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Synthesis and Antimicrobial Activity of some Methyl -4-(Benzo[*D*] Thiazol-2yl) Phenylcarbamodithioate Amine Derivatives

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Abstract:
In the present study, a new series of Methyl -4-(benzo[d]thiazol-2yl) phenylcarbamodithioate amine derived from Methyl -4-(benzo[d]thiazol-2yl) phenylcarbamodithioate (4TT1-TT6) have been synthesized by reaction of the thio methyl group with different amines in presence of ethanol. The structural assessment of the compounds (TT1- TT6) was made on the basis of spectral data. The synthesized compounds were screened for their in vitro growth inhibiting activity against strains of bacteria and fungi viz., *Bacillus subtilis* and *Candida albicans* were compared with standard agents such as Norfloxacin and Fluconazole using well plate method. Compounds exhibit moderate to high antibacterial and antifungal activity.
Keywords: Antimicrobial activity, Benzothiazole, Heterocyclic, Synthesis, Thiourea.

INTRODUCTION

The chemistry of thioureas and their derivatives has attracted a 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. Benzothiazole derivatives widely used in pharmaceutical industry [1], medicinal chemistry and drug development. Derivatives of benzothiazole have numerous biological activities such as antimicrobial [2-3], antibacterial [4], antifungal [5], anticancer [6], anti-inflammatory [7], anticonvulsant [8], antidiabetic [9], antipsychotic [10], protein tyrosine inhibitor [11] and diuretic [12], properties. Nitrogen-containing heterocyclic systems have a diverse spectrum of pharmacological properties. Different heterocyclic motifs can be incorporated to produce molecules with enhanced biological properties. A review of the literature suggests that there is the scope for the design of additional benzothiazole derivatives with antimicrobial activity, by examining the effect of a number of different functional groups. Thiocarbamide, thiourea derivatives have biological activity such as antibacterial [13-14] antimicrobial [15] antioxidant [16] anti HIV [17-18] anti malarial [19] and anticancer [20]. The review of benzothiazole and thiourea motivate to synthesize such derivatives.

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 (2:6) or methanol: chloroform (1:8) using iodine vapour and UV light as detecting agents. Melting points were taken in open capillaries in an electric melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer with DMSO and CDCl3 as solvents and TMS as internal standard. The chemical shifts were expressed in δ (ppm) values. The IR spectra were recorded on a Bruker alpha FT-IR spectrophotometer. The Mass spectra were recorded in terms of mass to charge ratio (m/z) on Waters LCMS-MS. All the chemicals used were of synthetic grade and were procured from S.D. Fine Chem. Ltd and Merck, Mumbai, India.

EXPERIMENTAL

Synthesis of Benzothiazole Thiourea Derivatives: Synthetic Scheme and all synthesise compounds are summarise in table1 and table 2 respectively.

Synthesis of 4-(benzo[d]thiazol-2-yl) benzenamine:
Equimolar quantities of of 2-aminobenzenethiol (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. The reaction mixture was heated slowly to 180°C. Heating was continued for 4 hours at 180°C (± 6°C). At the end of the reaction was cooled to 100°C and then poured on crushed ice with constant stirring. The product 4-(benzo[*d*]thiazol-2-yl) benzenamine was recrystallized from ethyl alcohol.

Synthesis of (4-benzo[d]thiazol-2-yl) phenyl) carbamodithioic acid:
To a solution of 0.15 moles of KOH in ethanol and 0.15 moles of 4-(benzo[*d*]thiazol-2-yl) benzenamine 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.

Synthesis of Methyl -4-(benzo[d]thiazol-2yl)phenylcarbamodithioate:
In a 100 mL round bottom flask, 0.0275 moles of (4-benzo[*d*]thiazol-2-yl)phenyl) carbamodithioic acid, 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.

Synthesis of amine derivative of Methyl -4-(benzo[d]thiazol-2yl) phenylcarbamodithioate (TT1-TT6):
To 0.1 Methyl -4-(benzo[*d*]thiazol-2yl)phenylcarbamodithioate was added 0.15 mole of appropriate amine (N-methylbenzamine , p-toiuidine, phenylmethanamine, 4-nitrobenzamine, naphthalene-1-amine, pyridine-2-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.

3-(4-(1H-benzo[d]imidazol-2-yl)-1-methyl-1-phenylthiourea, TT1:
Yield: 77%; Melting Point: >300°C; IR (KBr, cm-1): 1386.01(C-N str), 3511.13 (N-H str), 1687.04 (Aromatic C=N str), 1600.37 (C-C Aromatic) 3125.64 (C-H str), 2700.58 (CH_3 –N), 628.45 (C-Sstr), 1211.51 (C=S str); MS (FAB) m/z : 375.1, 376.2, 377; 1H-NMR(δ ppm): 8.18 (CH benzothiazole), 7.76 (CH benzothiazole), 6.77 (CH benzene), 4.01 (C-NH aromatic), 3.43 (CH_3).

1-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-3-p-tolylthiourea, TT2:
Yield: 80%; Melting Point: >300°C; IR (KBr, cm-1): 1376.01(C-N str), 3125.64 (N-H str), 1697.04 (Aromatic C=N str), 1620.33 (C-C Aromatic), 3030.61 (C-H str), 755.61 (C-str), 1211.31 (C=S str); MS (FAB) m/z **:** 375.20, 376.5; 1H-NMR (δ ppm): 8.17 (CH benzothiazole), 7.94 (CH benzothiazole), 6.96 (CH benzene), 4.02 (C-NH aromatic), 3.42 (CH_3).

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-benzylthiourea, TT3:
Yield: 66%; Melting Point: >300°C; IR (KBr, cm-1): 1386.01(C-N str), 3125.64 (N-H str), 1687.04 (Aromatic C=N str), 1600.33 (C-C Aromatic), 3000.61 (C-H str), 756.51 (C-S str), 1211.51 (C=S str); MS (FAB) m/z **:** 375.20 (100%) 376; 1H-NMR (δ ppm): 8.18 (CH benzothiazole), 7.73 (CH benzothiazole), 6.68 (CH benzene), 4.01 (C-NH aromatic), 4.71 (CH_3), 2.01 (NH aromatic).

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea, TT4:
Yield: 62 %; Melting Point: >300°C; IR (KBr, cm-1): 1396.01(C-N str), 3125.64 (N-H str), 1687.04 (Aromatic C=N str), 1600.31 (C-C Aromatic), 30030.51 (C-H str), 1532.62 (N=O str), 887.50 (C-NO_2 str), 756.61 (C-S str), 1211.31 (C=S str); MS (FAB) m/z **:** 406.10(100%), 407.2, 408.1; 1H-NMR (δ ppm): 8.14 (CH benzothiazole) 7.73 (CH benzothiazole), 6.70 (CH benzene) 4.01 (C-NH aromatic).

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-(naphthalen-1yl)thiourea, TT5:
Yield: 78 %; Melting Point: >300°C IR (KBr, cm-1): 1342.50 (C-N str), 3136.67 (N-H str), 1650.43 (Aromatic C=N str), 1600.72 (C-C Aromatic), 3000.36 (C-H str), 736.08 (C-S str), 1313.54 (C=S str); MS (FAB) m/z **:** 441.10 (100%) 442.2, 443; 1H-NMR (δ ppm): 8.16 (CH benzothiazole), 7.56 (CH benzothiazole), 6.99 (CH benzene), 4.01 (C-NH aromatic).

1-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-3-(pyridin-2-yl)thiourea, TT6:
Yield: 62 %; Melting Point: >300°C; IR (KBr, cm-1): 1386.01(C-N str), 3125.64 (N-H str), 1687.04 (Aromatic C=N str), 1600.33 (C-C Aromatic) 3070.61 (C-H str), 756.51 (C-Sstr), 1211.31 (C=S str); MS (FAB) m/z **:** 407.1 (100%), 408; 1H-NMR (δ ppm): 8.18 (CH benzothiazole), 7.77 (CH benzothiazole), 6.69 (CH benzene), 4.02 (C-NH aromatic), 8.07 (CH pyridine).

Table 1 **Synthetic Scheme**

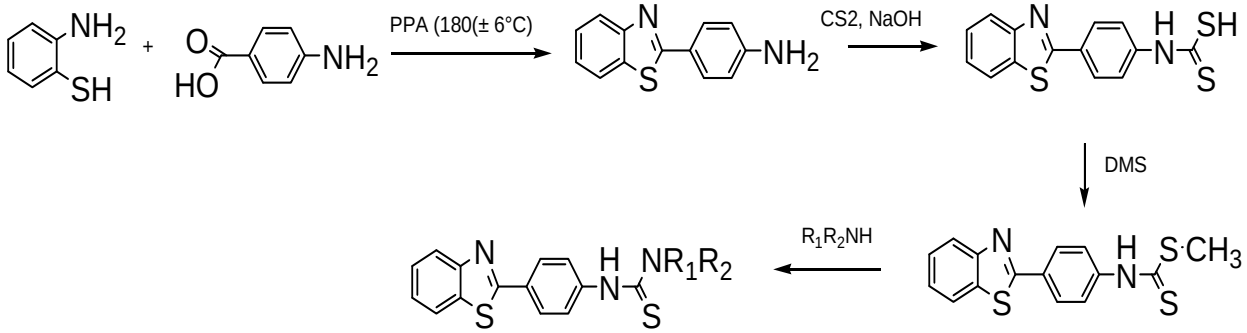
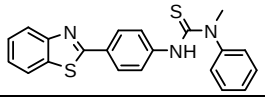
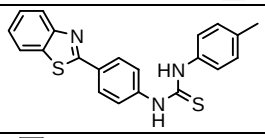
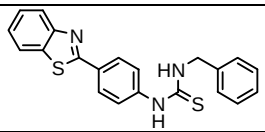
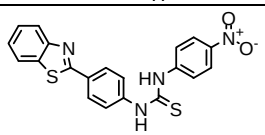
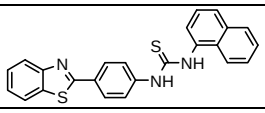
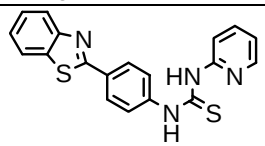


Table 2 **Summary of the Synthesized Compounds:**

Compound	Structure	Moleculer Formulae	Molecular Mass	% Yield
3-(4-(benzo[*d*]thiazol-2-yl)phenyl)-1-methyl-1-phenylthiourea, TT1:		$C_{21}H_{17}N_3S_2$	375.10	77
1-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3-*p*-tolylthiourea, TT2:		$C_{21}H_{17}N_3S_2$	375.20	80
1-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3-benzylthiourea, TT3:		$C_{21}H_{17}N_3S_2$	375.20	66
1-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea, TT4:		$C_{20}H_{14}N_4O_2S_2$	406.10	62
1-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3-(naphthalen-1-yl)thiourea, TT5:		$C_{24}H_{17}N_3S_2$	441.10	78
1-(4-(benzo[*d*]thiazol-2-yl)phenyl)-3-(pyridin-2-yl)thiourea, TT6:		$C_{19}H_{14}N_4S_2$	407.10	62

SCREENING FOR ANTIMICROBIAL ACTIVITY

The well diffusion method was used to determine the antimicrobial activity of the synthesized compound using standard procedure [21]. The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient and potato dextrose broth than incubated for 24 hours at 37^{o}C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the agar plates with loop full of microbes and further incubated for next 24 hours at 37^{o}C to obtain the pure culture and stored as stocks that are to be used in further research work. There were 3 concentration used which are 25 and 12.5, 6.25 µg/ml for each synthesized compound in antibiogram studies. The placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The antimicrobial screening results are presented in Table 3-4. The zone of inhibition of synthetic compounds and standard against bacteria and fungus are shown in Fig.1 and Fig.2.

Table 3 **Antibacterial Activity of Synthetic Compounds on *Bacillus Subtilis***

S.No	Compounds	Zone of inhibition In mm Mean for *Bacillus subtilis* at different concentration (µg/ml)		
		25 µg/ml	12.5 µg/ml	6.25 µg/ml
1	TT1	21±0.03	15±0.09	08±0.07
2	TT2	18±0.03	11±0.01	08±0.03
3	TT3	10±0.03	09±0.02	08±0.11
4	TT4	25±0.13	19±0.09	17±0.07
5	TT5	13±0.03	11±0.08	10±0.17
6	TT6	10±0.03	08±0.19	07±0.04
7	Standard	25±0.04	21±0.09	13±0.11

Table 4 Antifungal Activities of Compounds on *Candida Albicans*

S.No	Compounds	Zone of inhibition In mm Mean for *Candida Albicans* at different concentration (µg/ml)		
		25 µg/ml	12.5 µg/ml	6.25 µg/ml
1	TT1	21±0.13	17±0.09	12±0.17
2	TT2	22±0.03	17±0.09	12±0.05
3	TT3	19±0.03	17±0.19	13±0.07
4	TT4	24±0.13	16±0.09	15±0.04
5	TT5	20±0.13	14±0.29	11±0.07
6	TT6	23±0.03	21±0.09	20±0.06
7	Standard	28±0.11	20±0.09	16±0.04

Fig. 1 **Antibacterial Study of Synthetic Compounds on *Bacillus Subtilis***

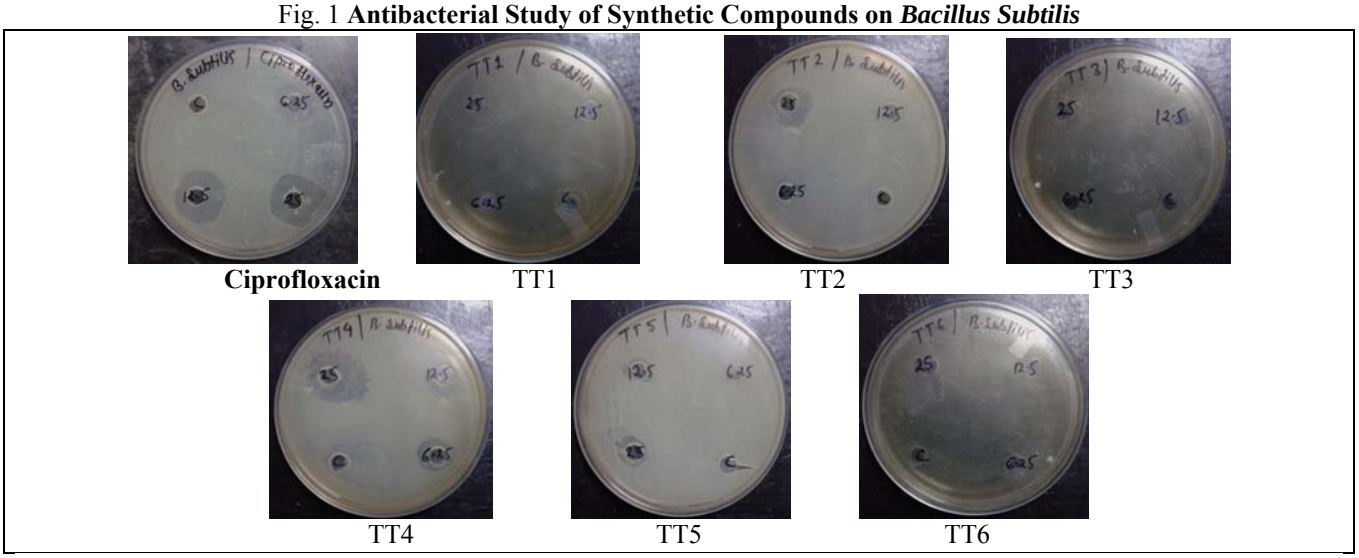
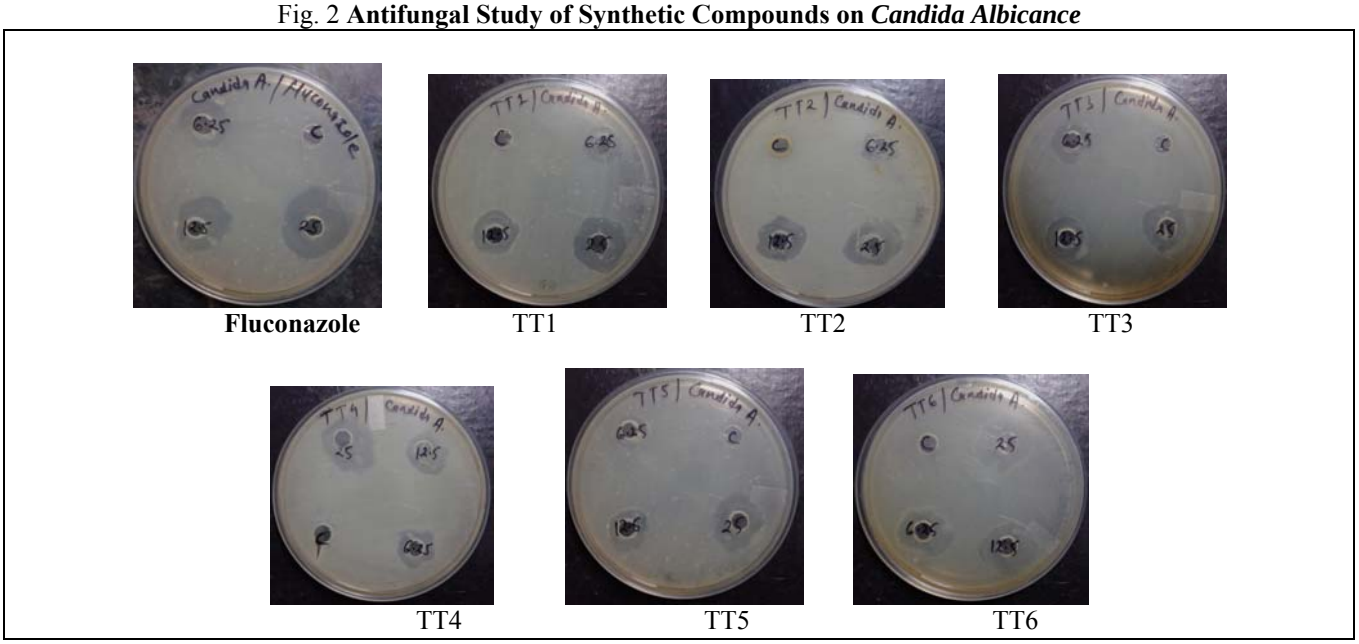


Fig. 2 **Antifungal Study of Synthetic Compounds on *Candida Albicance***



RESULTS AND DISCUSSION

In the present study, a new series of Methyl -4-(benzo[*d*]thiazol-2yl)phenylcarbamodithioate amine derived from Methyl -4-(benzo[*d*]thiazol-2yl)phenylcarbamodithioate (4TT1-TT6) have been synthesized by reacting the thio methyl group with different amines in presence of ethanol. The starting material 4-(benzo[*d*]thiazol-2-yl) benzenamine was synthesized by condensation of 2-aminobenzenethiol and *p*-amino benzoic acid, catalyzed by polyphosphoric acid. The structural assessment of the compounds 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., *Bacillus subtilis* and *Candida albicans*; were compared with known antibiotics Ciprofloxacin and Fluconazole against bacterial and fungal strains. Compounds exhibit moderate to high antibacterial and antifungal activity. Compounds TT1 and TT4 exhibit highest antibacterial activity other are moderate antibacterial activity. The compounds TT3, TT4 and TT6 having antifungal activity among them TT4 shows highest antifungal activity.

CONCLUSION

The biological evaluation of synthesized compounds of benzothiazole thiourea derivatives show moderate to high degree of antimicrobial as well as antifungal activity, but no one is better than standard drug norfloxacin and fluconazole. The synthesized compounds 1-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea and 3-(4-(1H-benzo[d]imidazol-2-yl)-1-methyl-1-phenylthiourea shows highest antibacterial activity. 1-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea shows highest antifungal activity. These studies provide researchers to synthesize more derivatives and evaluate biological activity including antimicrobial activity.

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