C-NH aromatic); Anal. Calcd for $C_{24}H_{17}N_3OS$ was C, 72.89; H, 4.33; N, 10.63; O, 4.05; S, 8.11; found C, 72.45; H, 4.12; N, 10.23; O, 3.96; S, 8.09;

2.1.4.6 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(pyridin-2-yl)thiourea, TO6

Yield: 82 %; Melting Range: 270-280°C; IR (KBr, cm^{-1}): 1379 (C-N), 3222.59 (H-N_{str}), 1644.55 (C=N_{str}), 1613 (C-C Aromatic), 1139 (C=O), 1053 (5-Membered C-O_{str}) 1222 (C=S_{str}); MS (FAB) m/z **:** 346 (M^+), 347(M^++1); 1H-NMR (δ ppm): 8.07 (CH Pyridine) 7.7, 7.5 (4H Benzoxazole) , 7.6, 7.9 (4H aromatic), 8.4 (1H CH), 4.01(C-NH Aromatic). Anal. Calcd for $C_{19}H_{14}N_4OS$ was C, 65.88; H, 4.07; N, 16.17; O, 4.62; S, 9.26; **f**ound C, 65.46 H, 3.97; N, 16.12; O, 4.43; S, 9.36;

Screening For Antimicrobial Activity

The antimicrobial potential of the synthesized compounds has been evaluated by determining the minimum inhibition concentration values by broth dilution method using Mueller-Hinton broth for culturing the pathogen. MIC is the minimum concentration of the test compound that exhibits no growth of microorganism. Both gram negative and gram positive bacteria were used in the study, as much of the resistance has been acquired by the gram positive bacteria against the currently available antibiotics. Though the resistant strains were not used in the study, the preliminary investigations reveal high potential of the synthesized compounds against all the four tested microorganisms. The results obtained indicate the role of nucleus in the antibacterial potential of the compounds.

On confirmation of the identity of the synthesized compounds, all the thiourea derivatives were subjected for antibacterial evaluation in vitro. The IC_{50} value of the compounds against gram negative and gram positive bacterial cultures is presented in table

Antibacterial and Antifungal potential of the thiourea derivatives

Compound	MIC (µg/mL)[a,b,c]			
	E.coli	**B. subtilis**	**P. aeuroginosa**	**S. aureus**
TO1	12.5	25	6.25	6.25
TO2	12.5	25	6.25	6.25
TO3	6.25	6.25	6.25	6.25
TO4	25	25	12.5	6.25
TO5	50	6.25	25	25
TO6	6.25	6.25	12.5	12.5
Norfloxacin	2.56	1.28	1.28	1.28

[a] A set of tubes with only the inoculated broth was used as control to determine MIC

[b]MIC is expressed by measuring the turbidity of test and control dilution tubes. A 50% decrease in turbidity was taken as MIC.

[c] All values are expressed as mean of a set of three experiments

The ED50 values obtained on the antifungal evaluation of the synthesized compounds are presented

Antifungal potential of the thiourea derivatives

Compound	ED_{50} values (µg/mL)	
	Candida albicans	**Cryptococcus neoformans**
TO1	580	760
TO2	540	575
TO3	550	450
TO4	230	330
TO5	475	650
TO6	530	700
Amphotericin B	95	75

III. RESULTS AND DISCUSSION

In the present study, a new series of methyl (4-(benzo[d]oxazol-2-yl)phenyl) carbamodithioate amine derived from methyl (4-(benzo[d]oxazol-2-yl)phenyl) carbamodithioate (4TO1-TO6) have been synthesized by reacting the thio methyl group with different amines in presence of ethenol. The starting material 4-Benzoxazol-2-yl-phenylamine was synthesized by condensation of o-aminophenol and p-amino benzoic acid, catalyzed by polyphosphoric acid. The structural assessment of the compounds (TO 1- TO 6) was made on the basis of spectral data. The synthesized compounds were screened for their in vitro growth inhibiting activity against different strains of bacteria and fungi viz., Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, S. aureus, Candida albicans and Cryptococcus neoformans were compared with standard agents such as Norfloxacin (10µg/ml) and Amphotericin B (10µg/ml) using broth dilution method. Compounds exhibit moderate to high antibacterial and antifungal activity. Compounds TO 3 and TO 6 exhibit highest antibacterial activity and compounds TO 4 and TO5 showed good antifungal activity.

IV. CONCLUSION

The biological evaluation of synthesized compounds of benzoxazole thiourea derivatives show moderate to high degrre of antimicrobial as well as antifungal activity, but no one is better than standard drug norfloxacin and amphotericin B. The synthesized compounds 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-benzylthiourea, and 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(pyridin-2-yl)thiourea show better antimicrobial activity. The compounds 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea and 1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(naphthalene-1-yl)thiourea shows better antifungal activity. These study provide researchers to synthesise more derivatives and evaluate biological activity including antimicrobial activity.

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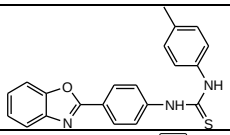
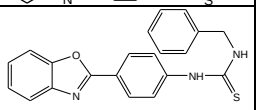
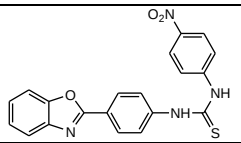
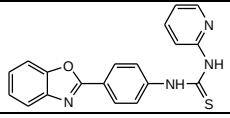
Table 1 Summary of the synthesized compounds

Compound	Structure	Moleculer ormulae	Molecular Mass	% Yield
3-(4-(benzo[d]oxazol-2-yl)phenyl)-1-methyl-1-phenylthiourea, TO1		$C_{21}H_{17}N_3OS$	359.11	71.56
1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-p-tolylthiourea, TO2:		$C_{21}H_{17}N_3OS$	359.11	83.0
1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-benzylthiourea, TO3:		$C_{21}H_{17}N_3OS$	359.11	65.25
1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea, TO4		$C_{20}H_{14}N_4O_3S$	390.08	73.40
1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(naphthalene-1-yl)thiourea, TO5		$C_{24}H_{17}N_3OS$	395.11	67.40
1-(4-(benzo[d]oxazol-2-yl)phenyl)-3-(pyridin-2-yl)thiourea, TO6		$C_{19}H_{14}N_4OS$	346.09	82.00

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