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SYNTHESIS AND STUDY OF ANTIMICROBIAL ACTIVITY OF N, N'-DISUBSTITUTED ACYL THIOUREA DERIVATIVES

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ABSTRACT

N, N' disubstituted acyl thioureas are of crucial importance due to their wide range of biological activities such as antiinfluenza, antifungal, antibacterial and anti-inflammatory, etc. Six disubstituted acyl thioureas, say N'-(3-nitrobenzoyl)-N-(4-fluorophenyl)thiourea (*I*), N'-(3, 5-dinitrobenzoyl)-N-(benzyl)thiourea (*II*), N'-(4-methoxybenzoyl)-N-(4-fluorophenyl)thiourea (*III*), N'-(3,5-dinitrobenzoyl)-N-(3-hydroxyphenyl)thiourea (*IV*), N'-(4-methoxybenzoyl)-N-(3-hydroxyphenyl)thiourea(*V*) and N'-(4-nitrobenzoyl)-N-(3-hydroxyphenyl)thiourea (*VI*) have been synthesized and characterized by spectral analyses. Synthesized compounds were screened for their antimicrobial activity against *Staphylococcus aureus*, *Candida albicans* and *Enterococcus faecalis* by Minimum Inhibitory Concentration (MIC) method. Among the synthesized compounds, N'-(3,5-dinitrobenzoyl)-N-(3-hydroxyphenyl)thiourea (*IV*) was found to be as comparable to that of the Minimum Inhibitory Concentration of 2µg/ml and 1µg/ml against *Staphylococcus aureus* and *Candida albicans* as compared to standard amikacin and nystatin, respectively, while compound N'-(4-methoxybenzoyl)-N-(3-hydroxyphenyl)thiourea(*V*) was found effective against *Enterococcus faecalis* at the minimum inhibitory concentration of 31.25 µg/ml.

INTRODUCTION

Current antibiotics are facing lots of problems related to human health and safety as microorganisms against which these antibiotics act develops resistance. Since, emergence of resistant strains of microbial flora to the conventional antibiotics is increasing day by day; there is an enormous need to design effective antibiotic drugs **[1-3]**. Henceforth, there is an exceedingly greatest necessity of designing such antibiotics without developing their resistance, side effects and also possessing remarkable antibiotic activity. Among several antimicrobial agents developed, quite recently, thiourea derivatives have grabbed the attention of researchers due to their simple structure and easy methods of preparation. Antimicrobial activity of few thiourea derivatives and their corresponding nickel, copper complexes against gram-positive bacteria, gram-negative bacteria and in vitro anti-yeast activity had already been reported **[4]**. Thioureas with benzthiazole moiety showing antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* was reported in 2010 **[5]**. N, N'-disubstituted thioureas with 2-hydoxyphenyl thiourea as antimicrobial agents particularly against gram positive bacteria has also been reported **[6]**. The general formula of disubstituted thioureas designed as antimicrobials is given in **Figure 1**.

Andree *et al*. **[6]** has reported the synthesis of disubstituted thioureas with different R' groups such as heterocycles and substituted phenyl rings used and X with CO, SO_2, CH_2 and represented R_1, R_2 and R_3 with hydrogen, lower alkyl, chlorine, bromine, nitro and carboxyl **(Figure 1)**. Nielsen *et al*. **[7]** also discovered 3, 5-bis-trifluorophenyl thiourea derivatives as anti-infective agents against different species of *Staphylococcus aureus* and *Escherichia coli.* Based on the above literature survey, our work focuses on synthesis of acyl thioureas from 4-fluorophenyl thiourea, benzyl thiourea and 3-hydoxyphenyl thiourea. Six such acyl thioureas were characterized and screened for antimicrobial activity against *Staphylococcus aureus (gram positive bacteria)*, *Candida albicans (fungi)* and *Enterococcus faecalis (gram negative bacteria).*

EXPERIMENTAL DETAILS

Materials

Reaction was carried out between substrates as various aromatic anilines and various aromatic acid chlorides. Aromatic anilines such as 4-Fluoroanilline, Benzyl amine and 3-Hydroxyaniline were used, while, in case of, aromatic acid chlorides, 3-Nitrobenzoyl

chloride, 3, 5-Dinitrobenzoyl chloride, 4-Methoxybenzoyl chloride and 4-Nitrobenzoyl chloride were used.

Step 1 - Synthesis of Substituted Thioureas

It involves synthesis of substituted thiourea **(Figure 2)** followed by synthesis of acyl thiourea compounds. Substituted thioureas were prepared as follows: 4-fluoroaniline (1mole) and 3-aminophenol (1mole) are used for the preparation of 4-fluorophenyl thiourea and 3-hydroxyphenyl thiourea respectively. The starting compounds were mixed 15% HCl to convert the amino groups to their corresponding hydrochlorides. Aqueous solution of ammonium thiocyanate (1 mole) was then added mixed, boiled, until a viscous layer was formed. It was then poured in to crushed ice and the resultant products were recrystallized from alcohol.

Benzyl thiourea **[8]** (2 mole) was prepared from hydrochloride salt of benzylamine (0.5 moles) in 200 ml. of water, refluxed for about 45 minutes, cooled and filtered when fine crystals of disubstituted thiourea derivatives were obtained. Mixture of mono-substituted and di-substituted thiourea thus formed was separated by boiling with water.

Step 2 - Synthesis of disubstituted acyl thiourea derivatives

This involves the synthesis of disubstituted acyl thiourea derivatives as depicted in **Figure 3**. Sun *et al* **[9]** has reported the synthetic scheme for nicotinyl chloride and substituted furoyl chloride with disubstituted pyrimidinyl thiourea to give subsequent acyl thiourea derivatives. It was carried out as 1 mole of substituted thiourea from step 1 was mixed with 1.1 moles of aromatic acid chlorides as 3-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride ,4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride in the presence of acetonitrile and triethyl amine. It was refluxed for 3-4h and cooled, poured into crushed ice. Resultant precipitate was washed with saturated $NaHCO_3$ solution, dried and recrystallized with acetonitrile.

Analytical Measurements

IR spectra were recorded on JASCO 460 plus FT/IR spectrophotometer.^{1}H NMR spectra were recorded on Varian Mercury YH 300 FT-NMR spectrometer at 300 MHz frequency in deuteriated DMSO using TMS as internal standard. Mass spectra of the compounds were performed on Micro mass Q-Top, YA-105. Reactions were monitored by TLC plate on Silica gel G using ethyl acetate: n-hexane (4: 1) mobile phase for the first step and ethyl acetate: n-hexane (4: 6) mobile phase for the second step.

Antimicrobial Screening

Six N, N'-disubstituted acyl thiourea compounds were screened for antimicrobial

activity. They were screened by serial dilution method which determines minimum inhibitory concentration of compounds under study. Procedure for MIC determination was followed as per antimicrobial susceptibility testing protocols as, 9 dilutions of each drug was done with brain heart infusion broth for minimum inhibitory concentration. In the initial tube 20 µl of drug was added into the 380 µl of brain heart infusion broth. For dilutions 200 µl of brain heart infusion broth was added into the next 9 tubes separately. Then from the initial tube 200 µl was transferred to the first tube containing 200 µl of brain heart infusion broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 µl was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5 µl was taken and added into 2 ml of brain heart infusion broth. In each serially diluted tube, 200 µl of above culture suspension was added. The tubes were incubated for 24 h and observed for turbidity **[10]**.

RESULT AND DISCUSSION

Six acyl thiourea derivatives were synthesized and characterized by FT-IR, ^{1}H-NMR and mass techniques and the data are depicted in **Table 1**. The structures on the basis of general formula (a) is given in **Table 2**.

Occurrence of IR absorption band nearer to 1670 showed the presence of acyl (CO) group, while at 1084 showed presence of thio (C==S) group. ^{1}H NMR spectra of the synthesized compounds exhibited the presence of two aromatic rings, along with two different –NH groups of thioureas.

The compounds were screened for antimicrobial activity carried out against *staphylococcus aureus*, *candida albicans* and *enterococcus faecalis*. MIC was determined by serial dilution method and are shown in **Table 3**. A comparison was made with MIC of antibiotics against strains used. Acyl thioureas were effective against *s.aureus*, *c.albicans*, *e.faecalis.* They show MIC ranging from 1 µg/ml to 500 µg/ml. MIC for potent compounds 4 and 5 is also shown in **Figure 4, Figure 5 and Figure 6**. A Comparative antimicrobial activity of disubstituted acyl thioureas is represented graphically in **Figure 7**.

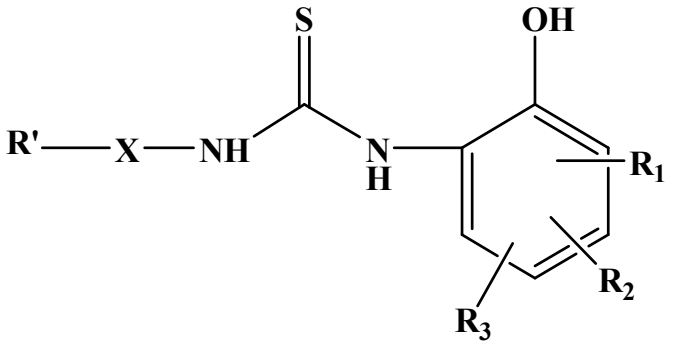

Figure 1: General formula of N, N' disubstituted thioureas designed as antimicrobials

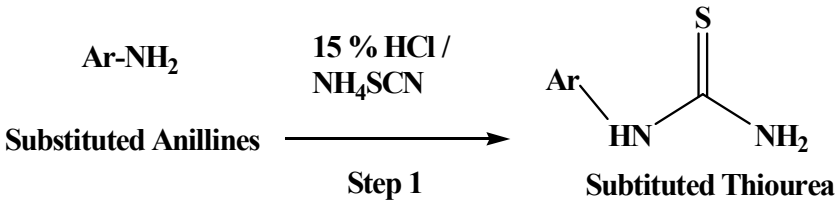

Figure 2: General reaction for synthesis of substituted thioureas

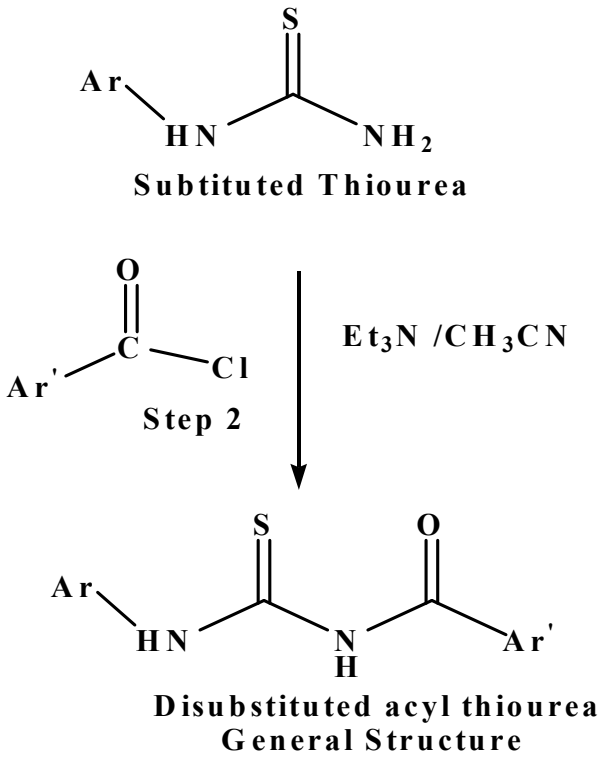

Figure 3: General reaction for synthesis of disubstituted acyl thioureas

Figure 4: MIC of 4 against *Candida albicans*

Figure 5: MIC of 5 against *e.faecalis*

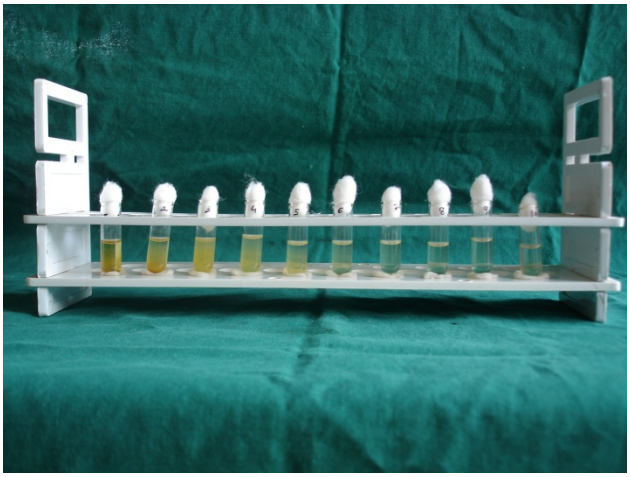

Figure 6: MIC of 4 against *s.aureus*

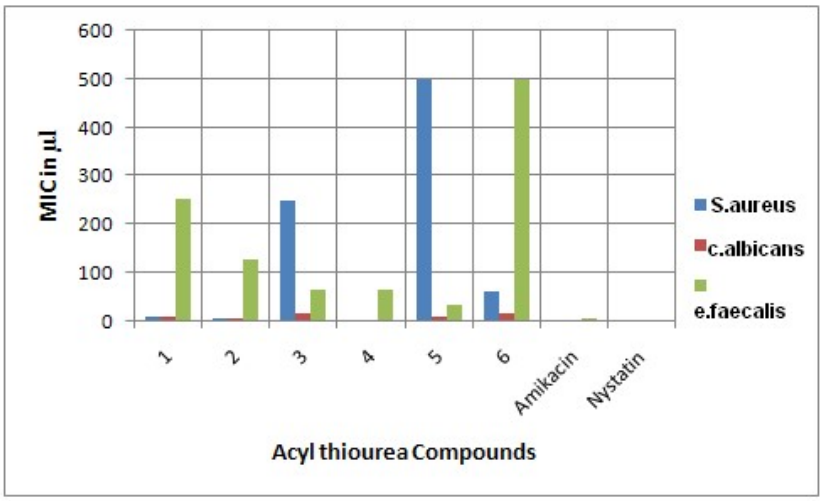

Figure 7: Antimicrobial activity of disubstituted acyl thioureas against *s.aureus, c.albicans* and *e.faecalis*

Table 1: Spectral data of synthesized compounds

Compound	Spectral data
1	IR, $\widetilde{\nu}$ /cm^{-1}: 1034.62 (C==S), 1673.91 (CO), 3453.28 (Secondary NH), 1172.51(C-F), 1523.21 (Asymmetric Ar-NO_2), 1330.61 (Symmetric Ar-NO_2). ^{1}H NMR (DMSO-d_6), δ: 8.22 (s, 1H, NH), 8.89 (s, 1H,NH),7.10–7.81 (m, 8H, 2Ph) MS, m/z (I_r/%): 319.04 (42) (M^+), 169.02(100), 150.01(33)
2	IR, $\widetilde{\nu}$ /cm^{-1}: 1037.64 (C==S), 1675.43 (CO), 3338.18 (Secondary NH), 1540.85 (Asymmetric Ar-NO_2), 2885.95 (Aliphatic CH stretch). ^{1}H NMR (DMSO-d_6), δ: 8.25 (s, 1H, NH), 8.9 (s, 1H, NH),7.14–7.98 (m, 8H, 2Ph), 3.40 (s, 2H, CH_2) MS, m/z (I_r/%): 360.05 (52) (M^+), 165.04(100), 195 (61)
3	IR, $\widetilde{\nu}$ /cm^{-1}: 1101.15 (C==S), 1671.73 (CO), 3334.22 (Secondary NH), 1266.4, 1024.1 (C-O-C stretch), 3023.84 (Aromatic CH stretch). ^{1}H NMR (DMSO-d_6), δ: 8.55 (s, 1H, NH), 8.81 (s,1H, NH), 3.88 (q, 3H, OCH_3) 7.45–7.82 (m, 8H, 2Ph) MS, m/z (I_r/%): 304 (56) (M^+), 169.02(100), 135.02(42)
4	IR, $\widetilde{\nu}$ /cm^{-1}: 1084.76(C==S), 1679.69 (CO), 3309.25 (Secondary NH), 1541.81 (Asymmetric Ar-NO_2), 3477.99 (OH stretch). ^{1}H NMR (DMSO-d_6), δ: 6.82 (s, 1H, OH), 7.39–7.91 (m, 7H, 2Ph), 8.21 (s, 1H, NH), 8.97 (s,1H, NH) MS, m/z (I_r/%): 362 (44) (M^+), 195(47), 167.02(100)
5	IR, $\widetilde{\nu}$ /cm^{-1}: 1071.45 (C==S), 1677.83 (CO), 3316.22 (Secondary NH), 1247.76, 1021.51 (C-O-C stretch), 3450.99 (OH stretch). ^{1}H NMR (DMSO-d_6), δ: 6.74 (s, 1H, OH), 3.72 (q, 3H, OCH_3), 7.40–8.12 (m, 8H, 2Ph), 8.15 (s, 1H, NH) MS, m/z (I_r/%): 302 (66) (M^+), 167.02(100), 135.04(54)
6	IR, $\widetilde{\nu}$ /cm^{-1}: 1078.01(C==S), 1675.84 (CO), 3351.68 (Secondary NH), 1527.35 (Asymmetric Ar-NO_2), 3551.68 (OH stretch). ^{1}H NMR (DMSO-d_6), δ: 6.87 (s, 1H, OH), 7.33–7.89 (m, 8H, 2Ph),8.67 (s,1H,NH) MS, m/z (I_r/%): 317 (58) (M^+), 167.02(100), 150.01(52)

Table 2: Characterization data of the synthesized compounds

Compound	Formula	M_r	Yield	m.p.
			%	°C
1	$C_{14}H_{10}N_3O_3SF$	319.04	75	158-160
2	$C_{15}H_{12}O_5N_4S$	360.05	78	170-172
3	$C_{15}H_{13}N_2O_2FS$	304.07	81	166-168
4	$C_{14}H_{10}N_4O_6S$	362.03	88	180-182
5	$C_{15}H_{14}O_3N_2S$	302.07	72	156-158
6	$C_{14}H_{11}N_3O_4S$	317.05	75	150-152

Table 3: Substitution pattern for synthesized disubstituted acyl thiourea

Compound	Ar	Ar'
1	4-fluorophenyl (4-f-C_6H_4)	3-nitrophenyl (3-NO_2-C_6H_4)
2	Benzyl (C_6H_5-CH_2)	3,5-dinitrophenyl(3,5-NO_2-C_6H_4)
3	4-fluorophenyl (4-f-C_6H_4)	4-methoxyphenyl(4-OCH_3-C_6H_4)
4	3-hydroxyphenyl (3-OH-C_6H_4)	3,5-dinitrophenyl(3,5-NO_2-C_6H_4)
5	3-hydroxyphenyl (3-OH-C_6H_4)	4-methoxyphenyl(4-OCH_3-C_6H_4)
6	3-hydroxyphenyl (3-OH-C_6H_4)	4-nitrophenyl (4-NO_2-C_6H_4)

Table 4: Minimum inhibitory concentrations (MICs) of synthesized compounds in µg/ml carried out by serial dilution method

Compound	*s.aureus*	*c.albicans*	*e.faecalis*
1	8.3	8.3	250
2	4.15	4.15	125
3	250	16.6	62.5
4	2	1	62.5
5	500	8.3	31.25
6	62.5	16.6	500
Amikacin	2	---	4
Nystatin	---	1	---

CONCLUSION

Syntheses of six acyl thiourea derivatives were carried out in two steps and were characterized. The acyl thioureas showed excellent antimicrobial activity as, compound N′-(3,5-dinitrobenzoyl)-N-(3-hydroxyphenyl) thiourea (*IV*), showed the minimum inhibitory concentration similar to amikacin and nystatin, as 2 µg/ml and 1 µg/ml, respectively, against S*taphylococcus aureus*, *Candida albicans*, while, compound N'-(4-methoxybenzoyl)-N-(3-hydroxyphenyl) thiourea (*V*) was found effective against *Enterococcus faecalis*. Acyl thioureas were effective against staphylococcus and candida than enterococcus on the basis of result shown in **Table 4**. Hence, we conclude that acyl thioureas have sufficient potential to be developed as efficient antimicrobial agents.

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