Original Research Article

Design, Synthesis, reactions and Antibacterial properties of 2-hydrazinyl-3-methyl-6-nitroquinoxaline Derivatives

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

Context: Studies on the synthesis of new biologically active heterocyclic compounds have gained a wide variety of interest to researchers globally.

Abstract

Aims: This aims of this study was to synthesis new quinoxaline-based heterocycles and study its antibacterial properties.

Objective: This study was designed to ~~synthesized~~ synthesis some 3-methyl-6-nitroquinoxaline-2-one with hydrazine moiety, characterize the synthesized compounds, and study their antibacterial properties on some bacterial strains.

Materials and methods: Six 3-methylquinoxaline-2-hydrazone derivatives were synthesized by reacting 2-hydrazinyl-3-methyl-6-nitroquinoxaline with various substituted acetophenones. The hydrazones were screened for their potential antibacterial properties.

Results: All the test compounds were found to possessed promising antibacterial ~~property~~ properties against a panel of bacterial strains screened for this study. The MIC values exhibited by these compounds ranged between 0.0313 and 0.250 mg/mL. The lowest MBC of the compounds against the test organism was 0.0625 mg/mL while the highest MBC was 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 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.

INTRODUCTION

Infectious diseases are the world's major threat to human health [1]. These microorganisms causing different kinds of life-threatening infections have increased and it is becoming a major concern to health workers all over the world. There is an urgent need to source for new antimicrobial compounds that will combat the spread of resistant to antimicrobial compounds by pathogens. This study is therefore geared towards the potency of 3-methylquinoxaline hydrazones on some pathogenic microorganisms associated with human infections. Quinoxalines has been reported to be an important structural unit among nitrogen containing heterocyclic compounds [2]. Quinoxalines are, in general, easy to prepare and numerous derivatives have been reported in the literature because of their biological activity, specifically as anti-viral [3-6], anti-inflammatory [7,8], antimicrobial [9-15], anti-cancer [16], antimalaria [17], antibacterial [18-19], anti-aminoceptive [20] agents. Quinoxalines have been found to possess well biological activities which includes anti-Trypanosoma activity [21], antiplasmodial activity [22], AMPA/GlyN receptor antagonist [23], anti-herps, trypanocidal Ca2+ uptake/release inhibitor [24], antihistaminic agents [25] and inhibitor of vascular smooth muscle cell proliferation. Although rarely described in nature, synthetic quinoxaline moiety is a part of number of antibiotics such as echinomycin [26], levomycin and actinomycin which are known to inhibit the

growth of Gram-positive bacteria and also active against various transplantable tumors [15,27]. Some derivatives of quinoxaline have been reported to constitute the basis of many insecticides, fungicides, herbicides, as well as being important in human health and as receptor antagonists. In addition, quinoxaline derivatives are reported for their application in dyes, efficient electroluminescent materials, organic semiconductors, and DNA cleaving agents [28].

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 and ^{13}C-NMR 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.

Synthesis of 3-methyl-6-nitroquinoxaline-2(1H)-one

4-nitrophenylenediamine (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 [29, 30]; 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(J=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.

Synthesis of 2-hydrazinyl-3-methyl-6-nitroquinoxaline **1**

3-methyl-6-nitroquinoxalin-2-(1*H*)-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-methyl-6-nitroquinoxaline (1): % yield: 90.00%; Melting point: 319-321°C lit. 318-320 °C [30] IR KBr (cm^{-1}): 3448 (N-H str.), 3308 (N-H_2 str.), 3007 (N- H_2 str.), 2966 (C-H sp2 str.), 2898 (C-H sp3 str.), 1568 (N-H bend), 1665 (C=N str.) ~~1H-NMR (DMSO-d6):~~ ^{1}H-NMR (DMSO-d_6): 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 NH_2); 2.42 (s, 3H, methyl proton). ~~13C-NMR (DMSO-d6):~~ ^{13}C-NMR (DMSO-d_6): 163 ~~ppm~~, 145 ~~ppm~~, 135 ~~ppm~~, 127 ~~ppm~~, 125 ~~ppm~~, 124 ~~ppm~~, 17 ~~ppm~~.

Synthesis of (Z)-3-methyl-6-nitro-2-(2-(1-phenylethylidene)hydrazinyl)quinoxaline 2

2-hydrazinyl-3-methyl-6-nitroquinoxaline (1.0 g, 5.67 mmol) and acetophenones (0.68 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 4 hours. The reaction mixture was allowed to 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-methyl-6-nitro-2-(2-(1-phenylethylidene)hydrazinyl)quinoxaline 2**.**

Recrystallization from DMF/water afforded **2** % yield: 75.40%; Melting point: 252-254°C lit. 250-253°C [30] IR KBr (cm-1): 3435 (N-H str.), 2899 (C-H sp3 str.), 1602 (C=C aromatic str.), 1665 (C=N str.), 1564 (N-H bend), 1008 (N-N str.). 1H-NMR (DMSO-d6): 10.43 (broad s, 1H, hydrazine NH), 7.95(d, 2H, aromatics protons), 7.93 (d, 1H, aromatic proton); 7.90 (d, 1H, aromatic proton); 7.80 (d, 1H, aromatic proton); 7.76 (t, 1H, aromatic protons); 7.55 (t, 1H, aromatics proton), 7.53 (t, 2H, aromatics protons), 2.94 (s, 3H, methyl proton), 2.40 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 168 ppm, 163 ppm, 145 ppm, 137 ppm, 135 ppm, 131 ppm, 127 ppm, 125 ppm, 124 ppm, 17 ppm.

Synthesis of (Z)-2-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl)-3-methyl-6-nitro quinoxaline 3

2-hydrazinyl-3-methyl-6-nitroquinoxaline (1.0 g, 5.67 mmol) and 4-bromoacetophenones (0.58 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 4 hours. The reaction mixture was allowed to cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded **3** % yield: 78.40%; Melting point: 252-254°C lit. 254-256 °C [30] IR KBr (cm-1): 3442 (N-H str.), 2910 (C-H sp3 str.), 1605 (C=C aromatic str.), 1664 (C=N str.), 1598 (N-H bend). 1H-NMR (DMSO-d6): 10.43 (broad s, 1H, hydrazine NH), 7.60-7.90 (m, 4H, aromatic protons); 7.67 (d, H, aromatic protons); 7.43 (J=8, 8, 2) (t, 1H, aromatic protons); 7.27 (J=8, 8, 2) (t, 1H, aromatic protons); 7.36 (d, 1H, aromatics protons) 2.94 (s, 3H, methyl proton), 2.40(s, 3H, methyl proton). 13C-NMR (DMSO-d6): 168 ppm, 164 ppm, 145 ppm, 135 ppm, 134 ppm, 133 ppm, 130 ppm, 127 ppm, 125 ppm 124 ppm, 123 ppm, 17 ppm.

Synthesis of (Z)-2-(2-(1-(2-fluorophenyl)ethylidene)hydrazinyl)-3-methy-6-nitroquinoxaline 4

2-hydrazinyl-3-methyl-6-nitroquinoxaline (1.0 g, 5.67 mmol) and 2-fluoro acetophenones (0.76 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 4 hours. The reaction mixture was allowed to 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** % yield: 71.10%; Melting point: 205-208 °C lit. 207-209°C [30]; IR KBr (cm-1): 3435 (N-H str.), 2966 (C-H sp3 str.), 1608 (C=C aromatic str.), 1662 (C=N str.), 1564 (N-H bend), 1008 (N-N str.). 1H-NMR (DMSO-d6): 10.46 (broad s, 1H, hydrazine NH), 7.60-7.94 (m, 4H, aromatic protons); 7.67 (d, H, aromatic protons); 7.48 (J=8, 8, 2) (t, 1H, aromatic protons); 7.27 (J=8, 8, 2) (t, 1H, aromatic protons); 7.36 (d, 1H, aromatics protons), 2.95 (s, 3H, methyl proton). 2.43 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 168 ppm, 159 ppm, 145 ppm, 135 ppm, 134 ppm, 133 ppm, 130 ppm, 127 ppm, 124 ppm, 118 ppm, 17 ppm.

Synthesis of (Z)-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)-3methylquinoxaline 5

2-hydrazinyl-3-methyl-6-nitroquinoxaline (1.0 g, 5.67 mmol) and 4-fluoroacetophenones (0.76 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 4 hours. The reaction mixture was allowed to cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded **5** % yield: 85.40%; Melting point: 251-253 °C lit. 250-252°C [30]; IR KBr (cm-1): 3458 (N-H str.), 2850 (C-H sp3 str.), 1618 (C=C aromatic str.), 1660 (C=N str.), 1548 (N-H bend). 1H-NMR (DMSO-d6): 10.46 (broad s, 1H, hydrazine NH), 7.27 (m, 2H, aromatic

protons); 7.67 (d, 1H, aromatic protons); 7.48 (J=8, 8, 2) (t, 1H, aromatic protons); 7.27 (J=8, 8, 2) (m, 1H, aromatic protons); 7.19 (m, 2H, aromatics protons), 2.93 (s, 3H, methyl proton), 2.41 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 168 ppm, 165 ppm (C-F), 163 ppm, 145 ppm, 135 ppm, 134 ppm, 133 ppm, 129 ppm, 127 ppm, 124 ppm, 115 ppm, 17 ppm.

Synthesis of (Z)-2-(2-(1-(2,4-dibromophenyl)ethylidene)hydrazinyl)-3-methyl-6-nitroquinoxaline 6

2-hydrazinyl-3-methyl-6-nitroquinoxaline (1.0 g, 5.67 mmol) and 2,4-dibromo acetophenones (1.56 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at 120 ^{o}C for 4 hours. The reaction mixture was allowed to cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded b % yield: 77.35%; Melting point: 185-187 °C lit. 183-184°C [30]; IR KBr (cm-1): 3309 (N-H str.), 3103 (C-H sp2 str.), 2897 (C-H sp3 str.), 1605 (C=C aromatic str.), 1669 (C=N str.), 1568 (N-H bend). 1H-NMR (DMSO-d6): 10.46 (broad s, 1H, hydrazine NH), 7.27 (m, 2H, aromatic protons); 7.67 (d, 1H, aromatic proton); 7.48 (J=8, 8, 2) (t, 1H, aromatic proton); 7.69 (J=8, 8, 2) (m, 2H, aromatic protons); 7.94 (m, 1H, aromatics protons) 2.94 (s, 3H, methyl proton), 2.40 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 168 ppm, 164 ppm, 145 ppm, 135 ppm, 134 ppm, 133 ppm, 130 ppm, 127 ppm, 124 ppm, 123 ppm, 17 ppm, 15 ppm.

Synthesis of (Z)-4-(1-(2-(3-methyl-6-nitroquinoxalin-2-yl) hydrazono) ethyl) aniline 7

2-hydrazinyl-3-methyl-6-nitroquinoxaline (1.0 g, 5.67 mmol) and 4-aminoacetophenones (0.77 g, 5.67 mmol) were added to glacial acetic acid (10 ml) in a round bottom flask and refluxed at

120 ^{o}C for 4 hours. The reaction mixture was allowed to cooled and poured into crushed ice with continuous stirring to obtain a solid product which was filtered and dried. Recrystallization from DMF/water afforded **7** % yield: 45.40%; Melting point: 250-251 °C lit. 252-254°C [30]; IR KBr (cm-1): 3435 (N-H str.), 2966 (C-H sp3 str.), 1608 (C=C aromatic str.), 1600 (C=N str.), 1564 (N-H bend), 1008 (N-N str.) 1H-NMR (DMSO-d6): 10.46 (broad s, 1H, hydrazine NH), 7.27 (m, 2H, aromatic protons); 7.67 (d, 1H, aromatic proton); 7.64 (d, 2H, aromatic proton); 6.88(d, 2H, aromatic protons); 7.94 (m, 1H, aromatics protons), 5.48 (s, 2H, NH2), 2.94 (s, 3H, methyl proton), 2.40 (s, 3H, methyl proton). 13C-NMR (DMSO-d6): 168 ppm, 164 ppm, 145 ppm, 135 ppm, 134 ppm, 133 ppm, 130 ppm, 127 ppm, 124 ppm, 123 ppm, 17 ppm, 15 ppm.

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).

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 [31] 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.

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 [32]. Two milliliter of different concentrations of solution of the compound was added to 18 ml of 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.

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. [33] 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.

Results

The 3-methyl-6-nitroquinoxalin-2-one was synthesized by reacting 4-nitrophenylenediamine and ethyl pyruvate in n-butanol (Scheme 1). The starting material was synthesized by reacting 3-methyl-6-nitroquinoxalin-2-one with hydrazine dihydrate under reflux for four hours to give 2-hydrazinyl-3-methyl-6-nitroquinoxaline (I) which is the precursor for this study. The precursor (I) was allowed to react with different substituted acetophenones to obtained the various 3-methyl-6-nitroquinoxalin-2-hydrazones.

Antimicrobial Studies

All the synthesized compounds were shown to active against all the bacteria investigated. The zones of inhibition observed for the synthesized compounds against the test organisms ranged between 13 mm and 40 mm. On the other hand, the zones of inhibition observed for streptomycin and tetracycline against the bacteria ranged between 10 and 25 mm (Table 1). These results obtained showed that the synthesized compounds compared favourably with the standard antibiotics – streptomycin and tetracycline used as positive control in this study. The MIC exhibited by the synthesized compounds against the bacterial strains ranged between

0.0313 mg/mL and 0.250 mg/mL (Table 2). The lowest MBC against the test organism was 0.0625 mg/mL while the highest MBC was 0.250 mg/mL (Table 3). On the other hand, the MIC exhibited by streptomycin against the organisms ranged between 0.0078 mg/mL and 0.500 mg/mL and those exhibited by tetracycline were between 0.313 mg/mL and 0.500 mg/mL (Tables 2 and 3). The lowest MBC observed for streptomycin was 0.0313 mg/mL and the highest MBC was 0.500 mg/mL while lowest MBC observed for tetracycline was 0.0313 mg/mL and the highest MBC was 0.500 mg/mL. In comparison, the synthesized compounds compared favourably with the two standard antibiotics used as positive controls.

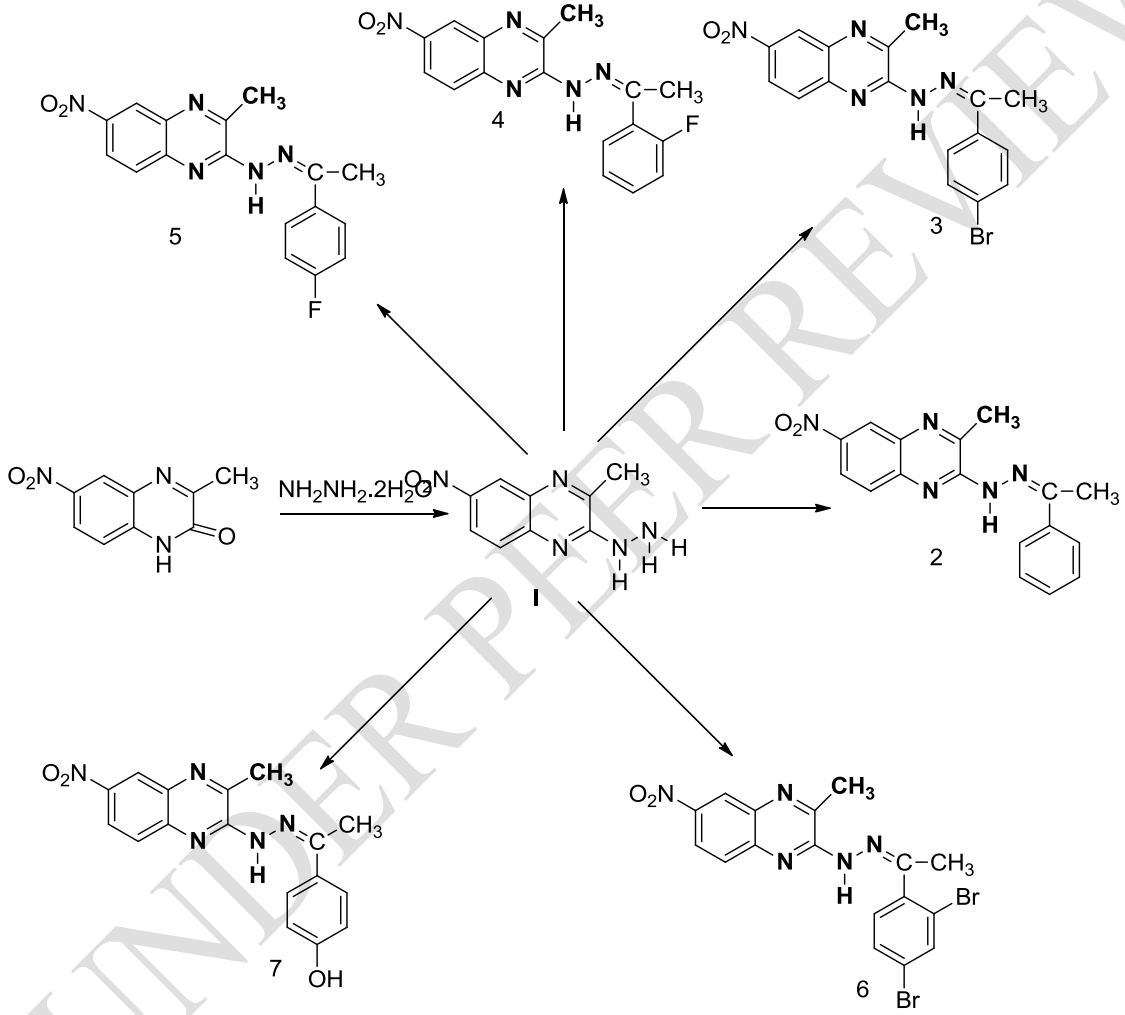
The lowest the MIC and MBC exhibited by antimicrobial compounds the better and more potent such antibiotics are. The synthesized compounds having exhibited low MIC and MBC is an indication that such compounds could be used to produce potent antimicrobial compounds that could be used to control the infections caused by pathogens that have now developed resistant against antibiotics.

Discussion

Chemistry

The 2-hydrazinyl-3-methyl-6-nitroquinoxaline (I) which was synthesized from the reaction of 3-methyl-6-nitroquinoxaline-2-one with hydrazine ~~di~~hydrate ~~with~~ by conventional heating under reflux was allowed to react with different substituted acetophenones to ~~obtained~~ obtain the various 3-methyl-6-nitroquinoxalin-2-hydrazones. 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.



Scheme 1: Reaction of 3-methylquinoxalin2-hydrazine (**I**) with various substituted acetophenones

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

The antimicrobial properties of the six synthesized compounds were investigated against panel of bacterial strains. The compounds were tested at a concentration of 2 mg/ml and they were found to inhibit the growth of both the Gram-positive and Gram-negative organisms. These results show that all the compounds possess broad spectrum antibacterial activities. These synthesized compounds exhibited appreciable antibacterial activity against all Gram-negative organisms tested in this study. It has been reported that Gram negative species are found to be more resistant to inhibition by most antibacterial compounds due to their outer membrane [34, 35]. Among the Gram-negative organisms that are susceptible to the antibacterial actions of these synthesized compounds are *Pseudomonas* species that have been reported to be more resistant to antimicrobial agents [36]. Any of such compounds that could inhibit the growth of *Pseudomonas* species could serve as a novel antimicrobial compound to manage infectious diseases that are caused by these opportunistic organisms. Similarly, some of the Gram-positive bacterial strains used for this study are known to cause various infections in man. For example, *Staphylococcus aureus* which are known to cause various infections in man and animal and predominates in surgical wound infections [37]. *Staphylococcus aureus* are also responsible for superficial skin infection and can as well cause some life-threatening diseases such as sepsis, respiratory and septicaemia [38]. This organism had been found to developed resistant towards many of the available antibiotics used as therapy to treat infections caused by this organism. It is widely known that methicillin and vancomycin which were adopted to treat the infections caused by *Staphylococcus aureus* are no longer showing potency towards the treatment of infections caused by this pathogen [39, 40]. Therefore, drugs formulated from these synthesized compounds could be used to manage infections caused by *Staphylococcus aureus* and other opportunist organisms. Other Gram-positive organisms that were susceptible to these compounds are *B. cereus* known 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 developed from these synthesized compounds and thus go a long way to help in combating disease causing organisms in healthcare delivery.

In this study the assay for MIC and MBC exhibited by the synthesized compounds were also investigated. It was observed from the assay that the compounds were found to exhibit low MIC and MBC against test bacterial strains used for this study. For example, the lowest MIC observed was 0.0313 mg/mL while the lowest MBC was 0.0625 mg/mL. Different Researchers have reported that, a low MIC value of antibacterial agents indicates a better antibacterial activity [41]. This lower MIC values shows that the synthetic compounds exhibited significant antibacterial activities against the bacterial strains and thus can be used to formulate 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 [42, 43] resulting in better or higher activity.

CONCLUSION

The synthesized quinoxaline hydrazones exhibited appreciable antimicrobial potency against panel of bacterial strains used for this study. The compounds exhibited broad spectrum activities

and thus showed a significant therapeutic action for the treatment of infections caused by pathogens. The study also confirmed the mode of action of these compounds through damage to the cytoplasm of the test bacterial strains and led to the leakage of cytoplasmic content. The bactericidal effects exhibited by these synthesized compounds thus caused the death of the test organisms. Such compounds could be used to formulate antimicrobial compounds which could be more potent than the available antibiotics used as therapy to treat infections caused by pathogens.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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Table 1. The Antibacterial Sensitivity Testing Exhibited by Quinoxaline-6-sulfonul hydrazones (2-7) Against Bacterial Strains

Bacterial Strains	Zones of Inhibition (mm*)							
	Compd. 2 (2 mg/mL)	Compd. 3 (2 mg/mL)	Compd. 4 (2 mg/mL)	Compd. 5 (2 mg/mL)	Compd. 6 (2 mg/mL)	Compd. 7 (2 mg/mL)	Strep (1 mg/mL)	Tet (1 mg/mL)
Gram-positive								
Bacillus polymyxa (LIO)	22±1.00	23±0.5	25±1.00	22±1.00	23±1.00	25±0.29	13±0.50	14±0.56
B. cereus (NCIB 6349)	22±1.00	19±0.29	26±0.29	18±0.56	25±1.00	17±1.00	25±0.56	16±1.00
Corynebacterium pyogenes (LIO)	19±1.00	18±0.50	19±1.00	19±0.29	24±0.50	25±0.50	23±0.56	20±1.00
Clostridium sporogenes (NCIB 532)	24±1.00	22±1.00	18±0.56	26±1.00	28±0.29	19±0.50	21±0.56	18±1.00
B. stearothermophilus (NCIB 8222)	26±1.15	25±1.00	28±1.00	28±1.00	26±1.00	27±1.00	20±0.56	20±1.00
Streptococcus pneumoniae (LIO)	18±1.05	20±0.50	19±1.00	28±0.29	14±0.50	35±1.00	22±0.56	22±0.56
Strep. Pneumoniae (PS)	19±1.00	20±1.00	18±1.00	23±1.00	18±1.00	23±1.00	23±0.50	12±0.56
B. subtilis (NCIB 3610)	26±1.00	26±1.00	26±0.50	27±0.50	25±1.00	19±0.50	25±1.00	20±0.56
Staphylococcus aureus (NCIB 8588)	24±1.05	19±0.50	26±0.29	19±1.00	20±1.00	25±1.00	21±1.00	12±0.56
Staphylococcus aureus (SW)	25±1.00	18±1.00	25±1.00	22±0.29	23±0.58	28±1.00	24±0.56	15±0.56
Enterococcus feacalis (NCIB 775)	26±1.15	28±0.50	28±0.29	25±0.56	19±1.00	27±0.50	20±0.56	23±0.50
Micrococcus luteus (NCIB 196)	27±1.00	24±1.00	24±1.00	27±1.00	25±0.29	16±1.00	20±0.50	20±1.00
Bacillus anthracis (LIO)	40±1.00	28±0.29	26±0.50	25±1.00	23±0.50	14±1.00	20±1.00	20±1.00
Gram-negative								
Escherichia coli (NCIB 86)	38±0.56	28±1.15	35±1.00	32±0.56	29±1.00	27±0.58	0	16±1.15
Citrobacter freundii (PS)	26±1.15	23±0.56	25±1.00	24±1.00	26±1.00	28±0.58	15±1.00	0
Pseudomonas fluorescence (NCIB 3756)	28±0.56	25±1.15	26±1.00	22±0.56	24±0.58	24±0.58	25±1.15	0
Klebsiella pneumoniae (418)	29±0.56	30±1.00	28±0.56	36±1.00	30±1.00	26±1.00	0	10±0.85
Pseudomonas aeruginosa (NCIB 950)	26±0.56	23±1.00	29±0.56	26±1.00	30±1.00	28±1.00	22±0.85	10±1.00
Pseudomonas aeruginosa (PS)	28±0.56	25±1.00	32±1.00	25±1.00	24±1.00	30±1.00	22±1.00	13±1.00
Pseudomonas aeruginosa (PS)	23±1.00	22±1.15	29±0.56	23±0.56	30±0.56	28±0.58	14±1.00	14±0.85
Pseudomonas aeruginosa (PS)	23±1.15	22±0.56	19±1.00	22±1.00	27±1.00	26±1.00	13±1.00	20±1.00
Shigella species (LIO)	29±1.00	27±0.56	28±1.00	30±1.00	24±1.00	25±1.00	20±0.85	0
Proteus vulgaris (NCIB 67)	22±1.15	24±0.56	26±1.00	28±0.58	25±0.58	19±0.58	10±1.00	20±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 MIC and MBC Exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones (2-7) against bacterial strains

Bacterial strain	Compounds (mg/mL)															
	2		3		4		5		6		7		Strep		Tet	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus polymyxa (LIO)	0.0625	0.125	0.0313	0.0625	0.125	0.250	0.0625	0.125	0.125	0.250	0.0625	0.125	0.125	0.250	0.0625	0.125
B. cereus (NCIB 6349)	0.125	0.250	0.125	0.250	0.0313	0.0625	0.125	0.250	0.0625	0.125	0.125	0.250	0.0313	0.0625	0.250	0.250
Corynebacterium pyogenes (LIO)	0.0625	0.125	0.0625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.0313	0.0625	0.0313	0.0625	0.0313	0.0625
Clostridium sporogenes (NCIB 532)	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.0313	0.0625	0.125	0.250	0.0078	0.0313	0.0313	0.0625
B. stearothermophilus (NCIB 8222)	0.0625	0.125	0.0625	0.125	0.125	0.250	0.125	0.250	0.0313	0.250	0.125	0.250	0.0625	0.125	0.125	0.250
Streptococcus pneumoniae (LIO)	0.125	0.250	0.0313	0.0625	0.0313	0.0625	0.0313	0.0625	0.125	0.0625	0.0313	0.0625	0.0625	0.125	0.125	0.250
Strep. Pneumoniae (PS)	0.0625	0.125	0.0625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.125	0.250
B. subtilis (NCIB 3610)	0.0625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.0313	0.0625	0.125	0.250	0.0625	0.125	0.250	0.500
Staphylococcus aureus (NCIB 8588)	0.125	0.250	0.0313	0.0625	0.0313	0.0625	0.0625	0.125	0.125	0.250	0.125	0.250	0.500	ND	0.0313	ND
Staphylococcus aureus (SW)	0.0625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.0313	0.0625	0.125	0.250	0.0625	0.125	0.125	0.250
Enterococcus feacalis (NCIB 775)	0.0625	0.125	0.0625	0.125	0.250	0.500	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.250	0.500
Micrococcus luteus (NCIB 196)	0.0625	0.125	0.0313	0.0625	0.0313	0.0625	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.250	0.500
Bacillus anthracis (LIO)	0.125	0.250	0.0625	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.500	ND	0.500	ND
Escherichia coli (NCIB 86)	0.125	0.250	0.125	0.250	0.0313	0.0625	0.125	0.250	0.125	0.250	0.0625	0.125	ND	ND	0.0313	0.0625
Citrobacter freundii (PS)	0.0625	0.125	0.0313	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	ND	ND	0.0313	0.0625
Pseudomonas fluorescence (NCIB 3756)	0.125	0.250	0.0313	0.0625	0.0313	0.0625	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND

Table 2. (contd.) The MIC and MBC Exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones (2-7) against bacterial strains

Bacterial strain	Compounds (mg/mL)															
	2		3		4		5		6		7		Strep		Tet	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Klebsiella pneumoniae (418)	0.625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.0625	0.125	ND	ND	0.500	ND
Pseudomonas aeruginosa (NCIB 950)	0.0625	0.125	0.0625	0.125	0.0625	0.125	0.0625	0.125	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND
Pseudomonas aeruginosa (PS)	0.0625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.0625	0.125	0.250	0.500	0.500	ND
Pseudomonas aeruginosa (PS)	0.0625	0.125	0.0625	0.125	0.125	0.250	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND
Pseudomonas aeruginosa (PS)	0.0625	0.125	0.125	0.250	0.0625	0.125	0.0625	0.125	0.0625	0.125	0.0625	0.125	0.250	0.500	0.500	ND
Shigella species (LIO)	0.125	0.125	0.0625	0.125	0.125	0.250	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND
Proteus vulgaris (NCIB 67)	0.0625	0.125	0.125	0.250	0.0625	0.125	0.0625	0.250	0.125	0.250	0.125	0.250	0.250	0.250	0.500	ND

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