https://doi.org/10.48047/AFJBS.5.4.2023.01-14

African Journal of Biological Sciences

Research Paper Open Access

Synthesis, Characterization and Antibacterial Activity Of Some Thiourea Derivatives

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Article Info

Volume 5, Issue 4, October 2023

Received:18 Aug 2023

Accepted :15 Sept 2023

Published: 05 Oct 2023

doi:10.48047/AFJBS.5.4.2023.01-14

Abstract: Four new thiourea derivatives have been synthesized, by two steps: Step (1): By the reaction one mole of ammonium thiocyanat with one mole of benzoyl chloride under reflux 3 hrs in acetone gave product (benzoylisothiocyanate). Step (2): By the reaction product with one mole of primary amines (Nicotinamide, Sulfamethoxazole, 2-Aminobenzoimidazole and 2-Aminobenzothiazole) under reflux 6 hrs in acetone. The synthesized compounds characterized by elemental microanalysis C.H.N.S, UV-Visible, FT-IR, and ^{1}H & ^{13}C NMR spectra. The biological effects of thiourea derivatives have been investigated on two types of bacteria species *Staphylococcus aureus*, *Pseudomonas aeruginosa* the results exhibited all the compounds have varsity anti-bacterial activities.

Key Word: thiourea, benzoylisothiocyanate, Nicotinamide, Sulfamethoxazole, 2-Aminobenzoimidazole and 2-Aminobenzothiazole, Antibacterial Activity

1. Introduction

In the recent years, thiourea derivatives have gained extensive applications in medicine, agriculture, and also as ligands in coordination chemistry[1], Because benzoyl thioureas have suitable C=O and C=S function groups, they can be considered as useful chelating agents due to their ability to encapsulate into their coordinating moiety metal ions[2].

Specialized literature reveals that thiourea derivatives show a broad spectrum of biological activities. The thiourea skeleton can be effectively used to prepare a large number of new compounds with biological activities such as antiviral [3], anticancer [4], anti-inflammatory [5], antimicrobial [6], anticonvulsant [7], and anti-helmintic activities [8].

Thiourea derivatives are used as corrosion inhibitors [9], and as intermediates to obtain a great variety of heterocyclic compounds [10].

The crystal X-ray diffraction study of thiourea derivatives allowed a better understanding of the nature of binding of these compounds and a valuable insight into their conformation [11]. Although antibiotics have saved countless millions of lives, over the last decades, the emergence of antimicrobial resistance has limited their efficiency, becoming a serious global health problem that requires the development of new antimicrobial agents effective against pathogenic microorganisms resistant to currently available treatments [12]. A distinguish biological activity was recorded for most investigated complexes especially with the presence of N, S and O heteroatom's [13, 14].

2.Experimental

2.1 Chemicals: All regents used were annular or chemically pure grade by (BHD),Merk and Fluka. Benzoyl chloride, ammonium thiocyanate , Nicotinamide, Sulfamethoxazole, 2-Aminobenzoimidazole, 2-Aminobenzothiazole , ethanol, acetone.

2.2 Instruments: ^{1}H and ^{13}C–NMR was recorded using Ultra Shield 300 MH_z Switzerl and at University of Al al-Bayt, Jordan. Melting point was recorded by using Stuart- melting point apparatus. FT-IR spectra were recorded as KBr disc using 3800 Shimadzu in the range of (4000-400) cm^{-1}. Electronic spectra were obtained using UV-160 Shimadzu spectrophotometer at 25°C for 10^{-3}M solution DMSO with 1.000 ± 0.001cm matched quartz cell. Digital Elemental micro analyses (C.H.N.S) were performed using Acrlo Erba 1106elemental analyzer.

3. Preparation of thiourea derivatives (a, b, c, d, e)[15]

1- Preparation of the (benzoyl isothiocyanate)

Mixture of benzoyl chloride (1.157ml, 0.01mole) and ammonium thiocyanate (0.76g,0.01mole) in 25ml acetone was refluxed with stirring for 3 hours and then filtered; the filtrate was used for further reaction.

2- Preparation of [1-(benzoyl)-3-(carbonyl Pyridin-2-yl)thiourea](a)

(1.22g, 0.01mole) of, Nicotinamide in 20ml acetone were rapidly added to benzoyl isothiocyanate solution and maintaining reflux for 6 hours. The resulting solid was collected, washed with acetone and recrystallized from ethanol. was prepared by two steps (scheme 1). (m.p = 242-245^{o}C), Yield (75%), FT-IR (KBr) υ(cm^{-1}): 3244-3124 m (N–H thioamide, sec amide), 3066 m(C–$H_{Ar,}$), 1666-1716 m, vs(C=O), 1600-1546 s(C=$C_{Ar,\ Py}$), 1485 s(C=N_{py}), 1385 s(C=S), 1400-1257 vs, m(C–N)[6,17], Fig.(1) showed the FTIR spectrum of (a). ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.57 (6H, t, CH DMSO), 7.60 (2H, d, CHAr), 7.76 (3H, t, CHAr), 8.126(H, t, CHpy), 8.95,9.07 (2H, d, CHpy), 9.38 (H, s, CHpy),10.83 (1H, s, NH thioamide), 11.40 (1H,s, NH sec amide) Fig.(4): showed the ^{1}H-NMR spectrum (a), ^{13}C NMR: 38.83 (2C, s, DMSO), 123.4 -137.8 (6C, s, CHAr), 144.2-159.2 (6C, s, CHpy), 162.3 (C=S), 176.7, 177.6 (2CONH)[18]. UV-Visible spectrum in DMSO (225nm, 44444cm^{-1}) which is due to (π→π*) transition, other band appeared at (262nm, 38167cm^{-1}) was expressed at the (n→π*)[17].Elemental analysis (%) for $C_{14}H_{11}N_3O_2S$: %C found (58.65) calc. (58.94), %H found (3.84) calc. (3.89), %N found (14.84) calc. (14.73) and %S found (11.13) calc. (11.24).

3- Preparation of [1-(benzoyl)-3-(N-5-methylisoxazol-3-yl) phenyl sulfonamide) thiourea] (b)

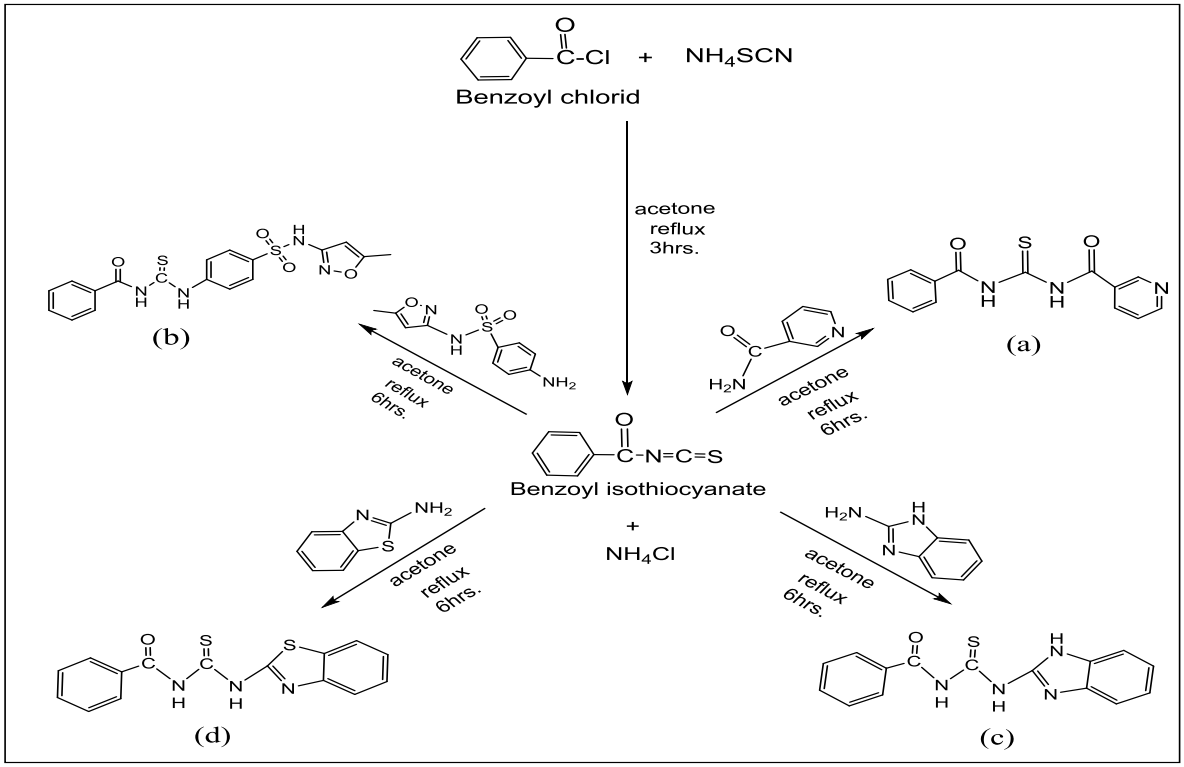
(2.53g, 0.01mole) of, Sulfamethoxazole in 30ml acetone were rapidly added to benzoyl isothiocyanate solution and maintaining reflux for 6 hours. The resulting solid was collected, washed with acetone and recrystallized from ethanol (m.p = 220-223^{o}C), Yield (86%), FT-IR (KBr) υ(cm^{-1}): 3417-3475 m, w (N–H thioamide, sec amide), 3055 m(C–$H_{Ar,}$), 1681 s(C=O), 1597-1550 m(C=C_{Ar}), 1315 m(C=S), 1384-1265 s, w (C–N), Fig.(2) showed the FTIR spectrum of (b), ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.30 (3H, s, CH_{aliph}), 3.36 (1H, s, $NH_{sulphonyl}$), 6.15 (1H, s, $CH_{Pyrazole}$), 7.50 (5H, t, CH_{Ar}), 8.01 (4H, t, CH_{Ar}), 10.63 (1H, s, NH thioamide), 11.38 (1H,s, NH sec amide), ^{13}C NMR: 12.02 (C, s, CH_{aliph}), 38.85 (2C, s, DMSO), 95.36, 143.52, 157.53 (3C, s, $CH_{Pyrazole}$), 119.97 -134.36 (12C, s, CH_{Ar}), 166.1 (C=S), 170.2 (CONH). Fig.(6): showed the ^{13}C-NMR spectrum (b), UV-Visible spectrum in DMSO (228nm, 43859cm^{-1}) which is due to (π→π*) transition, other band appeared at (268nm, 37313cm^{-1}) was expressed at the (n→π*). Elemental analysis (%) for $C_{18}H_{16}N_4O_4S_2$: %C found (51.91) calc. (51.58), %H found (3.89) calc. (3.81), %N found (13.45) calc. (13.63) and %S found (15.40) calc. (15.23).

4- Preparation of [1-(benzoyl)-3-(benzimidazol-2-yl) thiourea] (c)

(1.33g, 0.01mole) of, 2-Aminobenzoimidazole in 20ml acetone were rapidly added to benzoyl isothiocyanate solution and maintaining reflux for 6 hours. The resulting solid was collected, washed with acetone and recrystallized from ethanol (m.p = 245d), Yield (68%), FT-IR (KBr) υ(cm^{-1}): 3325 s ,3224 m, 3433 w (N–H thioamide, sec amide, Pyrazole), 3059 m(C–$H_{Ar,}$), 1678 s(C=O), 1585-1539 vs,m(C=C_{Ar}), 1385 m(C=S), 1458-1269 s(C–N), ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.49 (6H, t, CH DMSO), 7.93-8.25 (4H, m, $CH_{benzimidazol}$), 8.57-8.60 (5H, m, CH_{Ar}), 9.03 (1H, s, NH $_{Pyrazole}$), 10.78 (1H, s, NH thioamide), 12.12 (1H,s, NH sec amide), Fig.(5): showed the ^{1}H-NMR spectrum (c), ^{13}C NMR: 38.88 (2C, s, DMSO), 117.67- 125.34 (6C, m, $CH_{benzimidazol}$), 127.55-130.05 (6C, s, CH_{Ar}), 168.4 (C=S), 177.4 (CONH). 192.4 ($C_{Pyrazole}$). UV-Visible spectrum in DMSO (235nm, 42553cm^{-1}) which is due to (π→π*) transition, other band appeared at (262nm, 38167cm^{-1}) was expressed at the (n→π*). Fig.(8): U.V spectrum of (c). Elemental analysis (%) for $C_{15}H_{12}N_4OS$: %C found (60.80) calc. (60.68), %H found (4.08) calc. (4.01), %N found (18.91) calc. (19.12) and %S found (10.82) calc. (10.61).

5- Preparation of [1-(benzoyl)-3-(benzothiazol-2-yl)thiourea] (d)

(1.5g, 0.01mole) of, 2-Aminobenzothiazole in 20ml acetone were rapidly added to benzoyl isothiocyanate solution and maintaining reflux for 6 hours. The resulting solid was collected, washed with acetone and recrystallized from ethanol (m.p = 215d), Yield (57%), FT-IR (KBr) υ(cm^{-1}): 3313-3170 s, m (N–H thioamide, sec amide), 3062 m(C–$H_{Ar,}$), 1666 m(C=O), 1597-1550 m,s(C=C_{Ar}), 1392 vs(C=S), 1458-1269 s(C–N), Fig.(3) showed the FTIR spectrum of (d). ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.506 (6H, t, CH DMSO), 6.95 -7.31 (4H, m, $CH_{benzothiazol}$), 7.45-7.92 (4H, m, CH_{Ar}), 10.64 (1H, s, NH thioamide), 11.83 (1H,s, NH sec amide), ^{13}C NMR: 38.87 (2C, s, DMSO), 126.8 - 128.5 (6C, m, $CH_{benzothiazol}$), 128.6-131.8 (6C, s, CH_{Ar}), 161.3 (C=S), 178.6 (CONH). 118.6 ($C_{Pyrazole\ ring}$). Fig.(7): showed the ^{13}C-NMR spectrum (d). UV-Visible spectrum in DMSO (224nm, 44642cm^{-1}) which is due to (π→π*) transition, other band appeared at (262nm, 38167cm^{-1}) was expressed at the (n→π*). Elemental analysis (%) for $C_{15}H_{12}N_4OS$: %C found (57.49) calc. (57.34), %H found (3.54) calc. (3.49), %N found (13.41) calc. (13.62) and %S found (20.46) calc. (20.34).



Scheme (1) preparation of thiourea derivatives (a, b, c, d, e)

Fig.(1): Infrared spectrum of [1-(benzoyl)-3-(carbonyl Pyridin-2-yl)thiourea] (a)

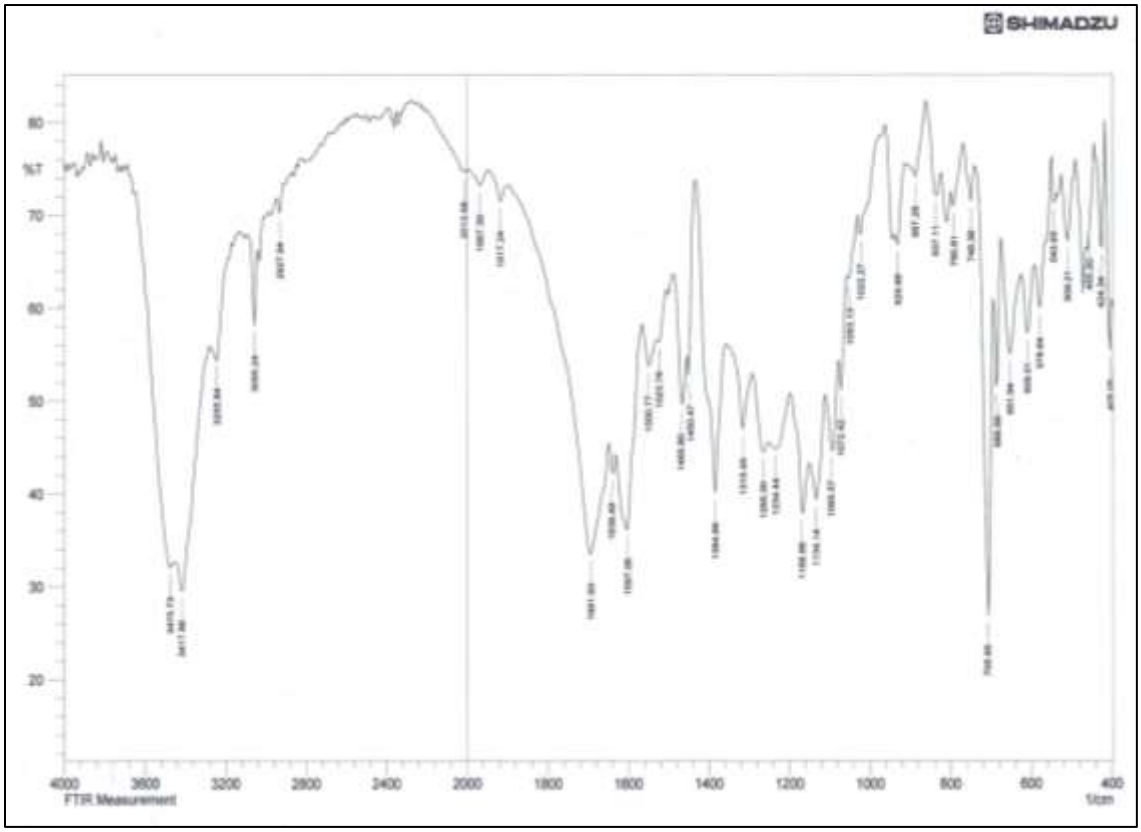


Fig.(2): Infrared spectrum of [1-(benzoyl)-3-(N-5-methylisoxazol-3-yl) phenyl sulfonamide) thiourea] (b)

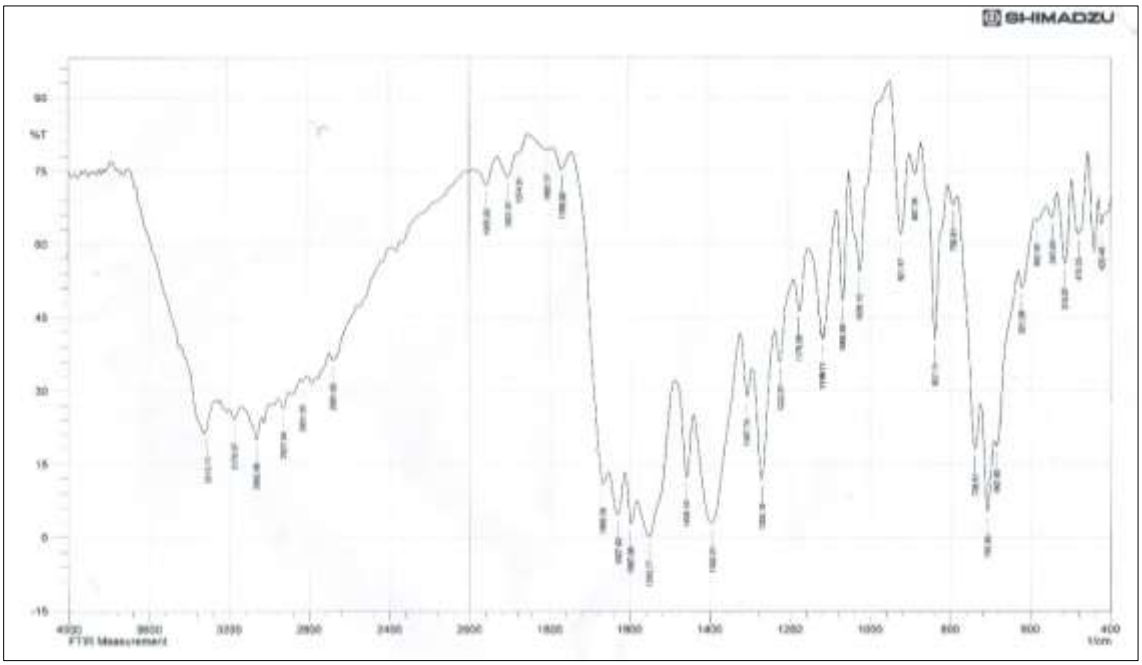


Fig.(3): Infrared spectrum of [1-(benzoyl)-3-(benzothiazol-2-yl)thiourea] (d)

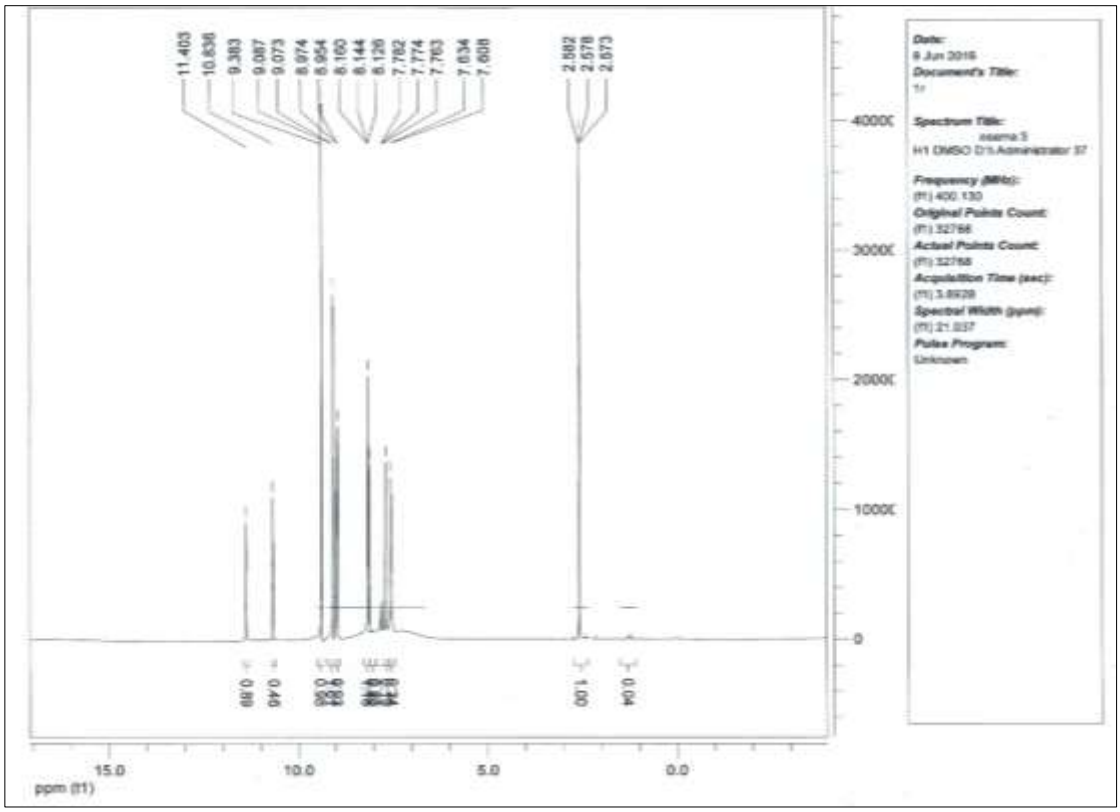


Fig.(4): ^{1}H-NMR spectrum of [1-(benzoyl)-3-(carbonyl Pyridin-2-yl)thiourea] (a)

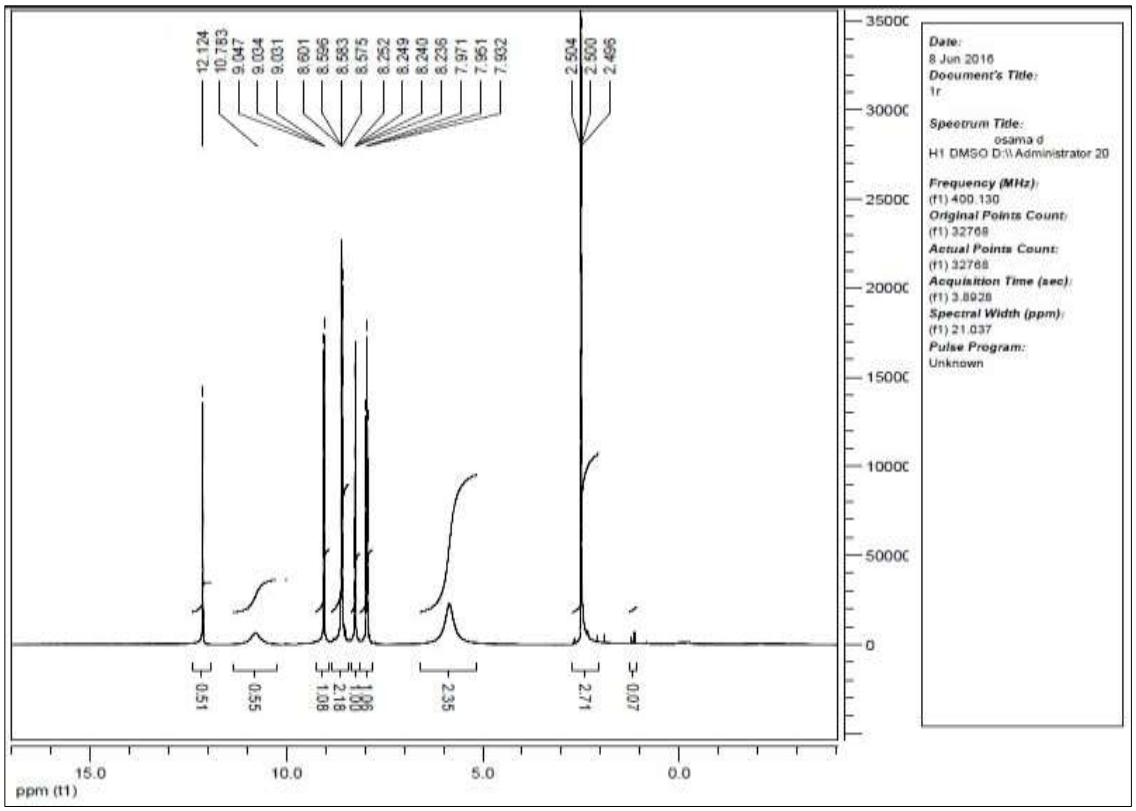


Fig.(5): ^{1}H-NMR spectrum of [1-(benzoyl)-3-(benzimidazol-2-yl) thiourea] (c)

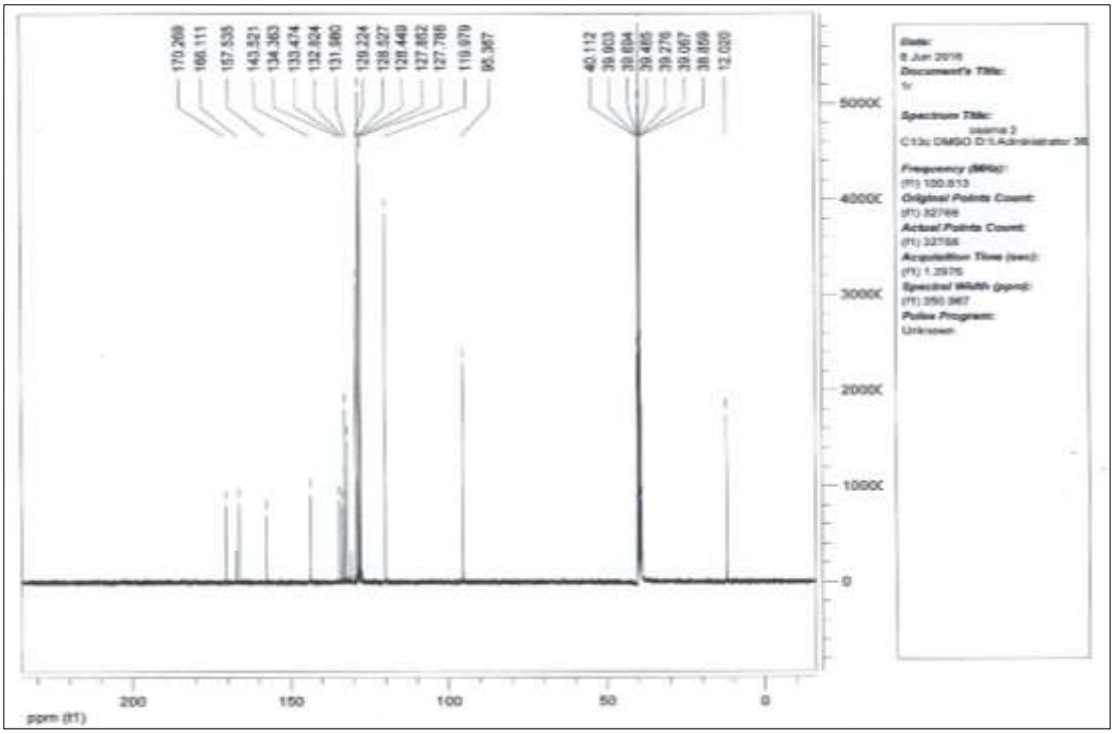


Fig.(6): ^{13}C-NMR spectrum of of [1-(benzoyl)-3-(N-5-methylisoxazol-3-yl) phenyl sulfonamide) thiourea] (b)

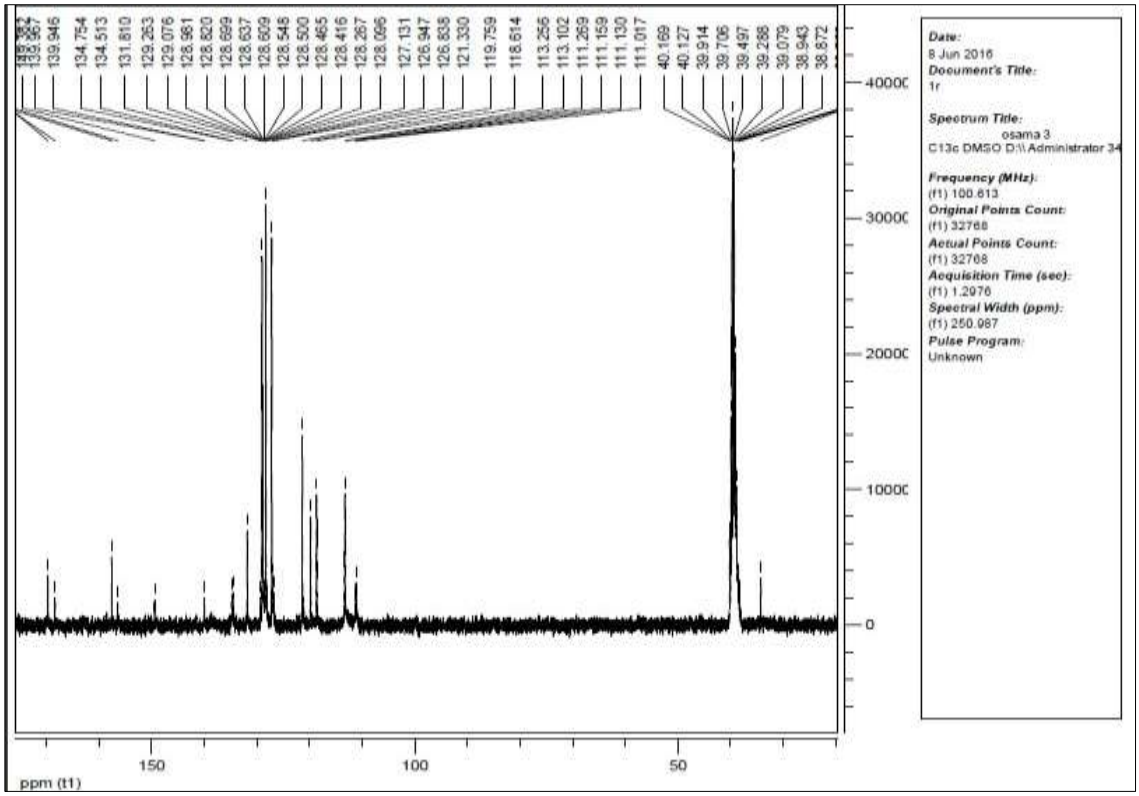


Fig.(7): ^{13}C-NMR spectrum of of [1-(benzoyl)-3-(benzothiazol-2-yl)thiourea] (d)

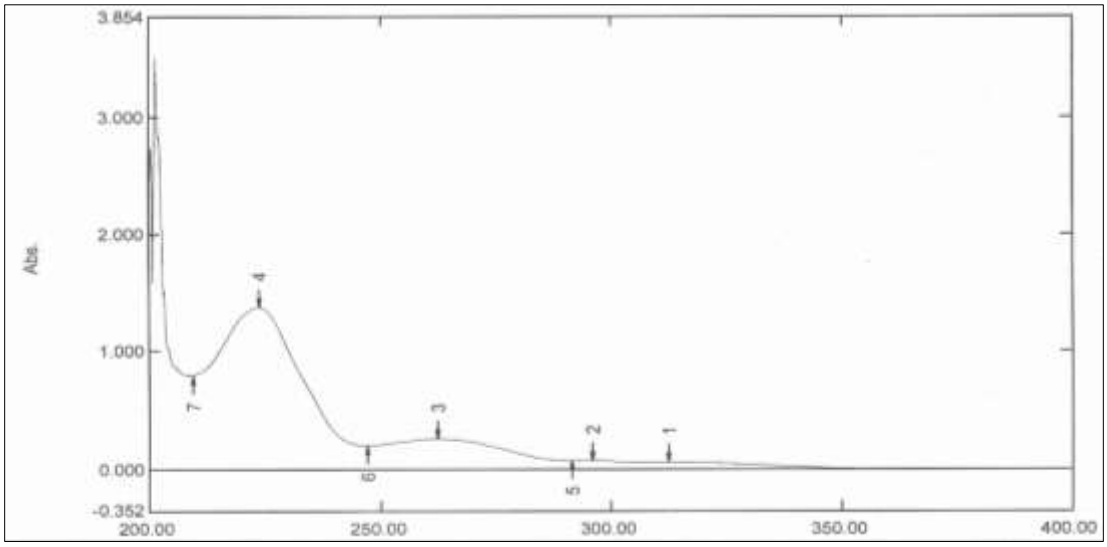


Fig.(8): U.V spectrum of [1-(benzoyl)-3-(benzimidazol-2-yl) thiourea] (c)

Table (1) physical properties for thiourea derivatives

Comp. N	M.Wt g/mol	Color	m.p °C or	Found, cal. (%)				UV-Visible		
				C	H	N	S	λ(nm	υ (cm	Transitio
a	285.32	White	242-245	58.65 (58.94)	3.84 (3.89)	14.84 (14.73	11.13 (11.2	225 262	44444 38167	π→π* n–π*
b	416.47	White	220-223	51.91 (51.40)	3.89 (3.81)	13.45 (13.63	15.40 (15.2	228 268	43859 37313	π→π* n–π*
c	296.35	light brown	245 dec	60.80 (60.68)	4.08 (4.01)	18.91 (19.12	10.82 (10.6	235 262	42553 38167	π→π* n–π*
d	313.39	yellow	215 dec	57.49 (57.34)	3.54 (3.49)	13.41 (13.62	20.46 (20.3	224 262	44642 38167	π→π* n–π*

dec.=decomposition

Table (2): The characteristic infrared of thiourea derivatives

Comp. No.	IR , (KBr, CsI), cm^{-1}						
	ν(NH) thioamide sec amide	ν(C=O)	ν(C-H) aromatic	ν(C=C)	ν(C=N)	ν(C=S)	ν(C-N)
a	3244 m 3124 m	1666 m 1716 vs	3066 m	1600 s 1546 s	1485 s	1385 s	1400 vs 1257 m

b	3417 m 3475 w	1681 s	3055 m	1597 m 1550 m	1465 m	1315 m	1384 s 1265 w
c	3325 s 3224 m	1678 s	3059 m	1585 vs 1539 m	1460 m	1385 m	1458 s 1269 s
d	3313 s 3170 m	1666 m	3062 m	1597 m 1550 s	1458 m	1392 vs	1458 s 1269 s

s= strong , vs=very strong , w = weak , m=middle

4. Antimicrobial activity:

In our study, Antimicrobial activity of the compounds (a, b, c, d) was examined by two types of bacteria species *Staphylococcus aureus* (Gram Positive), *Pseudomonas aeruginosa* (Gram Negative) by the agar diffusion technique [20]. Gentamicin were used as standard drug and DMSO as a solvent and as a control, for studying the potential activities of these compounds, the concentration of the compounds in this solvent was (5, 7.5, 10 mg/ml), This method involves the exposure of the zone of inhibition toward the diffusion of micro- organism on agar plate. The plates were incubated for 24hr. at 37C°[21] The inhibition zone diameters around each holes has been measured in milmeter.

The results exhibited most of the compounds have varsity anti-bacterial activities.show figs.(9 , 10) that compounds (b, c , d at concentration 10 mg/ml) have higher activity froagainst *Staphylococcus aureus* compared with standard drug (Gentamicin).except (a , c at concentration 5 mg/ml) with *Pseudomonas aeruginosa* has no biological activity [inhibition zone=0].

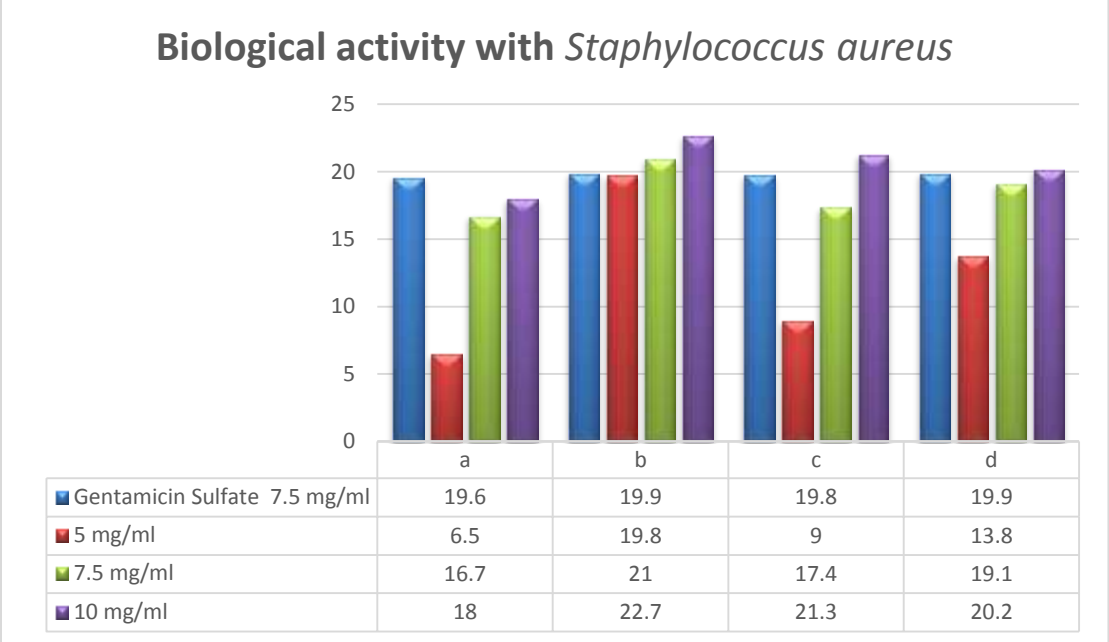
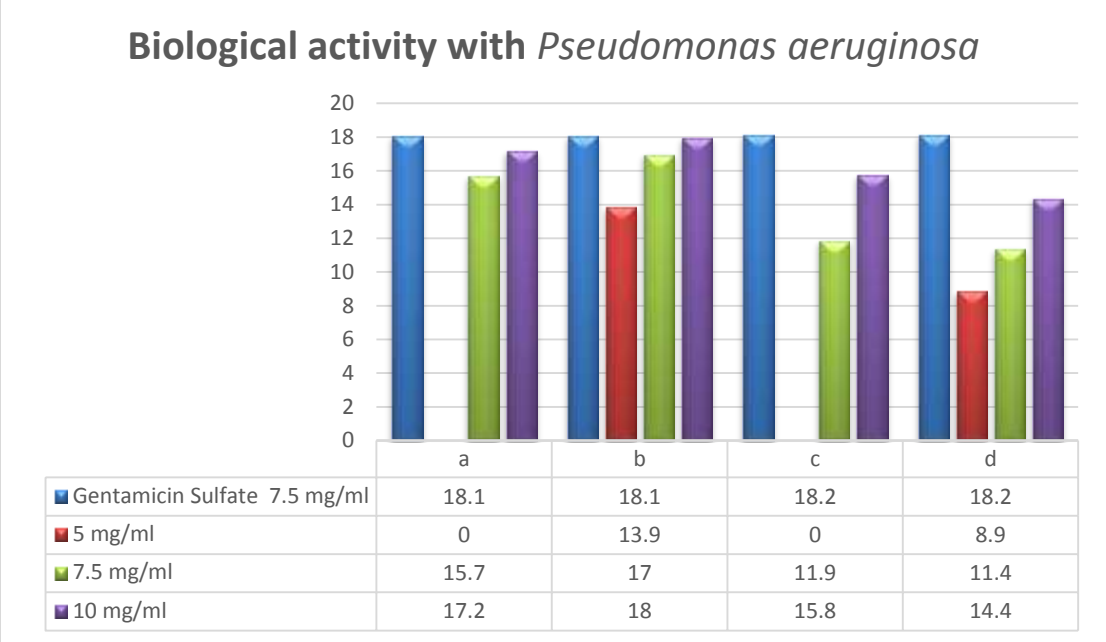


Fig.(9): Biological activity with *Staphylococcus aureus*



	a	b	c	d
Gentamicin Sulfate 7.5 mg/ml	18.1	18.1	18.2	18.2
5 mg/ml	0	13.9	0	8.9
7.5 mg/ml	15.7	17	11.9	11.4
10 mg/ml	17.2	18	15.8	14.4

Fig.(10): Biological activity with *Pseudomonas aeruginosa*

5.Conclusions

In this work, we have preparation a Four new thiourea derivatives from the basic compound (benzoyl isothiocyanate), was noticed a high stability of new the compounds were characterized by analytical and spectral data (FT-IR, ^{1}H-NMR, ^{13}C -NMR, C.H.N.S) proved the proposed structures. It can be concluded that thiourea derivatives has good biological activity against the bacteria (*Staphylococcus aureus*) compared with standard drug (Gentamicin)

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Cite this article as: **Osama Mohamed Mahdi (2023).**
Synthesis, Characterization and Antibacterial Activity Of Some Thiourea Derivatives
African Journal of Biological Sciences. 5(4), 01-14. doi: 10.48047/AFJBS.5.4.2023.01-14