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Synthesis, Characterization, and Antibacterial Study of New Ligands Derived from Nalidixic acid

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ABSTRACT
A novel compounds of nalidixic acid derivatives were synthesized by a coupling reaction with 2-aminothiazole-5-carbaldehyde using DIC as coupling reagent and HOBt as racemization suppressant then the product of reaction treated with different amine tail like 2-(methylthio) Ethan amine that result in compound 1 while refluxing with 2-(4-methyl piperazine-1-yl) Ethan amine and 2-(pyrrolidine-1-yl) Ethan amine results in compound 2 and 4 respectively. All the synthesized compounds were adequately identified by spectral studies utilizing IR and elemental analysis and assessed for their in vitro antibacterial activity in contradiction of different type of bacteria. All the tested molecules against bacterial strains compared with the nalidixic acid as a reference drug in this study. Results indicated that compounds 1, 2 & 4 have good antibacterial activities compared to standard drug especially as they were found to have promising activity against Bacillus and to be as effective as nalidixic acid toward psudomonus and E coli.

Keywords: *Nalidixic acid; coupling reaction; Schiff base; disc diffusion method; 2-aminothiazole-5-carbaldehyde;*

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INTRODUCTION

Quinolones form a broad class of antibacterial agents that are incredibly efficient in the therapy of various kinds of dangerous infections caused mainly by bacteria [1]. Nalidixic acid was the principal clinically valuable quinolone antibacterial agent structure illustrated in fig1. It was discovered serendipitously in 1962 as by product of chloroquine for the therapy of UTI in humans [2]. Nalidixic acid is thus estimated to be the antecedent of all members of the quinolone class, including the second, third and fourth generations generally recognized as fluoroquinolones. It acts versus bacteria by selectively hindering the topoisomerase type II & IV topoisomerase enzymes that perform a significant function in bacterial cell growth and movement [4]. As a rule, quinolones are amazingly dynamic against Gram-negative bacteria and are less potent against Gram-positive bacteria as resistance usually develops. Nalidixic acid, for example, is the most consumed antibacterial quinolone worldwide, and consequently most exposed to resistance. Thiazole containing molecules have been proved to be valuable in various remedial purposes such as treating viral infections, anticancer, and antiepileptic therapeutic agents [5–8] but their primary goal as antibacterial has not been thoroughly investigated.

Earlier research into thiazole structures incorporated by Cushman M, et al (2015)[9] display strong antimicrobial action on multidrug-resistant strains of Staphylococcus Aureus, including methicillin-resistant S. aureus (MRSA) [10, 11] The purposes of the research is to combine thiazoles to nalidixic acid and test the activity of the combined structure against different kind of bacterial isolates, to determine the probability of MRSA going active resistance to the composed compounds, and to determine if the structures could be used to re-sensitize MRSA to the effect of antibiotics.

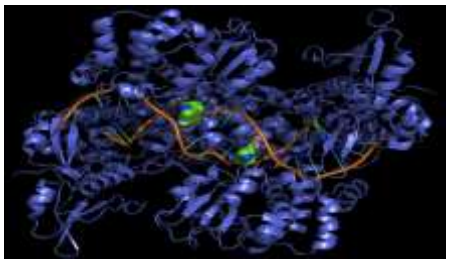

Figure 1: Structure of bacterial DNA gyrase complexed

MATERIALS AND METHOD

2.1 Chemical Synthesis

The compounds utilized throughout synthesis purchased from Sigma-Aldrich (Selangor, Malaysia). All compounds infrared readings expressed as cm-1 using a Nicolet 6700 FT-IR spectrophotometer (Thermo Nicolet Corp., Madison, WI, USA). NMR reading recorded with AVANCE III 400 MHz spectrometer (Bruker, Billerica, MA, USA), DMSO used as a solvent, **and the data reported in δ ppm. CHN analysis** performed on an Elemental analyzer Carlo Erba 1108 (Vario El III, SpA, Rodano, Italy).

Compounds synthesized by simple chemistry and yield from 70-84% final compounds purified by column chromatography applying dichloromethane/Methanol/ 2Mammonia in methanol as 8.5/1/0.5 fraction to get highly pure compounds the scheme for synthesis of intermediate as well as final compounds illustrated in figure 2:

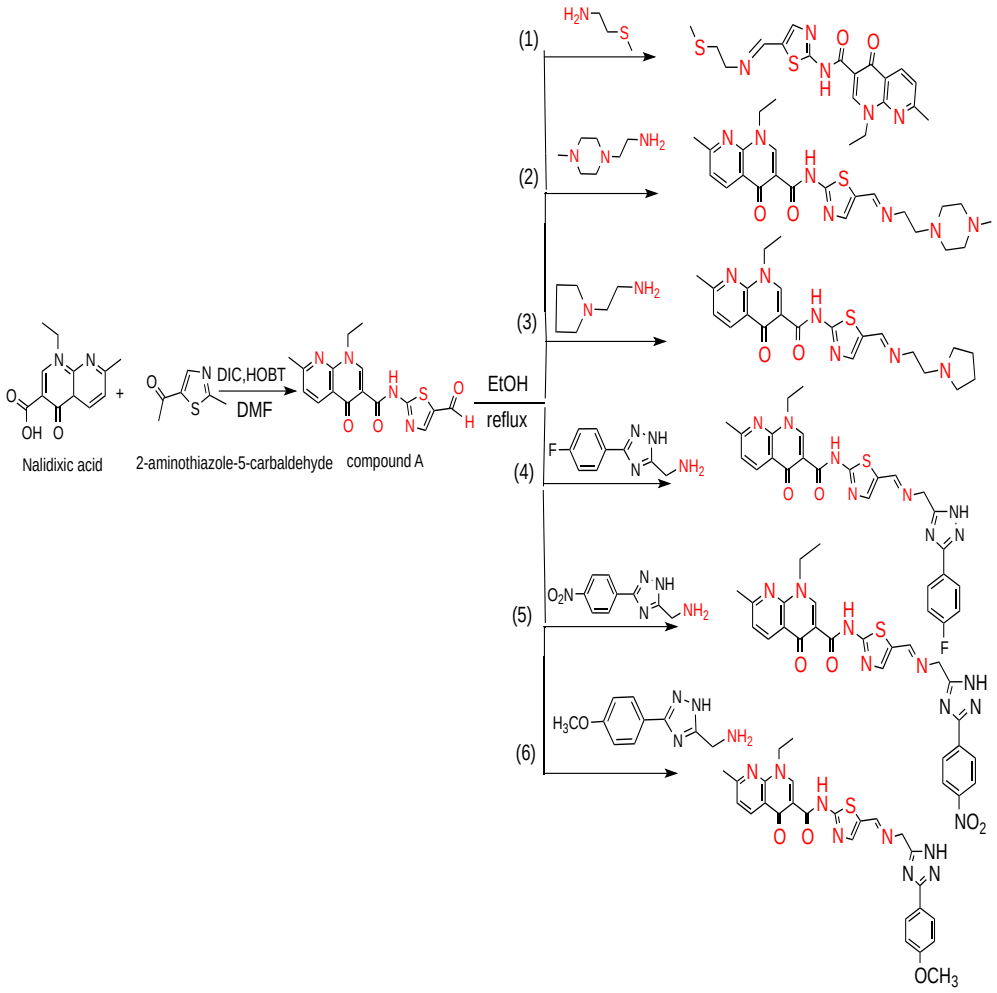

Figure 2: Scheme of synthesis of compounds (1-6)

Synthesis of (E)-1-ethyl-7-methyl-N- (5-(((2-(methyl thio) ethyl) imino) methyl) thiazol-2-yl)-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide (comp 1)

Mixtures of compound A (0.684 g, 2 mmol) and 2-(methylthio) Ethan amine (0.91 g, 1 mmol) were dissolved in 30 mL of absolute ethanol and boiled at refluxing temperature for 48 h. TLC monitored the progress of the reaction. Mixture transferred into iced water, and the solid collected was filtrated, concentrated, and recrystallized from ethanol.

^{1}H NMR (DMSO-d_6) δ 11.63 (s, 1H), 8.91 (s, 1H), 8.45 (d, J = 7.9 Hz, 1H), 8.01 (s, 1H), 7.91 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 4.44 (q, J = 8.0 Hz, 2H), 2.62 (s, 3H), 2.50 (t, J = 7.7 Hz, 2H), 2.08 (s, 3H), 1.70 (t, J = 7.7 Hz, 2H), 1.35 (t, J = 8.0 Hz, 3H), ^{13}C NMR (DMSO-d_6) δ 176.41, 164.06 (d, J = 7.2 Hz), 162.28, 149.88, 150.69, 147.03, 136.30, 136.05, 131.19, 122.93, 119.15, 111.65, 58.67, 45.75, 33.29, 23.70, 15.39 (d, J = 14.2 Hz), IR (cm−1): 3320 (NH stretching), 2840 (-CH2), 2910 (-CH3), 1715 (C=O), 1174 (C–S), 1568(C=N), 1446 (C=C), 1335 (C=C), 1129 (C=C), 717 (C-S). CHNS: C, 54.92%; H, 5.09%; N, 16.85%; S, 15.43%, Found: C, 54.87%; H, 5.15%; N, 16.80%; S, 15.49%. HRMS: m/z (+EI) calc. for $C_{19}H_{21}N_5O_2S_2$ 415.1137 ($M+H^+$), found 416.1159 ($M+H^+$)

Synthesis of (E)-1-ethyl-7-methyl-N- (5-(((2-(4-methylpiperazin-1-yl) ethyl) imino) methyl) thiazol-2-yl)-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide (comp 2)

Mixtures of compounds A (0.684 gm., 2 mmol) and 2-(4-methylpiperazin-1-yl) Ethan amine (0.143 g, 1 mmol) in 45 mL of absolute ethanol and bioled at refluxing temperature for 24 h. TLC monitored the progress of the reaction, and the solid got was filtered, dried out, and recrystallized in ethanol.

^{1}HNMR (DMSO-*d6*) δ 12.26 (s, 1H), 8.59 (d, J = 7.9 Hz, 1H), 8.34 (s, 1H), 8.02 (d, J = 11.2 Hz, 2H), 7.32 (d, J = 8.0 Hz, 1H), 4.70 (q, J = 8.0 Hz, 2H), 2.60 – 2.35 (m, 5H), 2.29 (t, J = 5.2 Hz, 5H), 2.24 (s, 3H), 2.11 (t, J = 5.3 Hz, 5H), 1.40 (t, J = 7.3 Hz, 2H), 1.35 (t, J = 8.0 Hz, 3H),^{13}C NMR (DMSO-*d6*) 172.61, 162.26 (d, J = 7.2 Hz), 160.72 , 159.58 , 152.89 , 150.60 , 136.30 , 136.05 , 131.19 , 122.93 , 119.15 , 111.65 , 59.22 , 54.41 , 54.09 , 53.17 , 45.75 , 44.94 , 23.70 , 15.33. IR (cm−1): 3330 (NH stretching), 2863 (-CH2), 2918 (-CH3), 1615 (C=O), 1174 (C–S), 1558(C=N), 1486 (C=C), 1375 (C=C), 1149 (C=C), 617 (C-S). CHNS: C, 59.08%; H, 6.25%; N, 20.97%; S, 6.86%, Found: C, 60.07%; H, 6.36%; N, 20.99%; S, 6.95%. HRMS: m/z (+EI) calc. for $C_{23}H_{29}N_7O_2S$ 467.2103 ($M+H^+$), found 468.2009 ($M+H^+$)

Synthesis of (E)-1-ethyl-7-methyl-4-oxo-N- (5-(((2-(pyrrolidin-1-yl) ethyl) imino) methyl) thiazol-2-yl)-1,4-dihydro-1, 8-naphthyridine-3-carboxamide: (comp 3)

A mixture of compounds A (0.684 g, 2 mmol) and 2-(pyrrolidin-1-yl) Ethan amine (0.143 g, 1 mmol) in 45 mL of absolute ethanol and refluxed for 72 h. TLC monitored the progress of the reaction, and the solid collected was filtrated, concentrated, and recrystallized from ethanol.

^{1}H NMR (DMSO-*d6*) δ 12.17 (s, 1H), 7.61 (d, *J* = 7.1 Hz, 1H), 7.37 – 6.78 (m, 3H), 6.32 (d, *J* = 6.8 Hz, 1H), 4.70 (d, *J* = 8.0 Hz, 2H), 3.13 – 1.89 (m, 11H), 2.01 – 1.09 (m, 11H) ^{13}C NMR (DMSO-*d6*) δ 168.61, 164.16 (d, *J* = 7.2 Hz), 160.52, 157.58, 154.89, 150.60, 136.30, 134.05, 128.19, 122.93, 119.04, 111.85, 58.42, 54.06 (d, *J* = 9.0 Hz), 45.65, 23.80, 15.43, IR (cm−1): 3340 (NH stretching), 2850 (-CH2), 2930 (-CH3), 1730 (C=O), 1164 (C–S), 1584(C=N), 1426 (C=C), 1365 (C=C), 1123 (C=C), 713 (C-S). CHNS: C, 60.25%; H, 5.98%; N, 19.16%; S, 7.31%, Found: C, 60.37%; H, 5.96%; N, 19.11%; S, 7.39%. HRMS: m/z (+EI) calc. for $C_{22}H_{26}N_6O_2S$ 438.1838 ($M+H^+$), found 438.1859 ($M+H^+$)

Synthesis of (*E*)-1-ethyl-7-methyl-*N*- (5-((((3-(4-nitrophenyl)-1*H*-1, 2,4-triazol-5-yl) methyl) imino) methyl) thiazol-2-yl)-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide (comp 4)

Mixtures of compound A (0.684 g, 2 mmol) and (3-(4-nitrophenyl)-1*H*-1,2,4-triazol-5-yl) methanamine (0.219 g, 1 mmol) were dissolved in 30 mL of absolute ethanol and heated at refluxing temperature for 10 h until TLC show complete reaction. Contents transferred into ice bath, and the crystals obtained was filtered, dried, and recrystallized from ethanol.

^{1}H NMR (DMSO-d_6) δ 12.45 (s, 1H), 12.44 (s, 1H), 7.91 (s, 1H), 7.45 (d, *J* = 7.0 Hz, 1H), 7.39 (d, *J* = 7.3 Hz, 2H), 7.07 (d, *J* = 9.7 Hz, 2H), 7.35 (d, *J* = 7.2 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 1H), 4.61 (s, 2H), 4.32 (q, *J* = 8.0 Hz, 2H), 2.72 (s, 3H), 1.45 (t, *J* = 8.0 Hz, 3H).

^{13}C NMR (DMSO-d_6) δ 178.61, 166.16 (d, *J* = 7.2 Hz), 160.42, 159.78, 158.49, 155.01, 150.60, 149.30, 147.84, 136.30, 136.05, 131.58, 131.19, 128.08, 125.23, 122.93, 119.15, 111.65, 55.68, 45.75, 23.70, 15.33. IR (cm−1): 3220 (NH stretching), 2860 (-CH2), 2945 (-CH3), 1633 (C=O), 1174 (C–S), 1577(C=N), 1466 (C=C), 1335 (C=C), 1129 (C=C), 717 (C-S),(1554- 1348) N-O. CHNS: C, 55.24%; H, 3.89%; N, 23.19%; S, 5.90%, Found: C, 55.27%; H, 3.98%; N, 23.24%; S, 5.93%. HRMS: m/z (+EI) calc. for $C_{25}H_{21}N_9O_4S$ 543.1437 ($M+H^+$), found 543.2839 ($M+H^+$)

Synthesis of (*E*)-1-ethyl-*N*- (5-((((3-(4-methoxy phenyl)-1*H*-1, 2,4-triazol-5-yl) methyl) imino) methyl) thiazol-2-yl)-7-methyl-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide (comp 5)

Mixtures of compound A (0.684 g, 2 mmol) and (3-(4-methoxy phenyl)-1*H*-1, 2,4-triazol-5-yl) methanamine (0.204 gm., 1 mmol) were dissolved in 50 mL of absolute ethanol and refluxed for 24 h. TLC monitored the reaction. Contents were covered into ice-cold water, and the solid obtained was filtered, dried, and recrystallized from ethanol.^{1}H NMR (DMSO-d_6) δ 12.73 (s, 1H), 11.63 (s, 1H), 7.91 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 7.3 Hz, 2H), 7.08 (d, *J* = 9.7 Hz, 2H), 6.95 (d, *J* = 7.2 Hz, 2H), 6.32 (d, *J* = 7.9 Hz, 1H), 4.81 (s, 2H), 4.44 (q, *J* = 8.0 Hz, 2H), 2.62 (s, 3H), 1.35 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (DMSO-d6) δ 168.98, 163.16, 161.01, 160.42, 158.28, 157.47, 149.70, 146.38, 140.05, 135.19, 132.37, 130.73, 124.93, 120.15, 118.77, 111.65, 55.68, 45.15, 35.75, 23.70, CHNS: C, 55.24%; H, 3.89%; N, 23.19%; S, 5.09%, Found: C, 55.17%; H, 4.06%; N, 23.31%; S, 5.19%. HRMS: m/z (+EI) calc. for $C_{26}H_{21}N_8O_4S$ 543.5571 ($M+H^+$), found 543.7689 ($M+H^+$)

Synthesis of (*E*)-1-ethyl-*N*-(5-((((3-(4-fluorophenyl)-1*H*-1,2,4-triazol-5-yl) methyl) imino) methyl) thiazol-2-yl)-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (comp 6)

^{1}H NMR (DMSO-d_6) δ 11.33 (s, 1H), 11.53 (s, 1H), 8.71 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.23 (s, 1H), 8.05 (s, 1H), 7.73 (t, *J* = 6.5 Hz, 2H), 7.35 – 7.24 (m, 3H), 4.44 (q, *J* = 8.0 Hz, 2H), 2.61 (d, *J* = 10.0 Hz, 4H), 1.35 (t, *J* = 8.0 Hz, 3H), ^{13}CNMR (DMSO-d_6) δ 178.61, 164.16 (d, *J* = 7.1 Hz), 159.82, 159.58, 157.46, 155.01, 150.30, 149.49, 136.52, 136.65, 131.27, 129.48 (d, *J* = 7.8 Hz), 122.89, 119.28, 116.15 (d, *J* = 20.0 Hz), 111.65, 55.68, 45.75, 23.70, 15.33. CHNS: C, 58.13%; H, 4.10%; N, 21.69%; S, 6.21%, Found: C, 58.17%; H, 4.16%; N, 21.72%; S, 6.19%. HRMS: m/z (+EI) calc. for $C_{25}H_{21}FN_8O_2S$ 516.1492 ($M+H^+$), found 516.1829 ($M+H^+$)

2.2 Antimicrobial Studies

In vitro antibacterial activity for the synthesized compounds was tested against different bacterial strains like *Staphylococcus Aureus*, *Bacillus Subtilus*, *Proteus Vulgaris*, *Klebsiella Pneumonia* and *E. Coli* with compared to standard antibiotic drug nalidixic acid and moxifloxacillin. Zone of inhibition (mm) values for analogues and positive control drug were determined by agar disc diffusion method [12]. Compounds (1-6) as well a standard drug were suspended in dimethyl sulfoxide (DMSO.; 125 µg/ mL, 250 µg/mL and 500 µg/mL respectively). Bacteria used in this study obtained as pure isolates from laboratories of Dr. Sabah Al-Fatlawi & Department of Clinical laboratory sciences were first sub cultured in Brain heart infusion broth at temperature 37°C for 18-24 hr. select 3-5 colonies of bacteria isolates by loop and transfer them to tube containing 3 mL normal saline and vortex well. Approximately one hundred microliters of the standardized inoculum bacterial suspension of around (1.5×108 CFU/mL) gained from McFarland turbidity standard (number 0.5) of each bacterium was used to inoculate by use glass spreader on the surface of Mueller Hinton Agar (MHA) plates. The additional liquid was air dried under a sterile hood or repeats the spreading process. The plates were allowed to dry and punched wells (five) in diameter 6 mm. into agar subsequently. In each agar plate of tested bacteria five wells were made and (100µl) of dilutions of derivatives compound introduced into wells on MHA plate. DMSO used as the negative controller. The plates were incubated at 37 °C for 24 hours and the antimicrobial action was estimated by determining the diameter of the inhibition zone (IZ) all over the place the disc in mm. as listed in Table 2.

RESULTS and DISCUSSIONS

The results showed a greater activity of compound 1 and 2 against *B. Subtilus and* S. Aureus compared to the rest of compound all compound show weak activity toward and standard drugs in the same concentrations while all compounds showing better activity toward *Bacillus Subtilus* that nalidixic acid have no activity and this may be attributed to positively charged amine tail that has ability of penetration of gram +ve bacteria comparable to nalidixic acid. Compound 2 is better than 1 and 4 against *Bacillus Subtilus* which may attributed to methyl piperazine ring further dilutions and bacterial test against wide range of bacteria is required to examine the activity of the recently synthesized compounds.

Table 2: In vitro Antibacterial Activity of Compounds using IZ and MIC (μg/mL) with Control drug Nalidixic acid (NA.) and MOX (Moxifloxacillin)

Comp.	CLogP	S. Aureus		*B. Subtilus*		P.Vulgaris		*K. Pneumonia*		*E. Coli*	
		IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
1	1.79	24	1.9	22	5.2	15	61	11	2.8	27	2.9
2	1.18	31	1.5	45	1.1	25	29	24	1.3	39	0.36
3	1.69	20	3.8	17	3.8	8	>128	12	8.8	20	6.4
4	2.33	17	2.5	24	4.7	17	33	19	3.2	22	15
5	2.39	15	3.3	33	1.2	8	>128	24	1.6	24	2.25
6	2.5	11	3.7	9	3.2	8	38	12	2.8	21	26
NALD	0.85	2	>128	NiL	>128	28	11	29	<0.5	26	<0.25
MOX	0.39	39	0.006	31	0.098	ND	ND	ND	ND	ND	ND

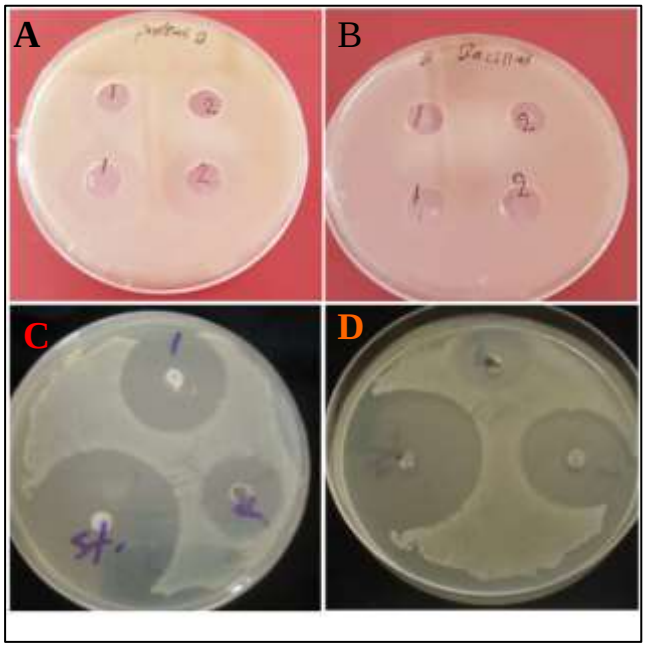

Figure 3: A) IZ of compounds 1& 2 against P.Vulgaris B) IZ of compounds 1 & 2 against Bacillus Subtlus C) IZ of compounds 1 & 2 against Staphylococcus aureus D) IZ of compounds 1&2 against Escherichia Coli

CONCLUSION

Thiazole derivatives of nalidixic acid were effectively synthesized and identified by spectroscopic methods (FT-IR and elemental microanalysis). Antimicrobial activities were evaluated by agar dilution method and the outcomes showed that they have valuable antibacterial activities compared to standard drugs specially Compounds 1 and 2 were found to have promising activity against S. Aureus and *B. Subtilus* better than the standard reference drugs used, and being as effective as nalidixic toward *K. Pneumonia* and E coli specially compound 2, and these results were encourage further study toward various gram positive and gram negative bacteria to see their effect. Compounds 1& 2 IZ against proteus species

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals or Humans were used during this study.

CONFLICT OF INTEREST

The authors have nothing to declare.

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