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SYNTHESIS AND BIOLOGICAL STUDIES OF AROYL THIOUREA AND 2,2,6,6- TETRAMETHYL-4-OXOPIPERIDINIUM 4-CHLORO-3-NITROBENZOATE

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ABSTRACT

The reaction of benzoylchloride, o-,m-,p-methylbenzoylchloride, o-,m- and p-nitrobenzoylchloride with ammonium thiocyanate followed by pyrrolidine all gave the corresponding *N*-(aroyl)-*N'*-(pyrrolidine-1-yl)thiourea. However, the same reaction but with 4- chloro-3-nitrobenzoyl chloride was found to give 2,2,6,6-tetramethyl-4-oxopiperidinium 4- chloro-3-nitrobenzoate. All the compounds were confirmed by elemental analysis. Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), UV-Vis and one of the synthesized compounds are analyzed by X-ray single crystal diffraction technique. The biological studies of the thiourea derivatives compounds were tested against a few types of bacteria and the results showed no activity against Methicilin Resistant Staphylococcus aureus (MRSA), S.Pyogenes, P.mirabilis and Enterobacter aerogenes. On the other hand, the ionic salt was active against all the bacteria.

Keywords: synthesis; biological studies; aroyl thiourea; 2,2,6,6-tetramethyl-4-oxopiperidium 4 chloro-3-nitrobenzoate

INTRODUCTION

The developing on research to find new antimicrobial and anticancer therapeutic are increased nowadays; in able to design new antimicrobial, we have to study the structure of compound relationships with the biological activity. Scientists have focused on thiourea derivatives compounds because the present of phenyl group, sulphur and oxygen contribute to the activity of biological studies and most medicine consists of heterocyclic compound [1]. Some of thiourea derivatives are biologically active, such as phenyl-ethyl-5-bromopyridylthiourea, *N*-[2- (2-methoxyphenylethyl)]-*N'*-[2-(5-bromopyridyl)] thiourea and *N*-[2-(2-chlorophenylethyl)]-*N'*- [2-(5-bromopyridyl)] thiourea potential act as anti-HIV (Dong et al. 2000) [2]. Thiourea derivatives are also potential as anticancer [3-6] and the combinations with benzothiazoles produced DNA

topoisomerase [7,8]. In the present study, we synthesis new thiourea derivatives and ion salt and report the preparation, characterization and antimicrobial assay of seven new compounds, *N*-(o-methylbenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea) (I), *N*-(m-methylbenzoyl)-*N'*- (pyrrolidin-1-yl)thiourea) (II), *N*-(p-methylbenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea) (III), *N*-(o- nitrobenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea (IV), *N*-(m-nitrobenzoyl)-*N'*-(pyrrolidin-1- yl)thiourea (V), *N*-(p-nitrobenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea (VI) and 2,2,6,6-tetramethyl-4- oxopiperidinium 4-chloro-3-nitrobenzoate (VII)

EXPERIMENTAL

Materials

o-methylbenzoyl chloride, m-methylbenzoyl chloride, p-methylbenzoyl chloride, o-nitrobenzoyl chloride, m-nitrobenzoyl chloride, p-nitrobenzoyl chloride, 4-chloro-3-nitrobenzoyl chloride and pyrrolidine was purchased from Aldrich. Ammonium thiocyanate and acetone were obtained from Merck. They are analytical grade and were used without purification.

Synthesis of N-(o-methylbenzoyl)-N'-(pyrrolidin-1-yl)thiourea)

A solution of o- methylbenzoyl chloride (1.56g, 0.01mol) was added into a flask containing potassium thiocyanate (0.76 g, 0.01 mol) in 30 ml acetone and was refluxed for 1h. Then, a solution of pyrrolidine (0.71g, 0.01mol) in anhydrous acetone (30mL) was added, and the mixture was refluxed for 1 hour. The solution was filtered-off and left to evaporate at room temperature. The colourless solid was obtained after one day of evaporation. Yield: 81.8%. M.p.: 445.1-446.0 K. Anal. Calc for $C_{13}H_{16}N_2OS$ (248.1): C, 62.87: H, 6.44 : N, 11.28 : S, 12.91 . Found: C, 64.20:H, 6.52: N, 12.70: S, 12.59. FT-IR (KBr pellet, cm^{-1}): ν(NH) 3154.87, ν(C=O) 1695, ν(C=S)722.71. ^{1}H NMR 7.23 (*d*,1H), 7.35 (*t*, 1H), 7.22 (*t*, 1H), 7.39 (*d*,1H), 2.35(*s*, 3H), 10.65 (*s*,1H), 2.49 (*t*,2H), 1.91 (*q*, 2H), 1.91 (*q*, 2H), 10.65 (*s*,1H).

Synthesis of N-(m-methylbenzoyl)-N'-(pyrrolidin-1-yl)thiourea)

This compound was prepared as above from equamolar of m-methylbenzoyl chloride, ammonium thiocyanate and pyrrolidine. Yield: 81.2%. M.p.: 448.0-449.5 K. Anal. Calc for $C_{13}H_{16}N_2OS$ (248.1): C, 62.87 : H, 6.44 : N, 11.28 : S, 12.91 . Found: C, 63.90: H, 6.45: N, 10.36: S, 12.21. FT-IR (KBr pellet, cm^{-1}): ν(NH) 3154.87, ν(C=O) 1695, ν(C=S) 722.71. ^{1}H NMR 7.69 (*s*,1H), 7.65 (*d*,1H),7.23 (*t*,1H), 7.39 (*d*,1H), 2.33(*s*,3H), 10.65 (*s*,1H), 2.32 (*t*,2H),1.91 (*m*,2H),1.91(*m*,2H), 2.32 (*t*,2H).

Synthesis of N-(p-methylbenzoyl)-N'-(pyrrolidin-1-yl)thiourea)

This compound was prepared as above from equamolar of p-methylbenzoyl chloride, ammonium thiocyanate and pyrrolidine. Yield: 82.1%. M.p.: 448.7-449.6 K. Anal. Calc for $C_{13}H_{16}N_2OS$ (248.1): C, 62.87 : H, 6.44 : N, 11.28 : S, 12.91 . Found: C, 63.9: H, 6.59 : N, 12.45 : S, 12.21. FT-IR (KBr pellet, cm−1): ν(NH) 3154.87, ν(C=O) 1695, ν(C=S) 722.71. ^{1}H NMR 7.74 (d,1H), 7.27 (d,1H), 7.27(d,1H), 7.74 (d,1H), 2.42

(s,3H), 10.60 (s,1H), 3.70 (t,2H), 2.04 (m,2H), 2.04 (m,2H),3.70 (t,2H).

Synthesis of N-(o-nitrobenzoyl)-N'-(pyrrolidin-1-yl)thiourea
A solution of o- nitrobenzoyl chloride (1.85g, 0.01mol) was added into a flask containing potassium thiocyanate (0.76 g, 0.01 mol) in 30 ml acetone and was refluxed for 1h. Then, a solution of pyrrolidine (0.71g, 0.01mol) in anhydrous acetone (30mL) was added, and the mixture was refluxed for 1 hour. The solution was filtered-off and left to evaporate at room temperature. The colourless solid was obtained after one day of evaporation. Yield: 80.8%. M.p.: 447.8-448.7 K. Anal. Calc for $C_{12}H_{13}N_3O_3S$ (279.1): C, 51.60 : H, 4.69 : N, 15.04 : O, 17.18 : S, 11.48 . Found: C, 52.30: H, 3.81: N, 12.47 : S, 12.00. FT-IR (KBr pellet, cm^{-1}): ν(NH) 3150.31, ν(C=O) 1681.50, ν(C=S) 715.48. ^{1}H NMR 8.19 (d,1H), 7.73 (t,1H), 7.63 (t,1H), 7.86 (d,1H), 10.7 (s,1H), 2.49 (t,2H), 1.23 (q,2H), 1.23 (q,2H), 2.49 (t,2H).

Synthesis of *N*-(m-nitrobenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea)
This compound was prepared as above from equamolar of m-nitrobenzoyl chloride, ammonium thiocyanate and pyrrolidine. Yield: 80.4%. M.p.: 447.6-448.0 K. Anal. Calc for $C_{12}H_{13}N_3O_3S$ (279.1): C, 51.60 : H, 4.69 : N, 15.04 : O, 17.18 : S, 11.48 . Found: C, 50.80: H, 3.93: N, 14.25: S, 11.96. FT-IR (KBr pellet, cm^{-1}): ν(NH) 3150.31, ν(C=O) 1681.50, ν(C=S) 715.48. ^{1}H NMR 8.68 (s,1H), 8.41 (d,1H),7.76 (t,1H), 8.27(d,1H), 11.20 (s,1H), 2.50(t,2H), 1.89 (q,2H), 1.89 (q,2H), 2.50 (t,2H).

Synthesis of N-(p-nitrobenzoyl)-N'-(pyrrolidin-1-yl)thiourea). This compound was prepared as above from equamolar of p-nitrobenzoyl chloride, ammonium thiocyanate and pyrrolidine. Yield: 81.1%. M.p.: 441.0-441.8K Anal. Calc for $C_{12}H_{13}N_3O_3S$ (279.1): C, 51.60 : H, 4.69 : N, 15.04 : O, 17.18 : S, 11.48 . Found: C, 57.89: H, 6.16: N, 13.09: S, 12.21. FT-IR (KBr pellet, cm^{-1}): ν(NH) 3150.31, ν(C=O) 1681.50, ν(C=S) 715.48. ^{1}H NMR 7.48(*d*,1H), 7.83 (*d*,1H), 7.83 (*d*,1H),7.48(*d*,1H), 10.73 (*s*,1H), 2.50 (*t*,2H), 1.87 (*q*,2H), 1.87 (*q*,2H), 2.50 (*t*,2H).

Synthesis of 2,2,6,6-tetramethyl-4-oxopiperidinium 4-chloro-3-nitrobenzoate
A solution of 4-chloro-3-nitrobenzoyl chloride (2.20g, 0.01mol) in 30 ml of acetone was added into a flask containing containing potassium thiocyanate (0.76 g, 0.01 mol) in 30 ml acetone and was refluxed for 1h. Then, a solution of pyrrolidine (0.71g, 0.01mol) in anhydrous acetone (30mL) was added, and the mixture was refluxed for 1 hour. The solution was filtered-off and left to evaporate at room temperature. The colourless solid was obtained after one day of evaporation. Yield: 81.3%. M.p. 447.3-448.3K. Anal. Calc for $C_{12}H_{12}ClN_3O_3S$ (313.03): C, 45.94: H, 3.85: N, 13.39: O, 15.30: S, 10.22. Found: C, 52.20: H, 5.28: N, 7.34. FT-IR (KBr pellet, cm^{-1}): ν(NH) 3150.31, ν(C=O) 1681.50, ν(C=S) 715.48. ^{1}H NMR 8.739 (t,1H), 8.33 (d,1H), 8.44 (d,1H), 7.80 (t,1H), 3.71(t,2H), 3.59 (t,2H), 1.94 (m,3H), 1.94 (m,3H), 1.94 (m,3H), 1.94 (m,3H).

Antimicrobial assay

Five pathogenic microbial were used to test the biological potential of the compounds: Methicilin Resistant Staphylococcus aureus (MRSA), S.Pyogenes, Esherichia coli, P.mirabilis and Enterobacter aerogenes cultures as obtained from the American Type Cultures Collection (ATCC). The stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO), with different concentrations. The concentrations of solution of compounds were 30, 15, 7.5, 3.75, 1.875 and 0.9375 μg/cm 3. 5 μl of solution were drifted on the filter paper (diameter 6mm) on a petri dish on Mueller-Hinton agar surface that inoculated with bacteria. DMSO was used as a negative control and Chloramphenicol was used as the positive control for all bacteria. All the inoculated plated were incubated at 35 °c for 24 hours. The diameters of zone of inhibition [mm] were been determined.

RESULTS AND DISCUSSION

All the products (compound (I)-(VII)) were obtained as colourless solids. The microelemental analysis data were in agreement with the expected *N*-(aroyl)-*N'*-(pyrrolidin)thiourea except compound (VII) which is the product of the reaction of 3-nitro-4-chloro-benzoylchloride. The existence of thiourea for compound I-VI can be confirmed by using FTIR spectroscopy, where the vibration of *v*(C=O) display peaks at between 1644.21-1695.59 cm^{-1}, *v*(C=S) at 1215.61- 1252.89 cm^{-1} and v(N-H) at 3103.82-3251.02 cm^{-1}. While, for UV-Vis spectroscopy show two intense broad bands with maxima in the range between 200 nm and 300 nm can be attributed to $\pi \rightarrow \pi^*$ transition on chromophore of C=O and C=S. In ^{1}H NMR spectra the substituent on the benzene group does not give significant affect for the amino proton chemical shifts except for *N*-(3- nitrobenzoyl) -*N*'- (pyrrolidin - 1 - yl)thiourea (V). The N-H proton for compound V were shifted to low field of 0.97 ppm and it shows that the electron-withdrawing effect of the nitro group is very effective in the meta position (Table 1). While for ^{13}C NMR spectra, its shows chemical shift for C=S at para position for compound III that shifted to high field at 176.54 ppm and at meta position for compound V, shifted to 176.78 ppm , based on this result it shows there are effect of position between electron withdrawing and electron donating groups (Table 2).

Table 1: Significant 1H NMR and J coupling spectral data of Aroyl Thiourea derivative compounds

Compound	Chemical shift, δ (ppm) & J coupling (Hz)		
	-NH-	-C-H (Aromatic)	-C-H (pyridine)
I	10.65	7.23(J=7.2),7.35(J=7.8), 7.22(J=7.2),7.39(J=7.2)	2.49(J=1.8),1.91(J=3.0), 1.91(J=3.0), 2.49(J=1.8)
II	10.65	7.69,7.65(J=7.8), 7.23(J=7.2), 7.39(J=7.8)	2.32(J=1.8),1.91(J=5.4), 1.91(J=5.4), 2.32(J=1.8)
III	10.60	7.74(J=7.8),7.27(J=8.4), 7.27(J=8.4), 7.74(J=7.8)	3.70(J=1.8),2.04(J=4.4), 2.04(J=4.4), 3.70(J=1.8)
IV	10.77	8.19(J=10.4),7.73(J=9.3), 7.63(J=11.0),7.86(J=11.6)	2.49(J=2.3),1.23(J=5.2), 1.23(J=5.2), 2.49(J=2.3)
V	11.20	8.68, 8.41(J=8.4), 7.76(J=8.0), 8.27(J=8.0)	2.50(J=2.5),1.89(J=5.4), 1.89(J=5.4), 2.50(J=2.5)
VI	10.73	7.75(J=10.6),8.28(J=9.8), 8.28(J=9.8), 7.75(J=10.6)	2.53(J=2.1),1.83(J=5.0), 1.83(J=5.0), 2.53(J=2.1)

Table 2: Significant ^{13}C NMR spectral data of Aroyl Thiourea derivative compounds.

Compound	Chemical shift, δ (ppm)		
	-C=O-	-C-CH (Aromatic)	-C=S
I	166.81	126.05-136.45	177.03
II	164.67	124.39-138.40	177.48
III	163.23	127.94-143.82	176.54
IV	167.10	128.80-145.64	177.91
V	162.50	123.53-148.01	176.78
VI	166.38	123.47-147.86	177.36

The reaction of 3-nitro-4-chloro-benzoylchloride with pyrrolidine gave salt compound (VII). The salt consists of 2,2,6,6-tetramethyl-4-oxopiperidin-1-ium 4-chloro-3-nitrobenzoate (Figure 1) indicates the opening of pyrrolidine ring involvement of acetone solvent in the reaction mechanism.

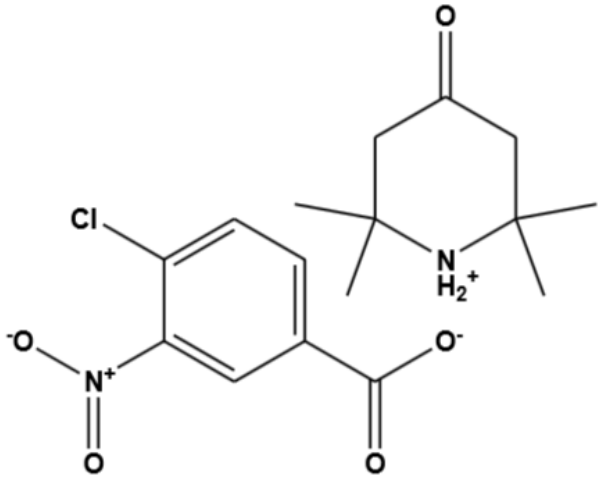

Figure 1: The molecular structure of 2,2,6,6-tetramethyl-4-oxopiperidin-1-ium 4-chloro-3- nitrobenzoate

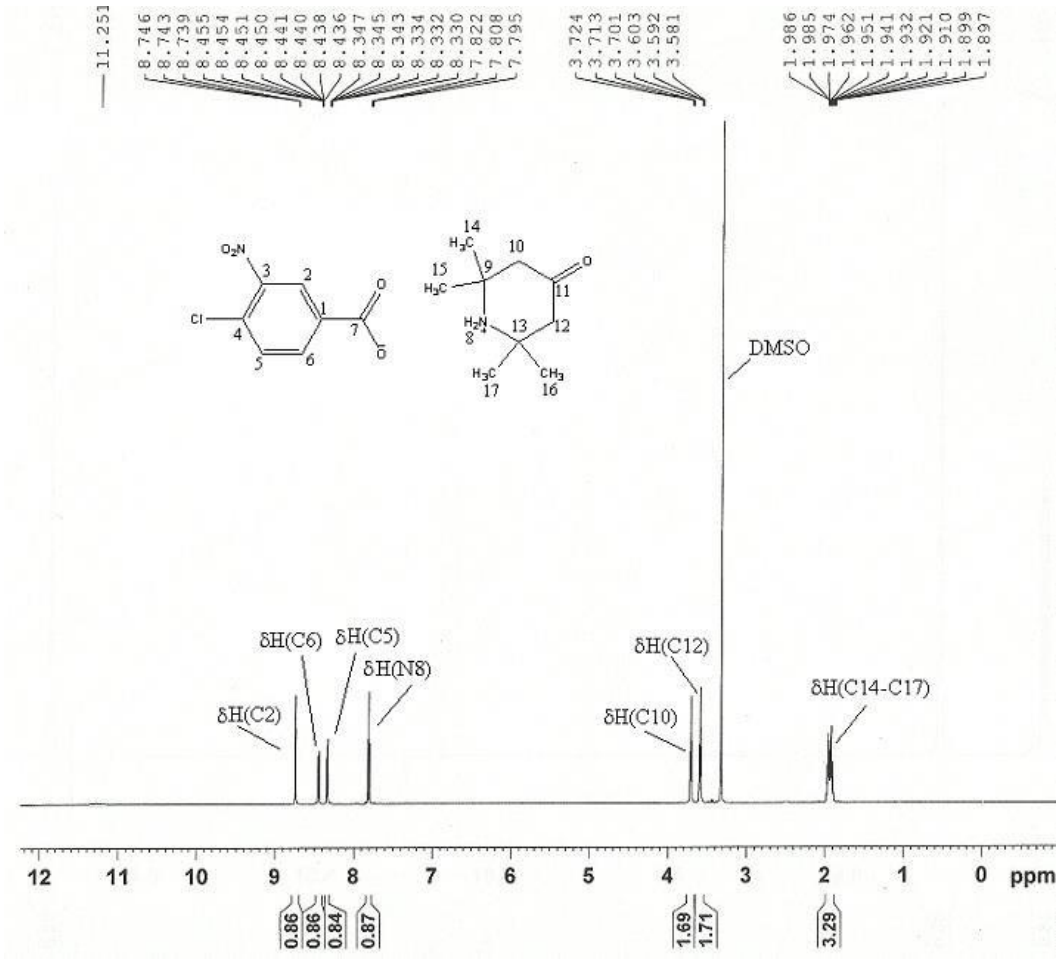

Figure 2: Spectrum of ^{1}H NMR of 2,2,6,6-tetramethyl-4-oxopiperidin-1-ium 4-chloro-3- nitrobenzoate

The FTIR data of compound VII were recorded at region 4000-400 cm^{-1}, and it display peaks at 1571.81 cm^{-1} due to *v*(C=C) aromatic and at 2828.11 cm^{-1} due to *v*(C-H). The peak of *v*(C-N) display around 1123.20 cm^{-1}. The FTIR agreed with the VII compound when the region of FTIR does not display any peaks for v(N-H) and v(C=O). The ^{1}H NMR spectra of compound VII was recorded in DMSO d^{6} solution using tetramethylsilane (TMS) as internal standard. The spectrum of ^{1}H NMR shift at 7.80 ppm as triplet represent of thioamide (Figure 2). Based on x-ray crystallography, clearly showed compound VII produce salt compound that crystallize in triclinic system with space group Pī, a = 7.9974(10)Å, b = 10.3267(13)Å, c = 11.9196(15)Å, α = 109.101(3)º, β = 96.785(3)º, γ = 104.720(3)º (Figure 3) [9].

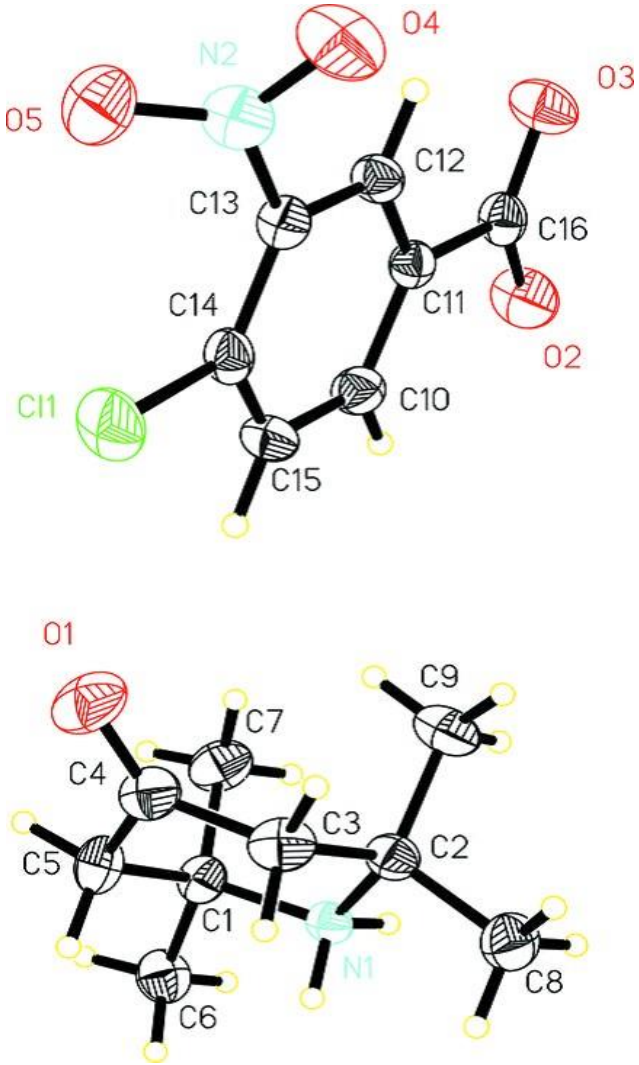

Figure 3: The ORTEP structure of 2,2,6,6-Tetramethyl-4-oxopiperidin-1-ium 4-chloro nitrobenzoate with displacement ellipsods drawn at the 50% probability level

Biological studies found that thiourea derivatives containing substituents on the benzene ring have no activity against the bacteria of gram-positive and gram negative. This results against the observation that the heterocyclic contribute to the activity. This might be due to the presence of pyrrolidine that decreased the delocalization of π-electrons and decreased the penetration of the compounds into the bacteria membranes. However, for 2,2,6,6-tetrametilpiperidin-ium 4-chloro- 3-nitrobenzoic (VII), found to inhibit bacteria methicillin-resistant Staphylococcus aureus (MRSA), S.Pyrogenes, E. coli and Enterobacter aerogenes P.mirabilis (Table 3).

Table 3: Antibacterial activity of the compounds I-VII of 30 mg/mL: zone of inhibition values [mm]

Compounds	Gram-positive		Gram-negative		
	MRSA	S. pyrogenes	E. coli	P. mirabilis	E. aerogenes
I	-	-	-	-	-
II	-	-	-	-	-
III	-	-	-	-	-
IV	-	-	-	-	-
V	-	-	-	-	-
VI	-	-	-	-	-
VII	10.6	11.00	8.33	9.00	9.00
Positive control	20	20	20	20	20

CONCLUSION

7 new compounds from the reaction of pyrrolidine with o-, m-, p-methylbenzoyl isothiocyanate, o-, m-, p-nitrobenzoyl isothiocyanate and 4-chloro-3-nitrobenzoyl isothiocyanate were obtained. All the reactions except 4-chloro-3-nitrobenzoylisothiocyanate with pyrrolidine gave thiourea derivatives as products. The products are *N*-(2-methylbenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea (I), *N*-(3-methylbenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea (II), *N*-(4-methylbenzoyl)-*N'*-(pyrrolidin-1- yl)thiourea (III), *N*-(2-nitrobenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea (IV), *N*-(3-nitrobenzoyl)-*N'*- (pyrrolidin-1-yl)thiourea (V), *N*-(4-nitrobenzoyl)-*N'*-(pyrrolidin-1-yl)thiourea (VI). The non- thiourea product is 2,2,6,6-tetramethyl-4-oxopiperidin-1-ium 4-chloro-3-nitrobenzoate (VII). All the 7 compounds were tested antibacterial studies against Methicilin Resistant Staphylococcus aureus (MRSA), S.Pyogenes, P.mirabilis and Enterobacter aerogenes. Only 2,2,6,6-tetramethyl-4-oxopiperidin-1-ium 4-chloro-3-nitrobenzoate (VII) was found active against the bacteria. Compounds VII was active against Methicilin Resistant Staphylococcus aureus (MRSA), S.Pyogenes, P.mirabilis and Enterobacter aerogenes.

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