

Synthesis and Antibacterial potency of some 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazone derivatives.

Abstract

Studies on the synthesis of some sulfa quinoxaline has generate a considerable reputation as a result of their outstanding biochemical properties. This recent study was designed to prepared some sulfa quinoxaline hydrazone derivatives and studying their antimicrobial potency. The 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazone derivatives were synthesized by the reactions of 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide and six substituted benzaldehydes and examined for their possible antibacterial potency. The synthetic compounds exhibited wide-ranging spectrum action counter to twenty-four bacteria strains with minimum inhibitory concentrations values 0.0313 - 0.250 mg/mL. This is a suggestion that such compounds could be used to formulate antibiotics to circumvent the problem of increasing resistance by pathogens to the existing synthetic antibiotics.

Keywords: 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide, Quinoxaline, Hydrazones, Antibacterial activity, quinoxaline-2,3-dione, Synthesis.

INTRODUCTION

The discovery of antibiotics in the course of human history led to the control or eradication of infections that hitherto ravaged mankind. The joy of discovery of antimicrobials did not last long as a result of the development of resistance to these antibiotics by pathogens. The trend at which these pathogens continue to develop resistance to the available antibiotics attract the attention of

scientists and thus brings about increase in searching for more potent antibiotics against these pathogens (Okeke *et al.*, 2007) The exploration of synthetic compounds as potential sources of novel antimicrobial compounds is one such studies in the development of potent antibacterial agents to reduce the trend of resistance to the existing antibiotics.

This study thus focused on derivatives of quinoxaline which have evoked considerable attention in recent years because of their biological activity. Synthesized quinoxaline part is a fragment of some antibiotics which are recognized to impede the growing of bacteria and likewise serves as an active agent to combat various trans-plantable lumps (Sato *et al.*, 1967; Dell *et al.*, 1975, Bailly *et al.*, 1999., Montana *et al*, 2019, Saleh *et al* 2022). These compounds are specifically known to possess antibacterial (Bailly *et al.*,1999; Burguete *et al.*, 2007; Taiwo, *et al*, 2008,, Taiwo *et al* 2021, Beheshtiha *et al.*, 2010), anti-cancer properties (Chen *et al.*, 2004), antimalaria (Rangisetty *et al.*, 2001), inhibitors of acetylcholinesterase (Taiwo *et al* 2021), anti-aminoceptive (Deepika and Nath, 2012), anti-viral (Michael *et al.*, 2002; Lindsley *et al.*, 2005; Geefhavani *et al.*, 2012), antimicrobial (Jaso *et al.*, 2003, ; Badran *et al.*, 2003, Taiwo *et al*, 2021, Taiwo *et al*, 2021, Taiwo *et al* 2021), ; Hearn and Cynamon 2004; Kaurase *et al.*, 2011; Aswartha *et al.*, 2012; Achutha *et al.*, 2013) ,and anti-inflammatory (Wagle *et al.*, 2008; Rajitha *et al.*, 2011) agents.

MATERIALS AND METHODS

General

Melting points were determined with open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. Infrared spectra were documented as KBr pellets on a Buck Spectrometer. The ^1H and ^{13}C NMR was run on a Bruker 600 MHz spectrometer (δ in

ppm relative to Me₄Si), The purity of the compounds were 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, except Glacial acetic acid, ethanol, oxalic acid and vanillin which were obtained from BDH Chemical Limited.

Synthesis of 3-methylquinoxaline-2(1H)-one

1,2-diaminobenzene (20 0.10 M) and ethyl pyruvate (22 g 0.10 M) in 200 ml of absolute ethanol was refluxed for 50 minutes on oil bath. The reaction mixture was allowed to cool to give some silvery white crystals which were collected by filtration, washed and purified by recrystallization from ethanol (Taiwo, *et.al.*,2016, Taiwo, *et.al.*,2021).

Preparation of 2-methyl-3-oxo-3,4-dihydroquinoxaline-6-sulfonyl chloride

3-methylquinoxaline-2(1H)-one (5.8 g, 30.9 mmol) was added portion-wise to chlorosulfonic acid (25 mL, 10 mmol equiv.) in an ice bath, the mixture obtained was refluxed at 110 °C for 8 h. The reaction mix obtained was chilled and transferred into ice to afford whitish solid which was filtered and washed with chilled water and sunction dried. The recrystallization was carried out in a mixture of dry toluene-acetone (50/50) to give 2-methyl-3-oxo-3,4-dihydroquinoxaline-6-sulfonyl chloride (m.p. >330 °C) 88% yield. IR (KBr): ν_{max} 3380 cm⁻¹, 1680 cm⁻¹, 1355 cm⁻¹, 1140 cm⁻¹.

Preparation of 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 1

Hydrazine dihydrate (25 ml, 0.460 mmol) in abs. methanol (300 ml) and 2-methyl-3-oxo-3,4-dihydroquinoxaline-6-sulfonyl chloride (28 g, 0.115 mmol) was stirred at room temperature for 18 h. This was then refluxed at 80°C for 2 hours. The clear solution was allowed to cooled to

room temperature and poured into chilled water to give compound 1. IR Spectra (KBr): 3347 cm⁻¹ (N-H), 3139 cm⁻¹ (N-H), 3050 cm⁻¹ (N-H), 3039 cm⁻¹ (N-H), 1669 cm⁻¹ (C=O), 1595 cm⁻¹ (C=N), 1391 (SO₂), 1159 cm⁻¹ (SO₂). ¹H NMR (DMSO-d₆): 3.37 (br s, 1H, NH), 4.12 (br s, 1H, NH), 12.10 (br s, 1H, NH), 7.60 (d, 1H, ArH), 7.49-7.50 (dd, 1H, ArH), 7.27 (d, 1H, ArH). ¹³C NMR (DMSO-d₆): 154.86 (C=O), 131.98, 128.95, 125.50, 122.33, 115.27, 114.96, 35.10 (CH₃).

Synthesis of N'-benzylidene-3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 2-7

A mixture of 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide **1** (39 mmol), the required benzaldehydes (39 mmol) and glacial acetic acid (25 mL) was refluxed at 120 °C for 3 hours. The resulting mixture was cooled and poured into crushed ice with continuous stirring. The solid obtained was filtered and washed with cold water, dried and recrystallized from DMF/water to afford the desired product.

N'-(2-hydroxybenzylidene)-3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 2

A yellow solid, m.p 247-248°C. IR Spectra (KBr): 3324 cm⁻¹ (OH), 3239 cm⁻¹ (N-H), 3100 cm⁻¹ (C-H aromatic), 1684 cm⁻¹ (C=O), 1599 cm⁻¹ (C=N), 1344 (ν_{max}SO₂), 1155 cm⁻¹ (ν_{max}SO₂), 1036 cm⁻¹ (N-N). ¹H NMR (DMSO-d₆): 12.14 (br s, 1H, NH), 12.17 (br s, 1H, NH), 7.66 (d, 1H, ArH), 7.55-7.57 (dd, 1H, ArH), 7.24 (d, 1H, ArH), 7.40 (dd, 2H, ArH), 6.76 (d, 2H, ArH), 7.79 (s, 1H, N=CH). ¹³C NMR (DMSO-d₆): 159.35 (C-O), 155 (C=O), 154 (C=N), 147.65, 132.86, 130.16, 129.36, 128.60, 125.76, 124.64, 122, 115.74, 115.56, 115.35, 114.35, 35.10 (CH₃).

N'-(3-hydroxybenzylidene)-3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 3

A yellow solid, m.p 239-240°C. **IR Spectra (KBr):** 3238 cm⁻¹ (N-H), 3215 cm⁻¹ (N-H), 3042 cm⁻¹ (CH aromatic), 1692 cm⁻¹ (C=O), 1603 cm⁻¹ (C=N), 1371 (v_{max}SO₂), 1163 cm⁻¹ (SO₂). **¹H NMR (DMSO-d₆)** 12.12 (br s, 1H, NH), 11.61 (br s, 1H, NH), 7.89 (s, 1H, ArH), 7.66 (d, 1H, ArH), 7.24 (d, 1H, ArH), 7.44 (m, 2H, ArH), 7.56 (m, 2H, ArH), 8.72 (s, 1H, N=CH). **¹³C NMR (DMSO-d₆):** 160.58 (C=O), 155.18 (C=O), 154.92 (C=N), 145.79, 134.54, 132.53, 132.66, 130.00, 129.52, 129.07, 128.84, 128.45, 125.85, 121.97, 115.42, 114.20, 35.10 (CH₃).

N'-(4-hydroxybenzylidene)-3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 4

A yellow solid, m.p 249-251°C, lit.250 °C (Taiwo and Obafemi, 2016).**IR Spectra (KBr):** 3247 cm⁻¹ (N-H), 3239 cm⁻¹ (N-H), 3077 cm⁻¹ (CH aromatic), 1680 cm⁻¹ (C=O), 1599 cm⁻¹ (C=N), 1341 cm⁻¹ (v_{max}SO₂), 1151 cm⁻¹ (SO₂). **¹H NMR (DMSO-d₆):** 12.20 (br s, 1H, NH), 12.13 (br s, 1H, NH), 8.27 (d, 1H, ArH), 8.15 (dd, 2H, ArH), 7.58 (dd, 1H, ArH), 7.26-7.28 (d, 1H, ArH), 7.66 (m, 1H, ArH), 6.76(m, 1H, ArH) , 8.97 (s, 1H, N=CH). **¹³C NMR (DMSO-d₆):** 158.65 (C=O), 155.19 (C=O), 154.91 (C=N), 148.85, 147.85, 133.91, 133.75, 132.14, 129.63, 129.41, 127.94, 127.79, 125.93, 124.76, 124.65, 115.55, 114.20. 35.10 (CH₃).

N'-benzylidene-3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 5

A yellow solid, m.p 274-276°C, lit. 272-273 °C (Taiwo and Obafemi, 2016). **IR Spectra (KBr):** 3239 cm⁻¹ (N-H), 3131 cm⁻¹ (N-H), 3066 cm⁻¹ (CH aromatic), 1680 cm⁻¹ (C=O), 1607 cm⁻¹ (C=N), 1322 (S=O), 1140 cm⁻¹ (S=O). **¹H NMR (DMSO-d₆):** 12.16 (br s, 1H, NH), 12.51 (br s, 2H, NH), 11.19 (br s, 1H, NH), 7.68 (s, 1H, ArH), 7.58-7.59 (m, 1H, ArH), 7.26 (d, 1H, ArH),

7.89-7.91 (m, 2H, ArH), 6.40 (m, 3H, ArH), 8.72 (s, 1H, N=CH). ^{13}C NMR (DMSO- d_6): 161.45 (C=O), 155.18 (C=O), 154.93 (C=N), 147.09, 133.75, 133.58, 132.72, 131.34, 130.09, 129.48, 128.88, 128.73, 128.32, 126.80, 125.82, 121.98, 115.39, 114.27, 35.10 (CH₃).

N'-(4-chlorobenzylidene)-3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 6

A yellow solid, m.p 240-242 °C, **IR Spectra (KBr)**: 3668 cm⁻¹ (N-H), 3459 cm⁻¹ (N-H), 3050 cm⁻¹ (CH aromatic), 1684 cm⁻¹ (C=O), 1599 cm⁻¹ (C=N), 1395 cm⁻¹ (C-O), 1322 (SO₂), 1151 cm⁻¹ (SO₂). ^1H NMR (DMSO- d_6): 12.18 (br s, 1H, NH), 12.13 (br s, 1H, NH), 7.77 (d, 1H, ArH), 7.67 (d, 1H, ArH), 7.24 (dd, 1H, ArH), 7.52 (d, 2H, ArH), 6.94 (d, 2H, ArH), 8.64 (s, 1H, N=CH), 3.75 (s, 3H, -OCH₃). ^{13}C NMR (DMSO- d_6): 160.77 (C=O), 160.46 (C=O), 155.19, 154.94 (C=N), 147.17, 132.81, 129.94, 129.40, 128.43, 126.51, 126.19, 125.77, 122.01, 115.35, 114.35, 114.31, 114.19, 55.23 (CH₃), 35.34 (CH₃).

3-methyl-N'-(4-nitrobenzylidene)-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 7

A yellow solid, m.p 260-262°C, **IR Spectra (KBr)**

3486 cm⁻¹ (N-H), 3212 cm⁻¹ (N-H), 3062 cm⁻¹ (CH aromatic), 1684 cm⁻¹ (C=O), 1586 cm⁻¹ (C=N), 1387 cm⁻¹ (C-O), 1310 (SO₂), 1155 cm⁻¹ (SO₂). ^1H NMR (DMSO- d_6) 12.16 (br s, 1H, NH), 11.50 (br s, 1H, NH), 7.80 (d, 1H, ArH), 7.58 (dd, 1H, ArH), 7.17 (d, 1H, ArH), 7.12 (m, 1H, ArH), 7.27 (dd, 1H, ArH), 7.32 (t, 1H, ArH), 6.96-6.98 (m, 1H, ArH), 7.68 (s, 1H, N=CH), 3.78 (s, 3H, -OCH₃). ^{13}C NMR (DMSO- d_6) 159.39 (C=O), 155.20 (C=O), 154.95 (C=N), 146.97, 134.97, 132.66, 130.30, 129.87, 129.50, 125.81, 125.58, 122.41, 122.02, 119.36, 115.85, 115.42, 114.31, 112.87, 111.61, 35.10 (CH₃).

Antibacterial Sensitivity Testing of some Synthesized Compounds

“All the synthetic compounds were tested for antibacterial potency using agar-well diffusion technique as defined by Akinpelu *et al.*, (2016). The trial bacteria were initially re-activated in nutrient broth for 18 hours earlier before use”. “Precisely 0.1 mL of standardized bacterial strains (10^6 cfu/mL of 0.5 McFarland standards) was transferred into Mueller-Hinton agar medium at 40°C. 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 previously cooled to 40°C and carefully mixed together and transferred into sterile Petri dishes which were correctly labeled. The medium was permitted to set and wells were bored rigid into it using 6 mm sterile cork borer. The wells were made 5 mm to the edge of the plates and filled-up with the solution of the compounds. Care was taken not to allow the solution to spill on the surface of the medium. Streptomycin phosphate and tetracycline were used as positive controls at a concentration of 1 mg/mL respectively. The plates were allowed to stand for about one hour on the bench to allow for proper in-flow of the solution into the medium and then incubated aerobically uprightly at 37°C for 24 hours. Care was taken not to stockpile the plates. The plates were later observed for zones of inhibition which is an indication of susceptibility of the organisms to the compounds”. [29]

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” (Akinpelu and Kolawole, 2004). “Two milliliters of different concentrations of solution of the compound were added to 18

ml of pre-sterilized molten nutrient agar at 40 °C to give final concentrations regimes of 0.0157 and 1.0 mg/mL. The same range of concentrations were also prepared for the two positive controls. The medium was then poured into sterile Petri dishes and allowed to set. The plates were left on laboratory bench overnight to ascertain their purity. 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 °C for up to 72 hours after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration of the test compounds that will prevent the growth of the susceptible bacterial strains tested”. [29]

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.* (1992) with some modifications. Samples were taken from line of streak in the plate with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar medium and incubated at 37 °C for 48 h. The MBC was taken as the lowest concentration of the compound that completely kills the susceptible test organisms”.

Determination of Killing Rate of the Susceptible Bacterial Strains

“The assay was carried out using each of the active synthesized compounds on the viability of *Enterococcus faecalis* representing Gram-positive and *Pseudomonas fluoresces* representing Gram-negative organisms” (Odenholt *et al.*, 2001). Viable counts of the test organisms were initially determined. A 0.5 mL volume of known cell density (by viable counts 10^6 cfu/mL) from

each organism suspension was added to 4.5 mL of different concentrations of the synthesized compounds. The suspension was thoroughly mixed and held at room temperature (28 – 30 °C) and the killing rate was determined over a period of 2 h. Exactly 0.5 mL of each suspension withdrawn at 15 minutes time interval and transferred to 4.5 mL nutrient broth recovery medium containing 3% “Tween 80” to neutralize the effect of the antimicrobial compounds carried over from the test organisms’ suspensions. The suspension was shaken properly and serially diluted up to 10^{-5} in sterile physiological saline. Exactly 0.5 mL of the final dilution of the test organism was transferred into pre-sterilized nutrient agar at 45 °C and plated out. The plates were allowed to set and incubated in inverted position at 37 °C for 72 h. The viable counts were made in triplicates for each sample. Depression in the viable counts indicated killing by the compounds.

RESULTS

Chemistry

The reactions of 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonylhydrazide **1** with some benzaldehyde derivatives in ethanol and glacial acetic acid gave the hydrazones **2-7** as shown in Scheme 2. The infrared spectra of the compounds exhibited absorption bands owing to the stretching vibrations of N-H and OH stretching frequency between 3135 and 3390 cm^{-1} , C=O stretching frequency between 1676 and 1692 cm^{-1} , C=C and C=N stretching frequency between 1607 and 1580 cm^{-1} , SO_2 stretching frequency at 1310 - 1391 cm^{-1} and 1140 - 1167 cm^{-1} for asymmetric and symmetric vibrations. The $^1\text{H-NMR}$ spectral data of compounds **2-7** in DMSO-d_6 showed NH signals between 8.37 ppm and 12.51 ppm, the CH=N signals between 7.68 ppm

and 9.59 ppm, the aromatic protons were observed between 6.40 ppm and 9.50 ppm, the methyl protons (CH₃) were observed at 2.50 ppm.

Antimicrobial Studies

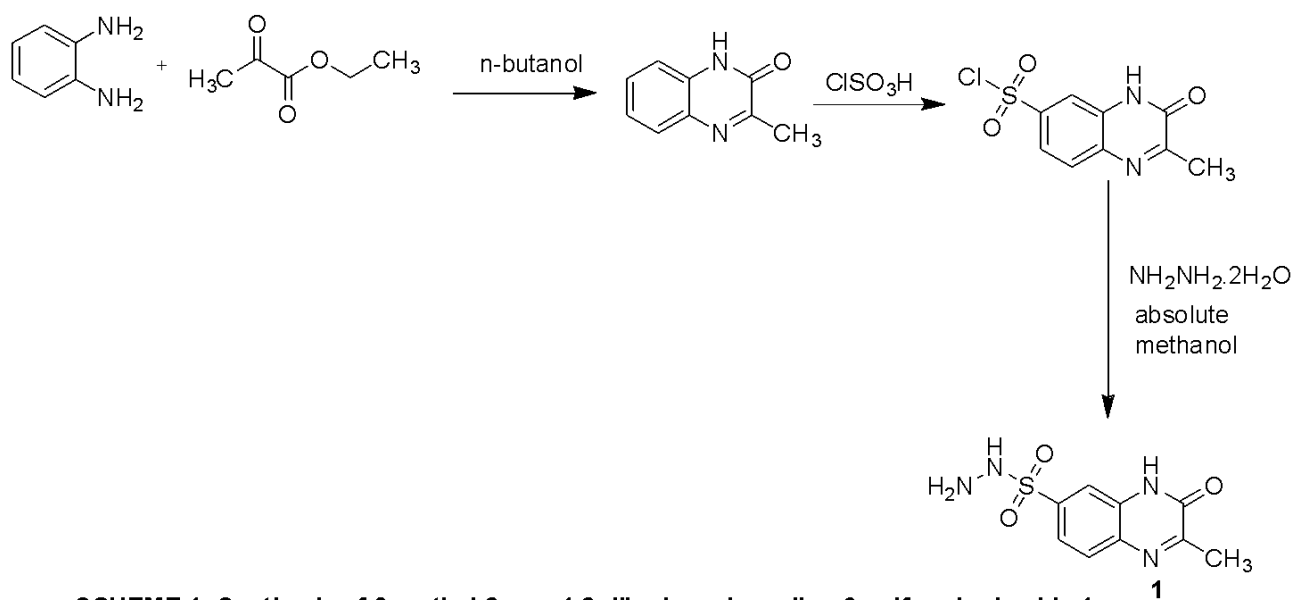
All the synthetic compounds were shown to be potent against all the bacterial strains studied. The zones of inhibition detected for the prepared compounds against the test organisms were 10 mm and 30 mm. On the other the hand, the zones of inhibition observed for streptomycin and tetracycline against the bacteria were 15 and 28 mm (Tables 1). The MIC observed for the synthetic compounds were 0.0625 mg/mL and 0.125 mg/mL, while that of the standard antibiotic, streptomycin, varied between 0.0078 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 synthetic compounds against Gram positive bacteria fall between 0.0625 mg/mL and 0.500 mg/mL, while streptomycin, varied between 0.0313 and 0.500 mg/mL and tetracycline varied between 0.0625 and 0.500 mg/ml. The results indicated that streptomycin has stronger 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).

The results of kill rate exhibited by the compounds showing that the test cells were eradicated or destroyed within the shortest exposure time and low concentration. For instance, 100% kill of the test organisms was accomplished within 120 minutes of exposure time with the prepared compounds (Figures 1, 2, 3 and 4) This is an indication of significant activity displayed by the synthetic compounds.

Discussion

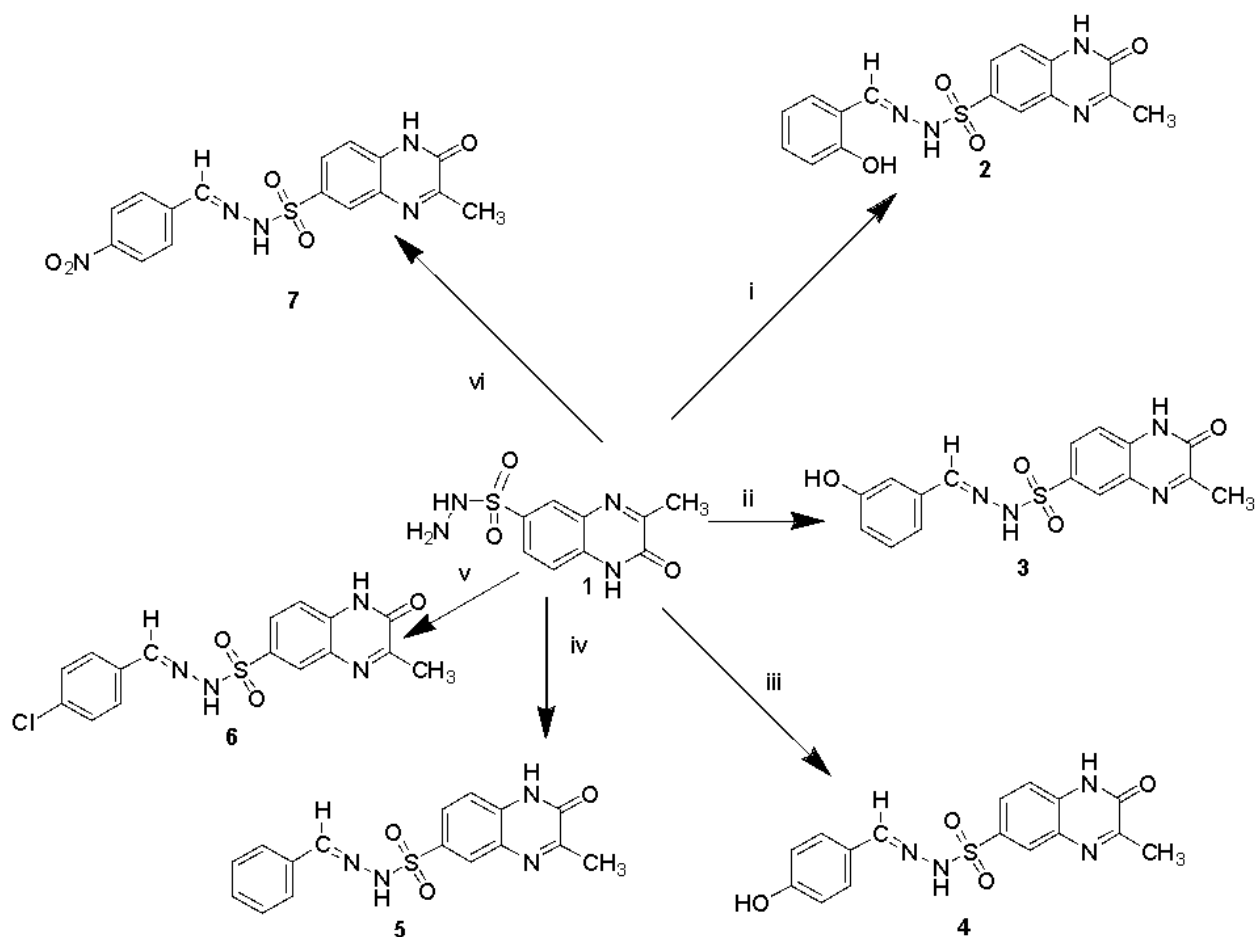
Chemistry

The 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide **1** was synthesized from the reaction of 2-methyl-3-oxo-3,4-dihydroquinoxaline-6-sulfonyl chloride with hydrazine dihydrate in absolute methanol under refluxing condition. The 2-methyl-3-oxo-3,4-dihydroquinoxaline-6-sulfonyl chloride was prepared from the chlorosulfonation of 3-methylquinoxalin-2(1H)-one synthesized from the reaction of 1,2-diaminobenzene with ethyl pyruvate in n-butanol with chlorosulfonic acid. (Taiwo, *et al.*, 2021) (Scheme 1)



SCHEME 1: Synthesis of 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 1

The reaction of different substituted benzaldehydes with 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide **1** in glacial acetic acid gave the different hydrazones **2 - 7** (Scheme 2).



- i. 2-hydroxybenzaldehydes Reaction condition: glacial acetic acid, reflux at 120 °C.
 ii. 3-hydroxybenzaldehydes Reaction condition: glacial acetic acid, reflux at 120 °C.
 iii. 4-hydroxybenzaldehydes Reaction condition: glacial acetic acid, reflux at 120 °C.
 iv. benzaldehydes Reaction condition: glacial acetic acid, reflux at 120 °C.
 v. 4-chlorobenzaldehydes Reaction condition: glacial acetic acid, reflux at 120 °C.
 vi. 4-nitrobenzaldehydes Reaction condition: glacial acetic acid, reflux at 120 °C.

SCHEME 2: The reaction of different benzaldehydes with 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide 1

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

The synthetic compounds exhibited significant antibacterial activities. The mode of action of the synthetic compounds were investigated through the ability of the synthetic compounds to kill or eliminate the test organisms within a specify period of time and concentrations. Elimination of

the test cells by the synthetic compounds might be through the damage of cytoplasmic membrane of these cells. As a results of impairment to the cytoplasm of these cells, leakages of protoplasmic inclusions may occur and thus led to the cells death as observed in this study. The noteworthy action of the compounds might be described on the basis of the contributions of fused aromatic ring which we know should enhance the lipophilicity of the compounds. This rise in lipophilicity would help their penetrability through the microbial cell wall and improve the interaction of the diverse functional groups existing in the compounds with the cellular membrane of the bacterial cell and thus weakened both its functions and integrity (Raccach, 1984; Blaszyk and Holley, 1998) resulting in enhanced activity.

CONCLUSION

The synthesis of some new 3-methyl-2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide derivatives were successful. The methodology employed for the synthesis of the compounds 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.

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								
<i>Bacillus polymyxa</i> (LIO)	20±1.00	20±0.5	22±1.00	20±1.00	20±1.00	20±0.29	15±0.50	20±0.56
<i>B. cereus</i> (NCIB 6349)	20±1.00	18±0.29	24±0.29	16±0.56	20±1.00	18±1.00	28±0.56	18±1.00
<i>Corynebacterium pyogenes</i> (LIO)	18	16±0.50	18±1.00	18±0.29	20±0.50	20±0.50	20±0.56	20±1.00
<i>Clostridium sporogenes</i> (NCIB 532)	20±1.00	20±1.00	16±0.56	24±1.00	24±0.29	16±0.50	25±0.56	20±1.00
<i>B. stearothermophilus</i> (NCIB 8222)	22±1.15	22±1.00	20±1.00	22±1.00	20±1.00	24±1.00	23±0.56	22±1.00
<i>Streptococcus pneumoniae</i> (LIO)	16±1.05	18±0.50	18±1.00	24±0.29	10±0.50	25±1.00	24±0.56	24±0.56
<i>Strep. Pneumoniae</i> (PS)	15±1.00	18±1.00	16±1.00	20±1.00	10±1.00	24±1.00	26±0.50	15±0.56
<i>B. subtilis</i> (NCIB 3610)	24±1.00	22±1.00	22±0.50	24±0.50	24±1.00	16±0.50	20±1.00	22±0.56
<i>Staphylococcus aureus</i> (NCIB 8588)	22±1.05	16±0.50	20±0.29	18±1.00	19±1.00	21±1.00	20±1.00	15±0.56
<i>Staphylococcus aureus</i> (SW)	24±1.00	16±1.00	22±1.00	20±0.29	20±0.58	22±1.00	21±0.56	17±0.56
<i>Enterococcus faecalis</i> (NCIB 775)	24±1.15	24±0.50	26±0.29	24±0.56	18±1.00	20±0.50	23±0.56	28±0.50
<i>Micrococcus luteus</i> (NCIB 196)	24±1.00	22±1.00	20±1.00	24±1.00	20±0.29	18±1.00	21±0.50	22±1.00
<i>Bacillus anthracis</i> (LIO)	22±1.00	24±0.29	24±0.50	24±1.00	22±0.50	18±1.00	22±1.00	25±1.00
Gram-negative								
<i>Escherichia coli</i> (NCIB 86)	30±0.56	26±1.15	30	28±0.56	28±1.00	24±0.58	0	18±1.15
<i>Citrobacter freundii</i> (PS)	20±1.15	21±0.56	23±1.00	21±1.00	22±1.00	20±0.58	18±1.00	0
<i>Pseudomonas fluorescense</i> (NCIB 3756)	22±0.56	22±1.15	24±1.00	20±0.56	22±0.58	20±0.58	30±1.15	0
<i>Klebsiella pneumoniae</i> (418)	26±0.56	28±1.00	26±0.56	28±1.00	28±1.00	26±1.00	0	12±0.85
<i>Pseudomonas aeruginosa</i> (NCIB 950)	20±0.56	20±1.00	27±0.56	20±1.00	28±1.00	24±1.00	25±0.85	12±1.00
<i>Pseudomonas aeruginosa</i> (PS)	22±0.56	23±1.00	29±1.00	23±1.00	21±1.00	28±1.00	20±1.00	15±1.00
<i>Pseudomonas aeruginosa</i> (PS)	20±1.00	20±1.15	27±0.56	20±0.56	28±0.56	24±0.58	10±1.00	16±0.85
<i>Pseudomonas aeruginosa</i> (PS)	20±1.15	20±0.56	15±1.00	20±1.00	25±1.00	22±1.00	17±1.00	21±1.00
<i>Shigella species</i> (LIO)	27±1.00	25±0.56	26±1.00	27±1.00	21±1.00	21±1.00	22±0.85	0
<i>Proteus vulgaris</i> (NCIB 67)	20±1.15	22±0.56	24±1.00	22±0.58	22±0.58	16±0.58	15±1.00	22±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)														Strep		Tet	
	2		3		4		5		6		7		MIC	MBC	MIC	MBC		
<i>Bacillus polymyxa</i> (LIO)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125		
<i>B. cereus</i> (NCIB 6349)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.125	0.250	0.031	0.0625	0.250	0.250		
<i>Corynebacterium pyogenes</i> (LIO)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.031	0.0625	0.031	0.062		
<i>Clostridium sporogenes</i> (NCIB 532)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.007	0.0313	0.031	0.062		
<i>B. stearothermophilus</i> (NCIB 8222)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.125	0.250		
<i>Streptococcus pneumoniae</i> (LIO)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.125	0.250		
<i>Strep. Pneumoniae</i> (PS)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.125	0.250		
<i>B. subtilis</i> (NCIB 3610)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.250	0.500		
<i>Staphylococcus aureus</i> (NCIB 8588)	0.125	0.250	0.125	0.250	0.125	0.250	0.0625	0.125	0.125	0.250	0.125	0.250	0.500	ND	0.031	ND		
<i>Staphylococcus aureus</i> (SW)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.125	0.250		
<i>Enterococcus faecalis</i> (NCIB 775)	0.062	0.125	0.062	0.125	0.250	0.500	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.250	0.500		
<i>Micrococcus luteus</i> (NCIB 196)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.250	0.500		
<i>Bacillus anthracis</i> (LIO)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.500	ND	0.500	ND		
<i>Escherichia coli</i>	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	ND	ND	0.031	0.062		

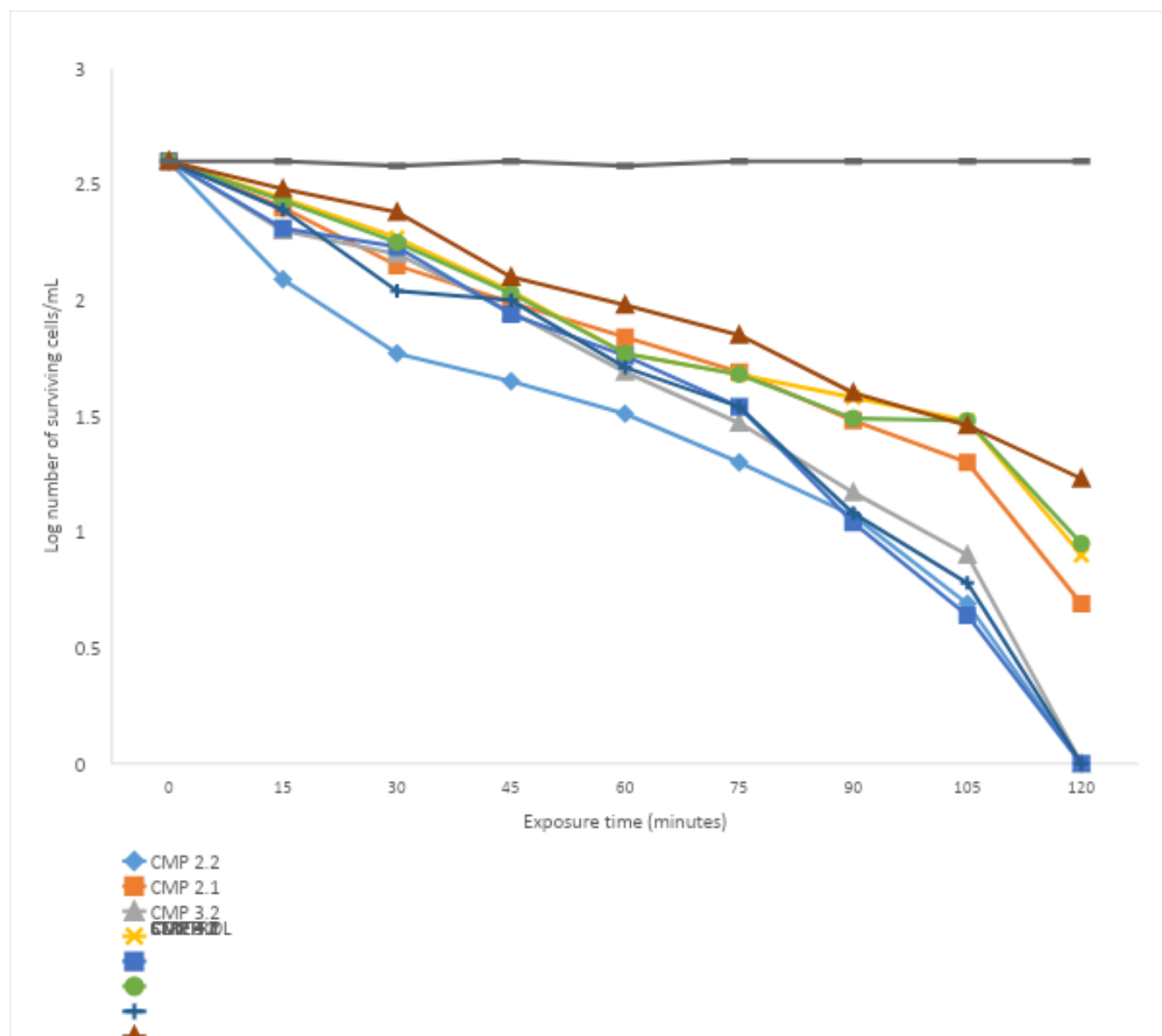
(NCIB 86)											5				3	5
<i>Citrobacter freundii</i>	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	ND	ND	0.031	0.062
(PS)											5				3	5
<i>Pseudomonas fluorescens</i> (NCIB 3756)	0.125	0.250	0.062	0.125	0.125	0.250	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	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
<i>Klebsiella pneumoniae</i> (418)	0.625	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.062	0.125	ND	ND	0.500	ND
<i>Pseudomonas aeruginosa</i> (NCIB 950)	0.125	0.250	0.062	0.250	0.125	0.250	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND
<i>Pseudomonas aeruginosa</i> (PS)	0.062	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.062	0.125	0.250	0.500	0.500	ND
<i>Pseudomonas aeruginosa</i> (PS)	0.125	0.250	0.062	0.125	0.125	0.250	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND
<i>Pseudomonas aeruginosa</i> (PS)	0.062	0.125	0.125	0.250	0.125	0.250	0.125	0.250	0.062	0.125	0.062	0.125	0.250	0.500	0.500	ND
<i>Shigella species</i> (LIO)	0.125	0.250	0.062	0.125	0.125	0.250	0.250	0.500	0.125	0.250	0.250	0.500	0.250	0.500	ND	ND
<i>Proteus vulgaris</i> (NCIB 67)	0.125	0.250	0.125	0.250	0.125	0.250	0.125	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 Done

Figure 1: Rate of Killing of *E. faecalis* by 1 X MIC and 2 X MIC of Compounds 2, 3 And 4



CMP 2.2 = Log of number of surviving cells for compound 2 at 2X MIC

CMP 2.1 = Log of number of surviving cells for compound 2 at 1X MIC

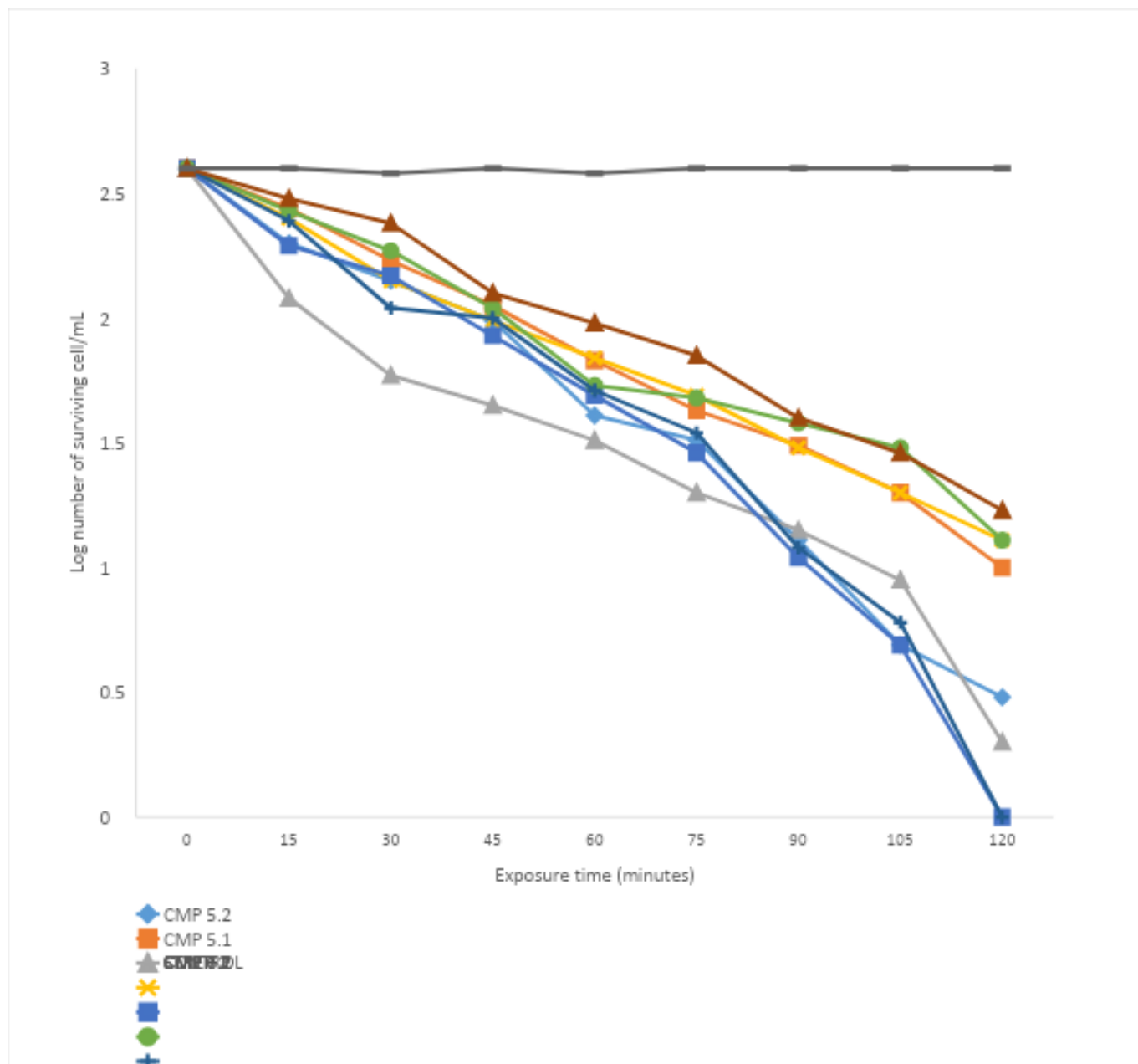
CMP 3.2 = Log of number of surviving cells for compound 3 at 2X MIC

CMP 3.1 = Log of number of surviving cells for compound 3 at 1X MIC

CMP 4.2 = Log of number of surviving cells for compound 4 at 2X MIC

CMP 4.1 = Log of number of surviving cells for compound 4 at 1X MIC

Figure 2: Rate of Killing of *E. faecalis* by 1 X MIC and 2 X MIC of Compounds 5, 6 and 7



CMP 5.2 = Log of number of surviving cells for compound 5 at 2XMIC

CMP 5.1 = Log of number of surviving cells for compound 5 at 1XMIC

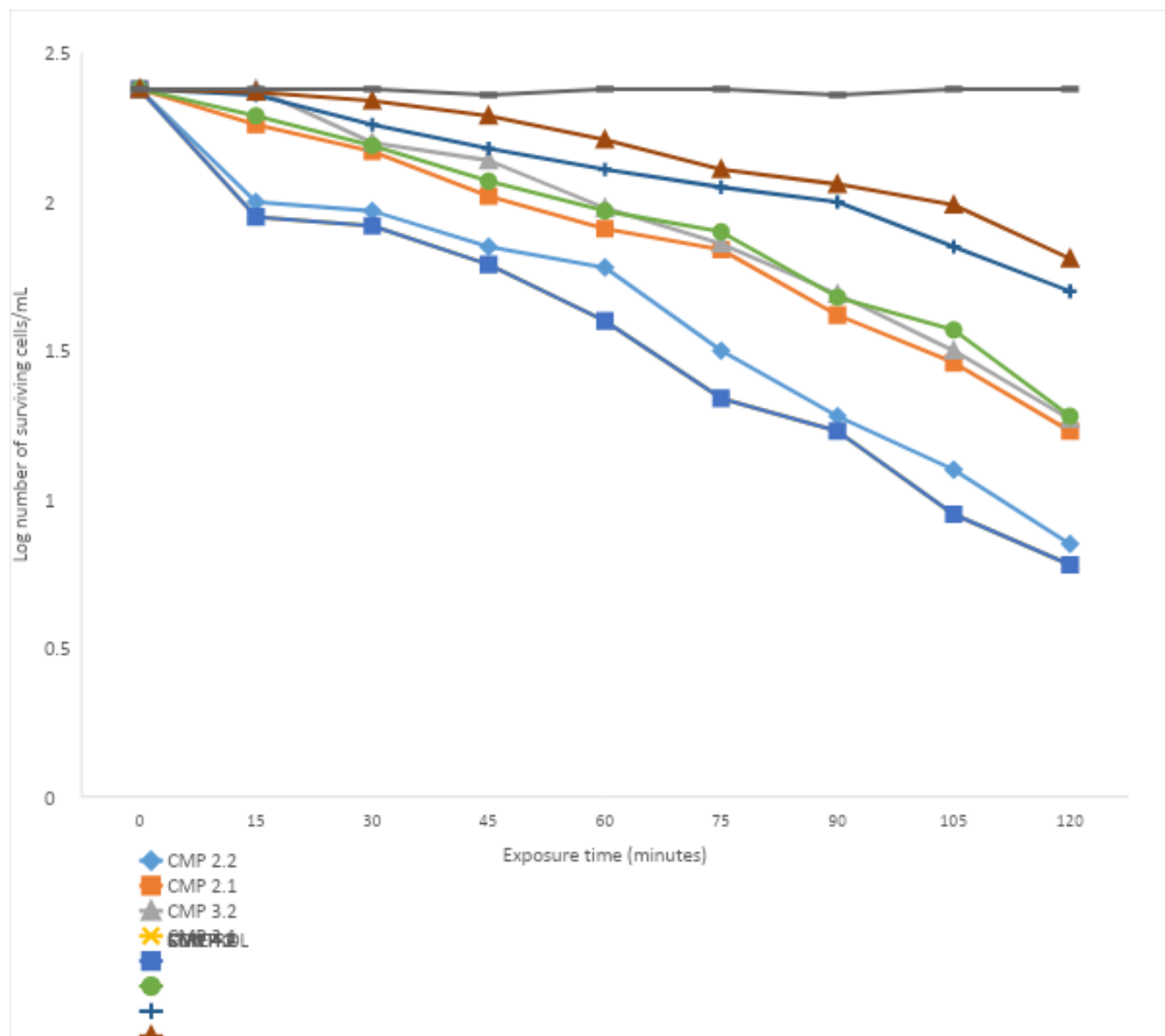
CMP 6.2 = Log of number of surviving cells for compound 6 at 2XMIC

CMP 6.1 = Log of number of surviving cells for compound 6 at 1XMIC

CMP 7.2 = Log of number of surviving cells for compound 7 at 2XMIC

CMP 7.1 = Log of number of surviving cells for compound 7 at 1XMIC

Figure 3: Rate of Killing of *P. fluorescens* by 1 X MIC And 2 X MIC of Compounds 2, 3 and 4



CMP 2.2 = Log of number of surviving cells for compound 2 at 2X MIC

CMP 2.1 = Log of number of surviving cells for compound 2 at 1X MIC

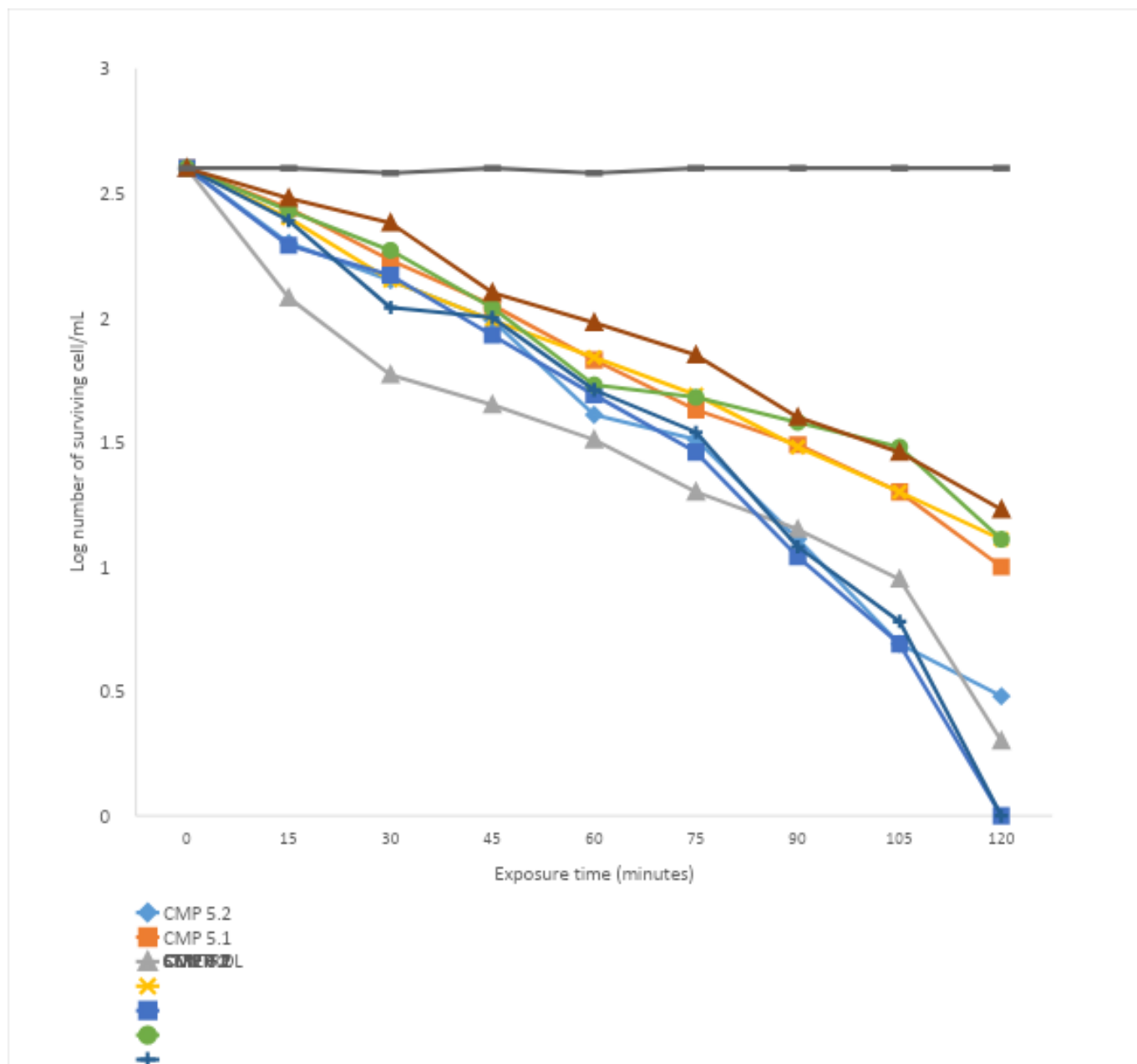
CMP 3.2 = Log of number of surviving cells for compound 3 at 2X MIC

CMP 3.1 = Log of number of surviving cells for compound 3 at 1X MIC

CMP 4.2 = Log of number of surviving cells for compound 4 at 2X MIC

CMP 4.1 = Log of number of surviving cells for compound 4 at 1X MIC

Figure 4: Rate of Killing of *E. faecalis* by 1 X MIC and 2 X MIC of Compounds 5, 6 and 7



CMP 5.2 = Log of number of surviving cells for compound 5 at 2X MIC

CMP 5.1 = Log of number of surviving cells for compound 5 at 1X MIC

CMP 6.2 = Log of number of surviving cells for compound 6 at 2X MIC

CMP 6.1 = Log of number of surviving cells for compound 6 at 1X MIC

CMP 7.2 = Log of number of surviving cells for compound 7 at 2X MIC

CMP 7.1 = Log of number of surviving cells for compound 7 at 1X MIC

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