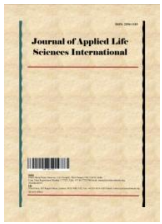

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Design, Synthesis and Antibacterial Activity of some 3,6-Dimethlyquinoxaline-2-Hydrazone Derivatives

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This aims of this study was to continue the effort to synthesis new quinoxaline-based heterocycles and study its antibacterial properties.
Objective: This study was designed to reacts 3,6-dimethylquinoxaline-2-hydrazine with some substituted aromatic ketones and study their antibacterial properties on some locally and clinically isolated bacterial strains.
Materials and Methods: Five 3,6-dimethylquinoxaline-2-hydrazone derivatives were synthesized from the reactions of 3,6-dimethylquinoxaline-2-hydrazine with various substituted aromatic ketones. The products were then tested for their potential antibacterial properties.
Results: All the synthesized compounds were found to be active against all the bacterial strains investigated in this study. It was observed that the zones of inhibition observed for the synthesized compounds against the test organisms ranged between 15 mm and 38 mm. The MIC observed for the synthesized compounds ranged between 0.0313mg/mL and 0.125 mg/mL, while that of the standard antibiotic, streptomycin, varied between 0.0313 mg/mL and 0.500 mg/mL and those observed for tetracycline falls between 0.0313 mg/mL and 0.500 mg/mL. The minimum bactericidal concentrations exhibited by the synthesized compounds ranged between 0.0625 mg/mL and 0.250 mg/mL
Discussion and conclusion: The study concluded that all the compounds exhibited appreciable bactericidal effects against all the bacterial strains, which is an indication that such

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synthetic compounds possessed broad spectrum activities and such compounds could be useful in formulation of antibacterial compounds which could be used to mitigates infections caused by pathogens that are now developing resistance against the available antibiotics.

Keywords: Quinoxalines; Antibacterial activity; Gram-positive bacteria; Gram-negative bacteria, quinoxaline-2-one; Synthesis; substituted acetophenone.

1. INTRODUCTION

Quinoxaline which are well known for their antimicrobial and pharmacological properties has attracted the attention of many scientists globally in their quest to search for potent antimicrobial compounds to combat the growing pandemic ravaging mankind. Quinoxalines have been reported to possess potent anti-viral [1-3] anti-bacterial [4-10] anti-inflammatory [11,12] anticancer [13-15]. These compounds have also found applications in the agricultural field where they are used as fungicides, herbicides, and insecticides to control weeds and pest [16]. This study focused more on the biological activity of quinoxaline on some bacterial strains known to cause human infections. Many of these pathogens have developed resistance against antibiotics. This has been creating a lot of headache in healthcare delivery which requires urgent solution. Thus, scientists need to move faster in researches on antimicrobials in order to develop more potent antimicrobials to take care of superbugs that are now "waging wars" against the available antimicrobials. This study is one of such efforts taken to develop potent antimicrobial compounds to combat the menace of these pathogens.

2. MATERIALS AND METHODS

Melting points were determined with open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. Infrared spectra were recorded as KBr pellets on a Buck Spectrometer. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) was run on a Bruker 600 MHz spectrometer (δ in ppm relative to Me_4Si) at the Department of Chemistry, Portland state University, Portland U.S.A. The purity of the compounds was routinely checked by TLC on silica gel G plates using n-hexane/ethyl acetate (1:1, v/v) solvent system and the developed plates were visualized by UV light. All reagents used were obtained from Sigma–Aldrich Chemical Ltd.

2.1 Synthesis of 3,6-Dimethylquinoxaline-2(1H)-one

4-Methyl-o-phenylenediamine (20 g; 0.10 M) and ethyl pyruvate (22 g; 0.10 M) in 200 mL of absolute ethanol was heated for 30 minutes on oil bath. The reaction mixture was allowed to cool to give a silvery white crystal which were collected by filtration, washed and purified by recrystallization from ethanol. % yield: 88.40%; Melting point: 246-247°C lit. 245-246°C [1, 17]; IR KBr (cm-1): 3103 (C-H sp2 str.), 1602 (C=C aromatic str.), 1660 (C=N str.), 2866 (C-H sp3 str.), 3462 (NH str.), 1568 (N-H bend), 1690 (C=O str.). 1H-NMR (DMSO-d6): 10.66 (broad s, 1H, quinoxaline NH), 8.27(*J*= 8, 2) (d, 1H, aromatic protons); 7.47(J=8, 2) (t, 1H, aromatic protons); 7.31(*J*= 8, 2) (t, 1H, aromatic protons); 7.09(=8, 2) (d, 1H, aromatic protons); 2.07 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 156 ppm, 154 ppm (C=O), 133 ppm, 131 ppm, 129 ppm, 125 ppm, 1253 ppm, 115 ppm, 21 ppm.

2.2 Synthesis of 2-Hydrazinyl-3,6-Dimethyl-1,2-Dihydroquinoxaline 1

3,6-dimethyl quinoxalin-2-(1H)-one (15 g, 0.0937 mol.) was added to hydrazine hydrate in 20 ml of water. The resulting mixture was refluxed for 6 hours. The reaction mixture was allowed to cool to room temperature to afford a brownish-yellow solid precipitate which was filtered, dried and recrystallized from ethanol. 2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (1): 90.00%; Melting point: 319-321°C lit. 318-320 °C [17] IR KBr (cm-1): 3448 (N-H str.), 3308 (N-H2 str.), 3007 (N- H2 str.), 2966 (C-H sp2 str.), 2898 (C-H sp3 str.), 1568 (N-H bend), 1665 (C=N str.) 1H-NMR (DMSO-d6): 8.46 (broad s, 1H, hydrazine NH), 7.94(J=8, 2) (d, 1H, aromatic protons); 7.82(J=8, 8, 2) (d, 1H, aromatic protons); 7.78(J=8, 8, 2) (t, 1H, aromatic protons); 7.67(J=8, 8, 2) (t, 1H, aromatic protons); 4.59 (broad s, 2H, hydrazine NH2) 2.42 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 163 ppm, 145 ppm, 135 ppm, 127 ppm, 125 ppm, 124 ppm, 17 ppm.

2.3 Synthesis of 2-(2-(3,6-Dimethylquinoxalin-2-yl)hydrazono)-1H-Indene-1,3(2H)Dione 2

2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (1.0 g, 5.67 mmol) and isatin (0,90 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 3 hours. The reaction mixture was cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded 2-(2-(3,6-dimethylquinoxalin-2-yl)hydrazono)-1H-indene-1,3(2H)dione 2.: 52.30%; Melting point: 217-220°C, lit. 219-221°C [17]; IR KBr (cm-1): 3451 (N-H str.), 1568 (N-H bend), 1608 (C=C aromatic str.), 2900 (C-H sp3 str.), 1590 (C=N str.) 1H-NMR (DMSO-d6): 10.45 (broad s, 1H, hydrazine NH), 7.93 (d, 1H, aromatic proton); 7.90 (d, 1H, aromatic proton); 7.80 (d, 1H, aromatic proton); 7.76 (t, 1H, aromatic protons); 7.72(d, 2H, aromatic protons); 7.67 (t, 1H, aromatics proton); 7.61 (t, 1H, aromatics proton), 2.40 (s, 3H, methyl proton) 13C-NMR (DMSO-d6): 163 ppm, 162 ppm (C=O), 145 ppm, 140 ppm, 135 ppm, 127 ppm, 125 ppm, 124 ppm, 122 ppm, 17 ppm.

2.4 Synthesis of 4,4'-((1E,3Z,5Z,6E)-3-hydroxy-5-(2-(3,6-Dimethylquinoxalin-2-yl) hydrazono)Hepta-1,3,6-triene-1,7-diyl)bis(2-methoxyphenol) 3

2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (1.0 g, 5.67 mmol) and Curcumin (2.07 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 3 hours. The reaction mixture was cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded 4,4'-((1*E*,3*Z*,5*Z*,6*E*)-3-hydroxy-5-(2-(3,6-dimethyl quinoxalin-2-yl)hydrazono)hepta-1,3,6-triene-1,7-iyl)bis(2-methoxyphenol) 3. % yield: 76.40%; Melting point: 172-173°C, lit. 174-179°C [17]; IR KBr (cm-1): 3442 (N-H str.), 2898 (C-H sp3 str.), 1607 (C=C aromatic str.), 1570 (N-H bend), 1662 (C=N str.) 1H-NMR (DMSO-d6): 10.45 (broad s, 1H, hydrazine NH), 7.93 (d, 1H, aromatic proton); 7.90 (d, 1H, aromatic proton); 7.80 (d, 1H, aromatic proton); 7.78 (s, 2H, aromatic protons); 6.99(d, 2H, aromatic protons); 6.78 (d, 1H, olefinic proton) 6.86 (d, 1H, olefinic proton); 6.80 (d, 1H, olefinic proton), 5.68 (d, 1H, olefinic proton), 5.33 (d, 1H, olefinic proton); 3.83 (s, 6H, OCH3 proton); 2.40 (s, 3H, methyl proton) 13C-NMR (DMSO-d6): 174 ppm (C=C-OH), 163 ppm, 156 ppm, 149 ppm, 149 ppm, 147 ppm, 140 ppm, 138 ppm, 135 ppm, 134 ppm, 127 ppm, 125 ppm, 124 ppm, 122 ppm, 119 ppm, 116 ppm, 111 ppm, 87 ppm, 56 ppm (OCH3), 17 ppm.

2.5 Synthesis of (Z)-3-(2-(3,6-dimethylquinoxalin-2-yl)hydrazono)indolin-2-one 4

2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (1.0 g, 5.67 mmol) and isatin (0.83g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 3 hours. The reaction mixture was cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded (*Z*)-3-(2-(36-dimethylquinoxalin-2-yl)hydrazono)I ndolin-2-one 4 % yield: 65.80%; Melting point: 260-261°C, lit. 260-262°C [17]; IR KBr (cm-1): 3435 (N- H str.), 2899 (C-H str.), 1608 (C=C aromatic str.), 1575(C=Nstr.) 1679(C=O str.) 1H-NMR (DMSO-d6): 10.45 (broad s, 1H, hydrazine NH), 10.05 (broad s, 1H, hydrazine NH),7.94 (d, 1H, aromatic proton); 7.92 (d, 1H, aromatic proton); 7.80 (d, 1H, aromatic proton); 7.77 (t, 1H, aromatic protons); 7.73(d, 1H, aromatic protons); 7.68 (d 1H, aromatics proton); 7.61 (t, 1H, aromatics proton); 7.35 (t,1H, aromatics proton), 2.41 (s, 3H, methyl proton) 13C-NMR (DMSO-d6): 168 ppm (C=O), 163 ppm, 145 ppm, 141 ppm, 135 ppm, 133 ppm, 131 ppm, 129 ppm, 127 ppm, 125 ppm, 124 ppm, 119 ppm, 17 ppm.

2.6 Synthesis of 2-(2-(1,3-Dihydro-2H-inden-2-ylidene)Hydrazinyl)-3,6-Dimethylquinoxaline 5

2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (1.0 g, 5.67 mmol) and 2-indanone (0.83 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 3 hours. The reaction mixture was cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded the hydrazones yield: 80.20%; Melting point: 248-250°C lit. 249--251°C [17]; IR KBr (cm-1): 3442 (N- H str.), 2968 (C-H sp3 str.), 1602 (C=C aromatic str.), 1570(C=N str.) 1008 (N-N str.) 1H-NMR (DMSO-d6): 10.45 (broad s, 1H, hydrazine NH), 7.93 (d, 1H, aromatic proton); 7.90 (d, 1H,

aromatic proton); 7.75 (d, 1H, aromatic proton); 7.76 (t, 1H, aromatic protons); 7.45 (d, 2H, aromatics proton); 7. 37(t, 2H, aromatics proton), 3.39 (d, 4H, aliphatic protons); 2.40 (s, 3H, methyl proton) 13C-NMR (DMSO-d6): 163 ppm, 155 ppm, 145 ppm, 141 ppm, 135 ppm, 128 ppm, 127 ppm, 125 ppm, 124 ppm, 38 ppm, 32 ppm, 17 ppm.

2.7 Synthesis of 1-(2,6-dimethylquinoxalin-3-yl)-2-((naphthalen-1-yl)methylene) hydrazine 6

2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (1.0 g, 5.67 mmol) and naphthaldehyde (5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120^0C for 3 hours. The reaction mixture was cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded 1-(2,6-dimethylquinoxalin-3-yl)-2-((naphthalen-1-yl)methylene)hydrazine **6**. % yield: 85.00%; Melting point: 158-160°C lit. 160-161°C [17]; IR KBr (cm-1): 3442 (N- H str.), 2900 (C-HSP3 str.), 1610 (C=C Aromatic str.), 1568(C=N str.) 1H-NMR (DMSO-d6): 10.45 (broad s, 1H, hydrazine NH), 8.55 (s, 1H, hydrazino proton); 8.50 (d, 1H, aromatic proton); 7.98 (t, 1H, aromatic proton); 7.93 (d, 1H, aromatic proton);7.93 (d, 2H, aromatic proton); 7.90 (d, 1H, aromatic proton); 7.77 (t, 1H, aromatic proton); 7.75 (d, 1H, aromatic proton); 7.76 (t, 1H, aromatic protons); 7. 45 (t, 2H, aromatics proton), 2.40 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 163 ppm, 145 ppm, 143 ppm, 135 ppm,133 ppm, 130 ppm, 128 ppm, 127 ppm, 126 ppm, 125 ppm, 124 ppm, 17 ppm.

2.8 Preparation of Experimental Bacterial Isolate

The following typed cultures were locally isolated organisms were obtained from culture collection of Professor D. A Akinpelu, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

These bacterial Isolates are: Gram-Positive: *Bacillus cereus* (NCIB 6349), *Bacillus polymyxa*(LIO), *Bacillus subtilis*(NCIB 3610), *Bacillus stearothermophilus* (NCIB 8222), *Clostridium sporogenes* (NCIB 532), *Corynebacterium pyogenese* (LIO), *Stapylococcus aureus* (NCIB 8588) and *Micrococcus luteus* (NCIB 196)

Gram-negative: *Escherichia coli* (NCIB 86), *Klebsiella pneumonia* (NCIB 418), *Pseudomnas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756) and *Proteus vulgaris* (LIO).

2.9 Antibacterial Sensitivity Testing of some Synthesized Compounds

All of the synthesized compounds were screened for antibacterial activity using agar-well diffusion method as described by Akinpelu and Kolawole [18] with little modifications. The medium employed was Mueller-Hinton agar medium. With the aid of a sterile 1 mL pipette, exactly 0.2 mL of the standardized broth culture of the test organism was added to 18 mL sterile molten agar medium which had already cooled down to 40 ^{o}C. This was well mixed and poured into previously sterilized Petri dishes, which were properly labelled. The medium was then allowed to set. With the aid of a sterile cork borer, the required numbers of holes were bored into the medium. The wells were made 5 mm to the edge of the plate and were filled-up with the solution of the compound using sterile Pasteur pipette. Streptomycin phosphate and tetracycline were used as the standard antibacterial agent at a concentration of 1 mg/mL. The plates were allowed to stand for about one hour on the bench to allow for proper diffusion of antibacterial agent into the medium and then incubated uprightly at 37 ^{o}C for 24 hours. Care was taken not to stockpile the plates. Clear zones of inhibition (mm) indicated the relative susceptibility of the bacteria to the compounds.

2.10 Determination of Minimum Inhibitory Concentrations (MICs) of the Test Compounds

Minimum inhibitory concentrations of the compounds and the standard antibiotics-streptomycin and tetracycline was carried out using a two-fold dilution method [19]. Two milliliter of different concentrations of solution of the compound was added to 18 mlLof pre-sterilized molten nutrient agar at 40 ^{o}C to give final concentrations regimes of 0.0157 and 1.0 mg/mL. The same concentrations were also prepared for the two positive controls. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry under a laminar flow chamber before streaking with 18 h old standardized bacterial cultures. The plates were

later incubated at 37 ^{o}C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration of the test compound and standard antibiotics that will prevent the growth of the susceptible test bacteria.

2.11 Determination of Minimum Bactericidal Concentrations (MBCs) of the Compounds and Standard Antibiotics

The minimum bactericidal concentrations of the compounds were determined as described by Oludare et al. [20]) with some modifications. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar medium and later incubated at 37 ^{o}C for 48 h. The MBC was taken as the lowest concentration of the compound that completely kills the susceptible test organisms.

3. RESULTS

3.1 Chemistry

The 2-hydrazinyl-3,6-dimethyl-1,2-dihydroquinoxaline (I) which was synthesized from the reaction of 3,6-dimethylquinoxaline-2-one with hydrazine hydrate using conventional heating method under refluxing condition. Compound **I** was allowed to react with different substituted acetophenones to obtained the various 3,6-dimethylquinoxalin-2-hydrazones. The compounds were partially characterized using Infrared, ^{1}H and ^{13}C Nuclear Magnetic Resonance spectroscopic methods. The spectroscopic data showed a diagnostic bands in the IR spectral for the formation of hydrazone bonds (C=N-N) which were observed between 1564 and 1679 cm^{-1}, The quinoxaline (NH) bands was observed to appeared between 3400 and 3451 cm^{-1}, while the CH-SP^{3} stretching bands appeared between 2899 and 2969 cm^{-1}. The ^{1}H-NMR spectral data of the compounds gave the proton of the methyl group diagnostic frequency in the region of 2.40 and 2.95 ppm upfield while the proton of the azomethine group CH=N-N appeared between 8.55 and 10.46 ppm downfield.

3.2 Antimicrobial Studies

All the synthesized compounds were found to be active against all the bacterial strains investigated in this study. It was observed that the zones of inhibition observed for the synthesized compounds against the test organisms ranged between 15 mm and 38 mm. On the other the hand, the zones of inhibition observed for streptomycin and tetracycline against the bacteria ranged between 11 and 38 mm (Tables 1). The MIC observed for the synthesized compounds ranged between 0.0313mg/mL and 0.125 mg/mL, while that of the standard antibiotic, streptomycin, varied between 0.0313 mg/mL and 0.500 mg/mL and those observed for tetracycline falls between 0.0313 mg/mL and 0.500 mg/mL (Tables 2). The minimum bactericidal concentrations exhibited by the synthesized compounds against Gram positive bacteria ranged between 0.0625 mg/mL and 0.250 mg/mL, while streptomycin, varied between 0.0625 and 1.000 mg/mL and tetracycline ranged between 0.0625 and 1.000 mg/ml. The results indicated that streptomycin has comparable activity against the test bacterial strains than those exhibited by the synthesized compounds. In comparison the synthesized compounds showed more antibacterial activity against some of the bacterial strains than tetracycline (Tables 2).

4. DISCUSSION

4.1 Chemistry

The 2-hydrazinyl-3-methyl-6-nitroquinoxaline (I) which was synthesized from the reaction of 3-methyl-6-nitroquinoxaline-2-one with hydrazine dihydrate with conventional heating under reflux was allowed to react with different substituted acetophenones to obtained the various 3-methyl-6-nitroquinoxalin-2-hydrazones according to the literature methods. Please give appropriate refrences here. The compounds were partially characterized using Infrared, ^{1}H and ^{13}C Nuclear Magnetic Resonance spectroscopic methods. The spectroscopic data used for the structural elucidation confirmed the structures of all the compounds synthesized. The diagnostic bands in the IR spectral for the formation of hydrazino-bond (C=N) were observed between 1564 and 1679 cm^{-1}, The quinoxaline (NH) bands appeared between 3409 and 3451 cm^{-1}, while the CH-SP$_3$ stretching bands appeared between 2899 and 2969 cm^{-1}. The ^{1}H-NMR spectral gave the proton of the methyl group diagnostic frequency in the region of 2.40 and 2.95 ppm upfield while the proton of the azomethine group CH=N- appeared between 8.55 and 10.46 ppm downfield.

Table 1. The sensitivity patterns exhibited by 3-methlyquinoxaline-2-hydrazone (2-6′) against Bacterial Strains

Test Organisms	Compounds /Zones of inhibition (mm) **						
	2 (2 mg/mL)	**3 (2 mg/mL)**	**4 (2 mg/mL)**	**5 (2 mg/mL)**	**6 (2 mg/mL)**	**Strep (1 mg/mL)**	**Tet (1 mg/mL)**
Bacillus polymyxa (LIO)	20±0.29	19±0.29	27±0.29	26±1.00	22±1.00	18±0.50	28±0.56
B. cereus (NCIB 6349)	23±1.00	28±0.50	22±1.00	24±0.50	19±0.50	30±0.56	19±1.00
Corynebacterium pyogenes (LIO)	20±0.50	27±1.00	24±0.29	20±1.00	22±1.00	25±0.56	26±1.00
Clostridium sporogenes (NCIB 532)	23±0.29	26±0.50	24±1.00	16±0.50	15±1.00	29±0.56	25±1.00
B. stearotherphilus (NCIB 8222)	18±0.50	24±1.00	23±0.29	28±1.00	28±1.00	26±0.56	24±1.00
Streptococcus pneumoniae (LIO)	18±0.50	28±0.29	24±1.00	27±1.00	24±0.50	25±0.56	28±0.56
Streptococcus pneumoniae (PS)	20±1.00	29±1.00	26±1.00	30±1.00	29±1.00	28±0.50	19±0.56
B. subtilis (NCIB 3610)	24±1.00	25±0.50	25±0.29	23±1.00	26±1.00	22±1.00	24±0.56
Staphylococcus aureus (NCIB 8588)	22±0.58	22±1.00	19±1.00	18±0.50	16±0.50	22±1.00	19±0.56
Staphylococcus aureus (SW)	29±1.00	24±0.29	22±0.50	23±1.00	18±1.00	26±0.56	19±0.56
Enterococcus feacalis (NCIB 775)	19±0.29	16±0.56	19±0.29	19±0.29	33±1.00	28±0.56	29±0.50
Micrococcus luteus (NCIB 196)	20±1.00	25±0.50	20±0.29	25±1.00	18±1.00	24±0.50	28±1.00
Bacillus anthracis (LIO)	20±0.29	22±1.00	24±1.00	23±1.00	25±0.50	26±1.00	29±1.00
Escherichia coli (NCIB 86)	28±1.00	29±0.85	30±1.00	30±1.00	32±1.15	0±0.00	20±1.15
Citrobacter freundii (PS)	22±1.00	22±0.85	24±1.00	29±1.00	29±1.15	23±1.00	0±0.00
Pseudomonas fluorescence (NCIB 3756)	20±0.85	25±0.58	28±1.00	28±1.00	28±0.85	38±1.15	0±0.00
Klebsiella pneumoniae (418)	28±0.85	28±0.58	30±1.00	28±1.00	24±1.15	0±0.00	22±0.85
Pseudomonas aeruginosa (NCIB 950)	28±0.85	30±1.00	25±1.15	33±1.00	27±1.15	28±0.85	22±1.00
Pseudomonas aeruginosa (PS)	30±1.00	25±1.00	28±1.00	38±1.00	21±1.15	25±1.00	18±1.00
Pseudomonas aeruginosa (PS)	26±0.85	23±1.00	29±1.00	34±1.00	26±1.15	20±1.00	18±0.85
Pseudomonas aeruginosa (PS)	20±0.85	30±1.00	29±0.58	29±1.00	26±1.00	19±1.00	14±1.00
Shigella species (LIO) 24±0.85	27±0.85	29±0.58	24±0.85	27±0.85	24±0.85	0±0.00	
Proteus vulgaris (NCIB 67) 25±0.85	25±1.00	20±0.58	26±0.85	23±1.15	17±1.00	28±1.00	

Key: NCIB = National Collection of Industrial Bacterial; LIO = Locally Isolated Organisms
PS = Pus Sample isolate; SW = Surgical wound isolate
Strep = Streptomycin; Tet = Tetracycline
0 = Resistant; mm = Mean of Three Replicates*

Table 2. The minimum inhibitory concentrations (MIC) exhibited by 3-methlyquinoxaline-2-hydrazone (2-6) against Susceptible Bacterial Strains

Bacterial Strains	Compounds (mg/mL)						
	2	**3**	**4**	**5**	**6**	**Strep**	**Tet**
Bacillus polymyxa (LIO)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0625
B. cereus (NCIB 6349)	0.0313	0.0625	0.0625	0.0625	0.0625	0.0313	0.250
Corynebacterium pyogenes (LIO)	0.0625	0.125	0.0313	0.125	0.125	0.0313	0.0313
Clostridium sporogenes (NCIB 532)	0.0625	0.125	0.0625	0.125	0.125	0.0313	0.0313
B. stearotherphilus (NCIB 8222)	0.0313	0.125	0.0625	0.125	0.0625	0.0625	0.125
Streptococcus pneumoniae (LIO)	0.0625	0.125	0.125	0.125	0.0625	0.0625	0.125
Streptococcus pneumoniae (PS)	0.0625	0.125	0.125	0.125	0.125	0.0625	0.125
B. subtilis (NCIB 3610)	0.0625	0.125	0.0313	0.0625	0.0625	0.0625	0.250
Staphylococcus aureus (NCIB 8588)	0.0313	0.125	0.0625	0.125	0.125	0.500	0.0313
Staphylococcus aureus (SW)	0.0625	0.125	0.125	0.125	0.125	0.0625	0.125
Enterococcus feacalis (NCIB 775)	0.0313	0.0625	0.0625	0.125	0.0625	0.0625	0.250
Micrococcus luteus (NCIB 196)	0.0313	0.125	0.0313	0.125	0.0625	0.0625	0.250
Bacillus anthracis (LIO)	0.0625	0.125	0.125	0.125	0.125	0.500	0.500
Escherichia coli (NCIB 86)	0.0625	0.125	0.0625	0.125	0.0625	ND	0.0313
Citrobacter freundii (PS)	0.0625	0.125	0.0625	0.125	0.0625	ND	0.0313
Pseudomonas fluorescence (NCIB 3756)	0.0625	0.125	0.125	0.250	0.125	0.250	ND
Klebsiella pneumoniae (418)	0.0625	0.125	0.0625	0.0625	0.0625	ND	0.50
Pseudomonas aeruginosa (NCIB 950)	0.0625	0.125	0.125	0.250	0.125	0.250	ND
Pseudomonas aeruginosa (PS)	0.0625	0.0625	0.0625	0.0625	0.0625	0.250	0.50

Table 2. (contd.) The Minimum Inhibitory Concentrations (MIC) 3-methlyquinoxaline-2-hydrazone (2-6) against Susceptible Bacterial Strains (Continued)

Bacterial Strains	Compounds (mg/mL)						
	2	**3**	**4**	**5**	**6**	**Strep**	**Tet**
Pseudomonas aeruginosa (PS)	0.0625	0.125	0.0625	0.250	0.125	0.250	ND
Pseudomonas aeruginosa (PS)	0.0625	0.0625	0.0625	0.0625	0.0625	0.250	0.50
Shigella species (LIO)	0.0625	0.0625	0.125	0.250	0.125	0.250	ND
Proteus vulgaris (NCIB 67)	0.0625	0.125	0.0625	0.125	0.0625	0.250	0.50

Key: NCIB = National Collection of Industrial Bacterial
LIO = Locally Isolated Organisms
PS = Pus Sample isolate
SW = Surgical wound isolate
Strep = Streptomycin
Tet = Tetracycline
ND = Not Done

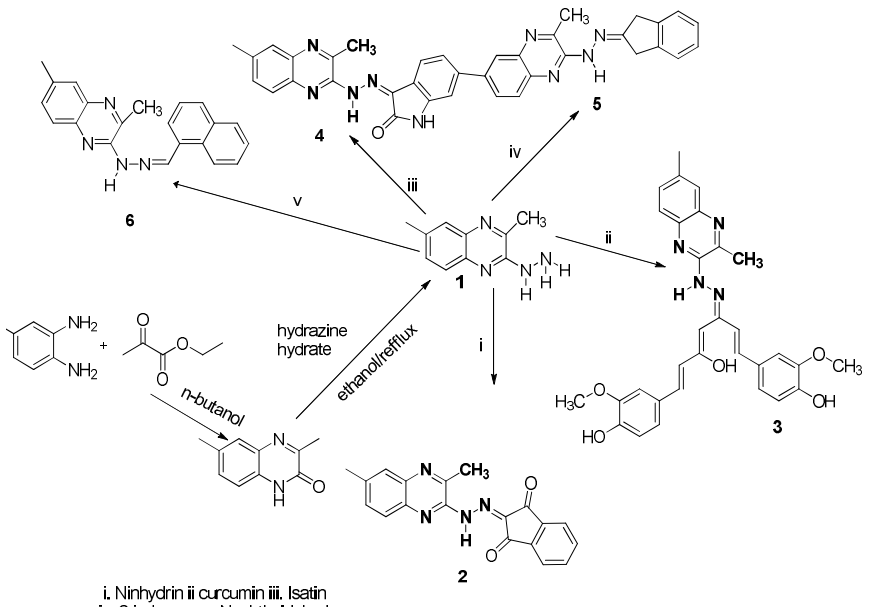
Table 3. The Minimum Bactericidal Concentrations Exhibited by 3-methlyquinoxaline-2-hydrazone 2-6 (2 mg/ml) against Susceptible Bacterial Strains

Bacterial Strains	Compounds (mg/mL)						
	2	3	4	5	6	Strep	Tet
Bacillus polymyxa (LIO)	0.125	0.250	0.250	0.250	0.250	0.250	0.125
B. cereus (NCIB 6349)	0.0625	0.125	0.125	0.125	0.125	0.0625	0.500
Corynebacterium pyogenes (LIO)	0.125	0.250	0.0625	0.250	0.250	0.0625	0.0625
Clostridium sporogenes (NCIB 532)	0.125	0.250	0.125	0.250	0.250	0.0625	0.0625
B. stearotherphilus (NCIB 8222)	0.0625	0.250	0.125	0.250	0.125	0.125	0.250
Streptococcus pneumoniae (LIO)	0.125	0.250	0.250	0.250	0.125	0.125	0.250
Streptococcus pneumoniae (PS)	0.125	0.250	0.250	0.250	0.250	0.125	0.250
B. subtilis (NCIB 3610)	0.125	0.250	0.0625	0.125	0.125	0.125	0.500
Staphylococcus aureus (NCIB 8588)	0.0625	0.250	0.125	0.250	0.250	1.000	0.0625
Staphylococcus aureus (SW)	0.125	0.250	0.250	0.250	0.250	0.125	0.250
Enterococcus feacalis (NCIB 775)	0.0625	0.125	0.125	0.250	0.125	0.125	0.500
Micrococcus luteus (NCIB 196)	0.0625	0.250	0.0625	0.250	0.250	0.250	0.500
Bacillus anthracis (LIO)	0.125	0.250	0.250	0.250	0.250	1.000	1.000
Escherichia coli (NCIB 86)	0.125	0.250	0.125	0.250	0.125	ND	0.0625
Citrobacter freundii (PS)	0.125	0.250	0.125	0.250	0.125	ND	0.0625
Pseudomonas fluorescence (NCIB 3756)	0.125	0.250	0.250	0.500	0.250	0.500	ND
Klebsiella pneumoniae (418)	0.125	0.250	0.125	0.125	0.125	ND	1.000
Pseudomonas aeruginosa (NCIB 950)	0.125	0.250	0.250	0.500	0.250	0.500	ND
Pseudomonas aeruginosa (PS)	0.250	0.125	0.125	0.125	0.125	0.500	1.00

Table 3. (contd.) The Minimum Inhibitory Concentrations (MIC) 3-methlyquinoxaline-2-hydrazone (2-6) against Susceptible Bacterial Strains (Continued)

Bacterial Strains	Compounds (mg/mL)						
	2	**3**	**4**	**5**	**6**	**Strep**	**Tet**
Pseudomonas aeruginosa (PS)	0.125	0.250	0.125	0.500	0.250	0.500	ND
Pseudomonas aeruginosa (PS)	0.125	0.125	0.125	0.125	0.125	0.500	1.00
Shigella species (LIO)	0.125	0.125	0.250	0.500	0.250	0.500	ND
Proteus vulgaris (NCIB 67)	0.125	0.250	0.125	0.205	0.125	0.500	1.00

Key: NCIB = National Collection of Industrial Bacterial
LIO = Locally Isolated Organisms
PS = Pus Sample isolate
SW = Surgical wound isolate
Strep = Streptomycin
Tet = Tetracycline
ND = Not Done



4.2 *In vitro* Antimicrobial Activities of the Compounds and Standard Antibiotics

The synthetic compounds exhibited significant antibacterial activities. The compounds were screened at a concentration of 2 mg/ml and they all inhibited the growth of both the Gram-positive and Gram-negative organisms. These results indicate that the compounds exhibit broad spectrum antibacterial activity. All the synthesized compounds exhibited appreciable antibacterial activity against all Gram-negative organisms tested. It is a common knowledge that Gram-negative species are found to be more resistant to inhibition by most antibacterial compounds due to their outer membrane of their cell wall [21,22]. Among the Gram-negative organisms that are susceptible to the antibacterial actions of these synthesized compounds are *Pseudomonas* species that have been found to be more resistant to antimicrobial agents [23]. Any synthetic compounds that could inhibit the growth of *Pseudomonas* species could be developed as a novel antimicrobial compound to mitigate infectious diseases that are caused by these opportunistic pathogens. It is noteworthy that some of the Gram-positive bacterial strains screened for this study are known to cause various infections in man. For example, *Staphylococcus aureus* which are causative agents for various infections in man and animal and such predominates in surgical wound infections [24]. *Staphylococcus aureus* has been implicated to be responsible for superficial skin infection and can also cause some life-threatening diseases such as sepsis, respiratory and septicaemia [25]. *Staphylococcus aureus* had been found to developed resistant towards many of the antibiotics used as therapy to treat infections caused by this organism. It is widely reported that methicillin and vancomycin which were often used to treat the infections caused by *Staphylococcus aureus* are no longer showing potency towards the treatment of infections caused by these pathogenic organisms [26,27]. Therefore, drugs that are developed from these synthesized compounds could be used to combat infections caused by *Staphylococcus aureus* and other opportunist pathogens. It was observed in this study that other Gram-positive organisms that were susceptible to these compounds are *B. cereus* which has been implicated to cause food infections among other diseases, *Streptococcus pneumoniae* the causative agent of pneumonia. The infections caused by these organisms can be treated using drugs formulated from these synthesized

compounds and thus go a long way to help in combating disease causing organisms in healthcare delivery.

The assay for MIC and MBC exhibited by these compounds were also investigated in this study. It was observed from the assay that the synthetic compounds were found to show low MIC and MBC against the test bacterial strains screened for this study. The lowest MIC observed was 0.0313 mg/mL while the lowest MBC was 0.0625 mg/mL. Various studies have showm that, a low MIC value of antibacterial agents indicates a better antibacterial activity [28]. This lower MIC values shows that the synthetic compounds exhibited significant antibacterial activities against the bacterial strains and thus can be used to develop potent antibacterial compounds that could be used to treat infections caused by pathogens that are known to be gradually developing resistant against antimicrobials.

The antibacterial activity of the compounds could be explained on the basis of the contributions of incorporated aromatic ring which we know should increase the lipophilicity of the compounds. It has been reported that increase in lipophilicity of antibacterial agents would help their permeability through the microbial cell wall and, thus, enhance the reaction of different functional groups present in the synthetic compounds to interacts with the cellular membrane of the bacterial cell and thus damaged both its functions and integrity [29,30] resulting in better or higher activity.

5. CONCLUSION

The synthesis of some new 3,6-dmethyquinoxaline-2-hydrazone derivatives were successful. The synthetic methodology employed in this study was efficient and environmentally friendly, this was due to the fact that the work-up stage was carried out in water.

It was found that all the test compounds exhibited good antimicrobial activity and that they all had a broad spectrum of activity. The use of this synthesized compounds will promote the effective treatment of infectious diseases that involves resistant pathogens and thereby help in circumventing the problem of increasing resistance by pathogens to the existing synthetic antibiotics.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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