Synthesis of Some quinoxaline sulfonamides as a potential Antibacterial agent

Keywords: Quinoxalines, Antibacterial activity, Gram-positive bacteria, Gram-negative bacteria, quinoxaline-2,3-dione, Synthesis, substituted benzaldehydes, sulfonamides.

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

Abstract

Aims: This studies aims at the synthesis of new heterocyclic systems and study its biological and pharmacological properties.

Objective: This study was designed to synthesized some quinoxaline-2,3-dione with sulfonamide moiety, characterize the synthesized compounds, and study the antimicrobial properties of the synthesized compounds on some bacterial strains.

Materials and methods: Six quinoxaline-6-sulfonohydrazone derivatives were synthesized by reacting quinoxaline-6-sulfonohydrazine with some substituted benzaldehydes and ketones. The compounds were tested for their potential antibacterial properties.

Results: All the test compounds possessed promising antibacterial property against a panel of bacterial strains used for this study. The MIC values exhibited by these compounds ranged between 0.0313 and 0.250 mg/mL. Among the compounds tested, compound **2** showed appreciable antibacterial activity.

Discussion and conclusion: The study concluded that all the compounds exhibited appreciable bactericidal effects towards all the bacterial strains, particularly, compound **2** This is an indication that such compounds possessing broad spectrum activities will be useful in formulating antimicrobial compounds which could be used to treat infections caused by pathogens that are now developing resistance against the available antibiotics.

INTRODUCTION

The synthesis and chemistry of quinoxalines have attracted considerable attention in the past twenty years. Quinoxaline molecules are nitrogen containing heterocycle derivatives which have broad spectrum biological and pharmaceutical applications. Quinoxaline are known for their therapeutic properties and as such attract the attention of many scientists searching for potent antimicrobial compounds. These compounds possess anti-viral [1-3] anti-bacterial [4-10] anti-inflammatory [11, 12] anticancer [13-15]. They are also used in the agricultural field as fungicides, herbicides, and insecticides [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.

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 recorded as KBr pellets on a Bruker 2000 Spectrometer. The ^{1}H and ^{13}C NMR was run on a Bruker 600 MHz spectrometer (δ in ppm relative to Me_4Si), Mass spectra were taken on a high-resolution ($m/\Delta m$ = 30 000)

Thermo Scientific LTQ-Orbitrap Discovery mass spectrometer (San Jose, CA) equipped with an electrospray ionization source 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, except Glacial acetic acid, ethanol, oxalic acid and vanillin which were obtained from BDH Chemical Limited.

Preparation of quinoxaline-2,3-(1*H*,4*H*)-dione-6-sulfonohydrazine 1

To hydrazine dihydrate (20 ml, 0.460mmol) in absolute methanol (400 mL) was added quinoxaline-6-sulfonylchloride (30 g, 0.115mol) portion wise with constant stirring for 15 minutes. The reaction mixture was stirred at room temperature for 24 hours. The mixture obtained was refluxed at 80 ^{o}C for 1 hour. The solution was cooled and poured into cold water to give **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 (SO_2), 1159 cm^{-1} (SO_2).

^{1}H NMR (DMSO-d_6): 3.37 (br s, 1H, NH), 4.12 (br s, 1H, NH), 12.10 (br s, 1H, NH), 8.37 (br s, 1H, NH), 7.60 (d, 1H, ArH), 7.49-7.50 (dd, 1H, ArH), 7.27 (d, 1H, ArH)**. ^{13}C NMR (DMSO-d_6):** 154.86 (C=O), 131.98 (Aromatic), 128.95 (Aromatic), 125.50 (Aromatic), 122.33 (Aromatic), 115.27 (Aromatic), 114.96 (Aromatic)**. MS *(m/z,* %):** 231 (M+, 15), 227 ([M - CHO]+, 100), 200 ([M – C_2O_2]+,14), 135 ([M – $C_2H_4N_2O_2SH$]+, 10), 111 (15).

General Procedure for the Reaction of Quinoxaline-6-sulfonohydrazide with Substituted Benzaldehydes and Aromatic Ketones

Quinoxaline-6-sulfonohydrazine (1.0 g, 39 mmol) and substituted benzaldehydes or aromatic ketones (39 mmol) were added to glacial acetic acid (25 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 N-(E)-(phenylideneamino)-6-(quinoxaline-2,3-(1H,4H)-dione)sulfonamide **2-7**.

Synthesis of N-(E)-(4-methoxybenzylideneamino)-6-(quinoxaline-2,3-(1*H*,4*H*)-dione)sulfonamide, 2

melting point 239-241 ^{o}C, lit 240-242 ^{o}C [17] **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 (SO_2), 1151 cm^{-1} (SO_2). **^{1}H NMR (DMSO-d_6)** 12.18 (br s, 1H, NH), 12.13 (br s, 1H, NH, D_2O exchangeable), 11.30 (br s, 1H, NH), 7.77 (d, 1H, ArH), 7.67 (d, 1H, ArH), 7.24-7.26 (dd, 1H, ArH), 7.52-7.54 (d, 2H, ArH), 6.94-6.96 (d, 2H, ArH), 8.64 (s, 1H, N=CH), 3.75 (s, 3H, -OCH_3). **^{13}C NMR (DMSO-d_6)** 160.77 (C=O), 160.46 (C=O), 155.19, 154.94 (C=N), 147.17 (Aromatic), 132.81 (Aromatic), 129.94 (Aromatic), 129.40 (Aromatic), 128.43 (Aromatic), 126.51 (Aromatic), 126.19 (Aromatic), 125.77 (Aromatic), 122.01 (Aromatic), 115.35 (Aromatic), 114.35 (Aromatic), 114.31 (Aromatic), 114.19 (Aromatic), 55.23 (CH_3), 55.34 (CH_3).

Synthesis of N-(E)-(3-methoxybenzylideneamino)-6-(quinoxaline-2,3-(1*H*,4*H*)-dione)sulfonamide 3

Melting point 261-263 ^{o}C, lit 262-263 ^{o}C [17]. **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 (SO_2), 1155 cm^{-1} (SO_2). **^{1}H NMR (DMSO-d_6)** 12.18 (br s, 2H, NH), 11.52(br s, 1H, NH), 7.88 (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, -OCH_3). **^{13}C NMR (DMSO-d_6)** 159.39 (C=O), 155.20 (C=O), 154.95 (C=N), 146.97 (Aromatic), 134.97 (Aromatic), 132.66 (Aromatic), 130.30 (Aromatic), 129.87 (Aromatic), 129.50 (Aromatic), 125.81 (Aromatic), 125.58 (Aromatic), 122.41 (Aromatic), 122.02 (Aromatic), 119.36 (Aromatic), 115.85 (Aromatic), 115.42 (Aromatic), 114.31 (Aromatic), 112.87 (Aromatic), 111.61 (Aromatic), 55.10 (CH_3).

Synthesis of N-(E)-((1-(4-dimethylamino)phenyl)methylideneamino)-6-(quinoxaline-2,3-*(1H,4H)*-dione)sulfonamide 4

Melting point 288-290 ^{o}C lit 286-288 ^{o}C [17] **IR Spectra (KBr)** 3193 cm^{-1} (N-H), 3135 cm^{-1} (N-H), 3035 cm^{-1} (CH aromatic), 1676 cm^{-1} (C=O), 1584 cm^{-1} (C=N), 1318 (SO_2), 1159 cm^{-1} (SO_2). **^{1}H NMR (DMSO-d_6)** 12.13 (br s, 1H, NH), 11.92 (br s, 1H, NH), 10.04 (br s, 1H, NH), 7.76 (s, 1H, ArH), 7.74 (d, 2H, ArH), 7.55 (ddd, 1H, ArH), 7.38-7.44 (dd, 2H, ArH), 7.23-7.32 (ddd, 1H, ArH), 8.61 (s, 1H, N=CH), 2.50 (s, 6H, CH_3). **^{13}C NMR (DMSO-d_6)**

155.18 (C=O), 154.92 (C=O), 137.27 (Aromatic), 132.79 (Aromatic), 132.05 (Aromatic), 129.72 (Aromatic), 129.40 (Aromatic), 129.02 (Aromatic), 128.40 (Aromatic), 128.34 (Aromatic), 126.44 (Aromatic), 126.01 (Aromatic), 125.66 (Aromatic), 125.58 (Aromatic), 122.40 (Aromatic), 14.67 (CH_3), 14.27 (CH_3)

Synthesis of N-(E)-((1-(5-methoxy-4-hydroxyl-)phenyl)ethylideneamino)-6-(quinoxaline-2,3-*(1H,4H)*-dione)sulfonamide 5

Melting point 230-231 ^{o}C lit 233 ^{o}C (decomposed)[17] **IR Spectra (KBr)** 3363 cm^{-1} (OH), 3239 cm^{-1} (N-H), 3054 cm^{-1} (CH aromatic), 1680 cm^{-1} (C=O), 1588 cm^{-1} (C=N), 1391 cm^{-1} (C-O), 1333 (SO_2), 1156 cm^{-1} (SO_2). **^{1}H NMR (DMSO-d_6)** 12.13 (br s, 2H, NH), 11.20 (br s, 1H, NH), 9.50 (s, 1H, ArH), 7.56-7.57 (dd, 1H, ArH), 7.66 (d, 1H, ArH), 6.98 (dd, 1H, ArH), 7.24-7.26 (d, 1H, ArH), 7.10 (d, 1H, ArH), 7.77 (s, 1H, N=CH), 3.78 (s, 6H, OCH_3)**. ^{13}C NMR (DMSO-d_6)** 154.89 (C=O), 148.79 (C=N), 147.73 (C-O), 132.72 (Aromatic), 129.32 (Aromatic), 125.68 (Aromatic), 124.96 (Aromatic), 121.99 (Aromatic), 121.08 (Aromatic), 115.35 (Aromatic), 115.29 (Aromatic), 114.31 (Aromatic), 109.50 (Aromatic), 55.47 (CH_3).

Synthesis of N-(E)-(2-oxoindole-3-ylideneamino)-6-(quinoxaline-2,3-(1*H*,4*H*)-dione)sulfonamide 6

Melting point 268-270 ^{o}C lit 273-274 ^{o}C [17]. **IR Spectra (KBr)** 3324 cm^{-1} (N-H), 3104 cm^{-1} (N-H), 1680 cm^{-1} (C=O), 1595 cm^{-1} (C=N), 1383 cm^{-1} (C-O), 1322 (SO_2), 1163 cm^{-1} (SO_2). **^{1}H NMR (DMSO-d_6)** 12.21 (br s, 1H, NH), 12.17 (br s, 1H, NH), 10.73 (br s, 1H, NH), 7.72 (d, 1H, ArH), 7.87 (d, 1H, ArH), 7.63-7.65 (dd, 1H, ArH), 6.85-6.86 (d, 1H, ArH), 7.27-7.29 (d, 1H, ArH), 7.37 (t, 1H, ArH), 7.06 (t, 1H, ArH). **^{13}C NMR (DMSO-d_6)** 171.93 (C=O), 163.61 (C=O), 155.16 (C=O), 154.86 (C=N), 143.84 (Aromatic),141.84 (Aromatic), 133.07 (Aromatic), 131.58 (Aromatic), 129.86 (Aromatic), 126.58 (Aromatic), 125.67 (Aromatic), 122.75 (Aromatic), 121.60 (Aromatic), 115.36 (Aromatic), 115.12 (Aromatic), 115.02 (Aromatic) 110.50 (Aromatic).

Synthesis of N-(E)-(-1-phenylethylideneamino)-6-(quinoxaline-2,3-(1*H*,4*H*)-dione)sulfonamide 7

Melting point 288-290 ^{o}C lit 290-292 ^{0}C [17]. **IR Spectra (KBr)** 3347 cm^{-1} (N-H), 3139 cm^{-1} (N-H), 3039 cm^{-1} (CH aromatic), 2927 cm^{-1} (CH aliphatic) 1676 cm^{-1} (C=O), 1595 cm^{-1} (C=N), 1314 (SO_2), 1167 cm^{-1} (SO_2). **^{1}H NMR (DMSO-d_6)** 12.17 (br s, 2H, NH), 8.36 (br s, 1H, NH), 8.35 (s, 1H, ArH), 7.91 (m, 1H, ArH), 7.25 (d, 1H, ArH), 7.60 (d, 1H, ArH), 7.46 (m, 3H, ArH), 2.50 (s, 3H, CH_3). **^{13}C NMR (DMSO-d_6)** 158.56 (C=O), 155.12 (C=O) 154.88 (C=N), 151.13, 148.17 (Aromatic), 143.28 (Aromatic), 132.89 (Aromatic), 129.23 (Aromatic), 128.09 (Aromatic), 125.65 (Aromatic), 124.58 (Aromatic), 121.96 (Aromatic), 120.51 (Aromatic), 115.23 (Aromatic), 114.31 (Aromatic), 111.93 (Aromatic) 111.84 (Aromatic), 20.95 (CH_3).

Antibacterial Sensitivity Testing of Synthesized Compounds

The synthesized compounds were screened for antibacterial activity using agar-well diffusion method as described by Akinpelu *et al*., (2016) [18]. The test organisms were first re-activated in nutrient broth for 18 hours before use. Exactly 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^{o}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 already cooled down to 40^{o}C and thoroughly mixed together and poured into sterile Petri dishes which were properly labeled. The medium was allowed to set and wells were bored 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^{o}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.

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

Minimum inhibitory concentrations of the compounds and the standard antibiotics-streptomycin and tetracycline were carried out using a two-fold dilution method [19]. 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 range of concentrations was 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 ^{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 compounds that will prevent the growth of the susceptible bacterial strains tested.

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)[19] with some modifications. Samples were taken from line of streak in the 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.

Determination of Killing Rate of the Susceptible Bacterial Strains

The assay was carried out using each of the synthesized compounds on the viability of *Enterococcus feacalis* representing Gram-positive organism and *Pseudomonas fluorescens* representing Gram-negative organism [20]. 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 ^oC) 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 (Lab. M) recovery medium containing 3% "Tween 80" to neutralize the effect of the antimicrobial compounds carried over from the test 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 (Biolab.) at 45 ^oC and plated out. The plates were allowed to set and incubated in inverted position at 37 ^oC 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 quinoxaline-6-sulfonyl hydrazide 1 was synthesized by reacting quinoxaline-2,3-dione with excess chlorosulfonic acid to obtain the corresponding quinoxaline-6-sulfonyl chloride, which was then reacted with hydrazine hydrate in methanol to give the expected quinoxaline-6-sulfonohydrazine, 1. The sequence of reactions is shown in Scheme 1.

The reactions of quinoxaline-6-sulfonohydrazine 1 with some substituted benzaldehyde under refluxing condition in glacial acetic acid afforded the hydrazones **2-5** as shown in Scheme 2. Furthermore, N-(E)-(2-oxoindole-3-ylideneamino)-6-(quinoxaline-2,3-(1H,4H)-dione) sulfonamide 6 was prepared by the reaction of quinoxaline-6-sulfonohydrazine, 1 with isatin as shown in Scheme 2. The reaction of quinoxaline-6-sulfonohydrazine 1 with acetophenone under refluxing condition in glacial acetic acid afforded the hydrazone 7. The sequence of this reaction is shown in Scheme 2. The infrared spectra of the compounds showed absorption bands due to the stretching vibrations of N-H and OH between 3135 and 3390 cm^{-1}, C=O between 1676 and 1692 cm^{-1}, C=C and C=N between 1607 and 1580 cm^{-1}, SO_2 at 1310 - 1391 cm^{-1} and 1140 - 1167 cm-1 for asymmetric and symmetric vibrations. The ^{1}H-NMR spectral data of compounds 2-7 in DMSO-d^6 showed signal for NH between 8.37 ppm and 12.51 ppm, the signals for CH=N between 7.68-ppm and 9.59 ppm, the signals for aromatic protons were observed between 6.40 ppm and 9.50 ppm, the signals for methyl protons (CH_3) were seen at 2.50 ppm and the signals for methoxy protons (OCH_3) were observed between 3.75 ppm and 3.78 ppm. The compounds 2-7 showed signal for C-O between 159.3 ppm and 162.79 ppm, the signals for C=O between 154.86 ppm and 171.93 ppm, the signals for C=N were observed between 148.79 ppm and 155.98 ppm, the signals for aromatic carbons were seen between 109.50 ppm and 154.92 ppm, while the signals for methoxy carbom (OCH_3) were observed between 55.10 ppm and 55.47 ppm and methyl carbon appeared between 14.27 ppm and 20.97 ppm.

Antimicrobial Studies

All the synthesized compounds were active against all the bacteria investigated. The zones of inhibition observed for the synthesized compounds against the test organisms ranged between 10

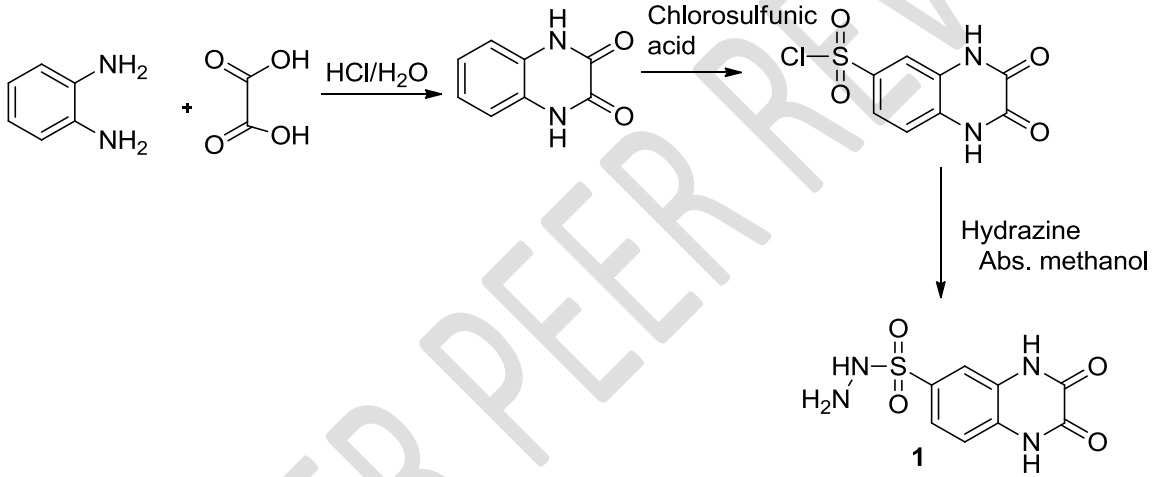
mm and 30 mm. On the other hand, the zones of inhibition observed for streptomycin and tetracycline against the bacteria ranged between 15 and 28 mm (Table 1). This is an indication that the synthesized compounds compared favourably with the standard antibiotics – streptomycin and tetracycline used as positive control. The MIC exhibited by the synthesized compounds against the bacterial strains ranged between 0.0625 mg/mL and 0.125 mg/mL (Table 2). The lowest MBC against the test organism was 0.125 mg/mL while the highest MBC was 5.00 mg/mL (Table 3). On the other hand, 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 tetracycline exhibited a range between 0.0313 mg/mL and 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. The results of killing rate exhibited by the synthetic compounds revealed that the test cells were eliminated or killed within the shortest contact time and low concentration. For example, 100% kill of the test organisms was achieved within 120 minutes of contact time with the synthetic compounds (Figures 1, 2, 3 and 4) This is an indication of significant activity displayed by the synthetic compounds.

Discussion

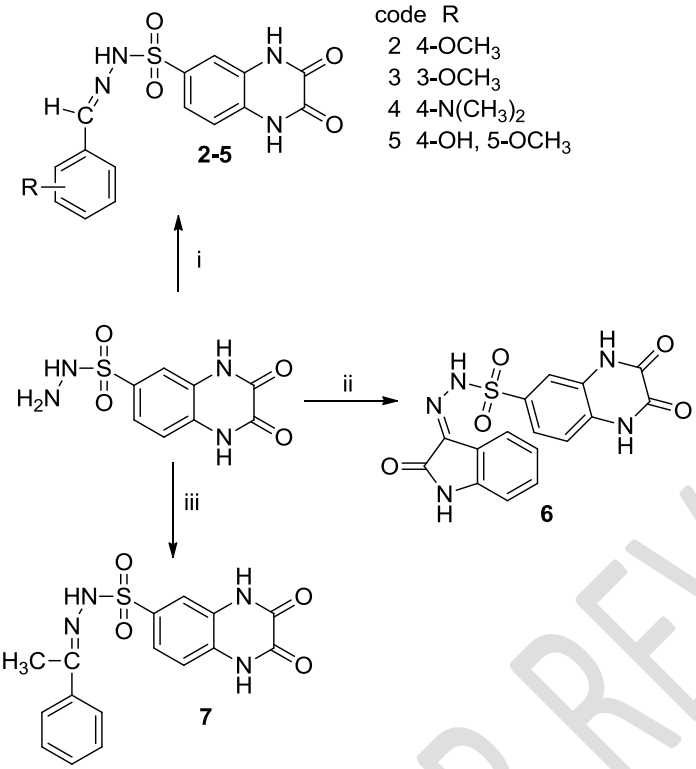
Chemistry

The quinoxaline-2,3-dione was prepared by reacting o-phenylenediarnine with oxalic acid dihydrate thermally or by microwave irradiation in acidified water. The quinoxaline-6-sulfonohydrazine was synthesize by reacting quinoxaline-6-sulfonyl chloride obtained from the reaction of quinoxaline-2,3-dione with excess of chlorosulfonic acid with hydrazine hydrate in absolute methanol(Scheme 1).



Scheme 1

The treatment of equimolar amount of **1** with some aromatic aldehydes under refluxing condition in glacial acetic acid afforded the hydrazones **2 - 5** (Scheme 2). Treatment of equimolar amount of compound **1** and isatin in glacial acetic acid led to the formation of N-(E)-(2-oxoindole-3-ylideneamino)-6-(quinoxaline-2,3-(1H,4H)-dione) sulfonamide **6.** The synthesis of N-(E)-(phenylideneamino)-6-(quinoxaline-2-(1H,4H)-dione)sulfonamide **7** was achieved by the condensation of compound **1** and equimolar amount of acetophenone in glacial acetic acid.



i. substituted benzaldehydes (**2-5**) ii). isatin(**6**) (iii).acetophenone (**7**). Reaction condition: glatial acetic acid, reflux at 120 ^{0}C.

In vitro Antimicrobial Activities of the Compounds and Standard Antibiotics.

The antimicrobial properties of all the synthesized compounds used in this study were investigated against panel of bacterial strains. These compounds at a concentration of 2 mg/ml were found to inhibit the growth of both Gram-positive and Gram-negative organisms. This shows all the compounds to possess broad spectrum activities. These synthesized compounds showed appreciable antibacterial activity against all Gram-negative organisms used for this study. Gram negative species are reported to be more resistant to inhibition by most antibacterial compounds due to their outer membrane [21, 22]. Among the Gram-negative organisms inhibited by these synthesized compounds are *Pseudomonas* species that are known to be more resistant to antimicrobial agents [23]. Such compounds that could inhibit the growth of *Pseudomonas*

species could serve as a novel antimicrobial compound to manage infections caused by these opportunistic organisms. Some of the Gram-positive bacterial strains used for this study are known to cause various infections in man. For example, *Staphylococcus aureus* are known to cause various infections in man and animal and predominates in surgical wound infections [24]. *Staphylococcus aureus* are also responsible for superficial skin infection and can as well cause some life-threatening diseases such as sepsis, respiratory and septicaemia [25]. This organism has developed resistant towards many of the antibiotics used as therapy to treat infections caused by this organism. For example, methicillin and vancomycin were adopted to treat the infections caused by *Staphylococcus aureus* and these drugs are no longer showing potency towards the treatment of infections caused by this pathogen [26, 27]. Thus, drugs formulated from these synthesized compounds could be used to manage infections caused by *Staphylococcus aureus* and other 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 in healthcare delivery.

The assay for MIC and MBC exhibited by the synthesized compounds were also investigated. The results obtained from the assay showed that the compounds exhibited 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., it has been reported that, a low MIC value of antibacterial agents indicates a better antibacterial activity [28]. This observation shows that the synthetic compounds exhibited significant antibacterial activities and thus can be used to

formulate potent antibacterial compounds that could be used to manage infections caused by pathogens that are gradually developing resistant against antimicrobials.

The significant 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. This increase in lipophilicity would help their permeability through the microbial cell wall and enhance the reaction of different functional groups present in the synthetic compounds to reacts with the cellular membrane of the bacterial cell and thus impaired both its functions and integrity [29, 30] resulting in higher activity. The synthetic compounds may be considered as the analogue of sulfonamides (a known antimicrobial) due the presence of the $R\text{-}SO_2\text{-}NHR^1$ group. Also the synthetic compounds contain quinoxaline and hydrazone (CH=N-NH-) group in their structure. These classes of compounds have been known to possess interesting antibacterial activities.

CONCLUSION

The synthesized quinoxaline sulfonamides 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.

REFERENCES

1. Michael, J. W., Ben-Hadda, T., Kchevan, A.T., Ramdani, A., Touzani, R., Elkadiri, S., Hakkou,

A., Boukka, M. and Elli, T., (2002). 2,3-bifunctionalized quinoxalines: Synthesis, DNA Interactions and Evaluation of anticancer, anti-tuberculosis and anti-fungal activity. *Molecules,* 7; 641-656.

2. Lindsley, C.W., Zhao, Z., Leister, W. H., Robinson, R. G., Barnett, S. G. and Defeo-Jones, R. E., (2005). Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors.. *Bioorganic and Medicinal Chemistry Letters,* 15; 761-764.

3. Geefhavani, M., Reddy, J. and Sathyanarayana, S., (2012). Synthesis, Antimicrobial and wound

healing activiies of diphenyl quinoxaline derivatives. *International Journal of Pharmacy and Technology,* 4(3); 4700-4710.

4. Jaso, A., Zarranz, B., Aldana, I. and Monge, A., (2003). Synthesis of new 2-acetyl and 2-benzoyl

quinoxaline-1,4-di-N-oxide derivatives as anti-mycobacterium tuberculosis agents. *European Journal of Medicinal Chemistry,* 39; 791-800.

5. Badran, M., Abonzid, K. and Hussein, M., (2003). Synthesis of certain substituted quinoxalines as antimicrobial agents. *Part ii. Archieves of Pharmarcy Reserves,* 26; 107-113.

6. Hearn, M. and Cynamon, M., (2004). Design and synthesis of anti-tuberculars: preparation and

evaluation against Mycobacterium tuberculosis of an isoniazid schiff base. *Journal of Antimicrobial Chemotherapy,* 55; 185-191.

7. Taiwo, F., Akinpelu, D. and Obafemi, C., (2008). Synthesis and antibacterial activity of some quinoxaline derivatives. *Ife Journal of Science,* 10 (1); 19-25.

8. Kaurase, S., Wadher, N. and Yeole, P., (2011). Microwave assisted Synthesis of hydrazone derivatives of quinoxalinone and evaluation of their antimicrobial activity. *International Journal of Universal Pharmacy and Life Sciences,* 1 (2); 117-126.

9. Aswartha U, M., Sreeramulu, J. and Puna, S., (2012). Synthesis and antimicrobial activity of a novel series of quinoxaline-2,3-dione derivatives. *International Journal of Advances in Pharmaceutical Research,* (7); 1010 - 1020.

10. Achutha, L., Parameshwar, R., Madhava Reddy, B. and Babu, H., (2013). Microwave-assisted

synthesis of some quinoxaline-incoporated schiff bases and their biological evaluation. *Journal of Chemistry,* 578438; 1-5.

11. Wagle, S., Adhikari, A. and Kumari, N., (2008). Synthesis of some new 2-(3-methyl-7-substituted-2-oxoquinoxalinyl)-5-(aryl)-1,34-oxadiazoles as potential non-steroidal anti-inflammatory and anagesic agents.. *Indian Journal of Chemistry,* 47; 439-448.

12. Rajitha, G., Saideepa, N. and Praneetha, P., (2011). Synthesis and evaluation of N-(x-benzamido cinnamoyl)-aryl hydrazone derivatives for anti-inflammatory and antioxidant activities. *Indian Journal of Chemistry and Biology,* 50; 729-733.

13. Sato, S., Shiratori, O. and Katagiri, K., (1967). The mode of action of quinoxaline antibiotics. Interaction of quinomycin a with deoxyribonucleic acid.. *Journal of Antibiotics,* 20, 270 - 277.

14. Dell, A., William, D. H., Morris, H. R., Smith, G. A., Feeney, J. and Robert, G. C, K, (1975). Structure revision of the antibiotic echinomycin. *Journal of American Chemical Society,* Volume 97; 2497- 2501.

15. Bailly, C., Echepare, S. Gago, F., and Waring, M., (1999). Recorgnition elements that determine affinity and sequence-specific binding DNA of 2QN a biosynthetic bis quinoline analogue of echinimycin. *Anti-Cancer Drug Descriptions,* 15; 291.

16. Deepika, Y. and Nath P.S, (2012). Design, Synthesis of Novel quinoxaline derivatives and their antinoceptive activity. *Asian Journal of Pharmaceutical and Health Sciences*, 2(1); 261-264.

17. Taiwo, F. O and Craig A. Obafemi (2016), Design, green synthesis and reactions of 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-Sulfono- hydrazide derivatives. International Journal of Physical Sciences (In press)

18. Akinpelu, D. A. and and Kolawole, D. O., (2004). Phytochemical and antimicrobial activity of

leave extract of Piliostigma thonniggii(Shum.). *Science Focus,* 7; 64-70.

19. Akinpelu D. A., Odewade J. O., Aiyegoro O. A., Ashafa A. O. T., Akinpelu O. F. and Agunbiade M. O. (2016). Biocidal effects of stem bark extract of *Chrysophyllum albidium* G.

Don. On vancomycin-resistant *Staphylococcus aureus. BMC Complementary and Aternative Medicine* 16: 105-113.

20. Oludare, E. E., Emudianugbe, T. S., Khaar, G. S., Kuteyi, S. A., Irobi, D. N., (1992). Antibacterial Properties of Leaf Extract of Cassia alata.. *Biology Reserves Communications,* 4; 1137-1142.

21. Odenholt, I., Owdi, E. and Cars, O., (2001). Pharmacodynamics of telithromycin in vitro against respiratory tract pathogens. *Antimicrobial Agents Chemotherapy,* 45; 23-29.

22. Longbottom C. J., Carson C. F., Hammer K. A., Mee B. J., and Riley T. V., (2004). Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. *Journal of Antimicrobial Chemotherapy.* 54, 386-92.

23. Nikaido, H., (1996). Outer membrane. In: Neidhardt FC, editors. *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*.. Washington: American Society for Microbiology.

24. Pelczar, M. J, Chan, E. C and Kruz, N. R (2006). *Microbiology*, 5th Edt. Teta, McGraw-Hill Publishing Company Ltd., New Delhi. p. 119- 123.

25. Prescott L. M., Harley J. P. and Klein D. A. (2002). Microbiology 5th Edition, McGraw-Hill Inc.

26. Livermore, D. M and Brown, J. D., (2001). Detection of Beta-lactmase-mediated resistance. Journal of Antimicrobial Chemotherapy. 4; 59-64

27. Lowry, F.D., (1998). Staphylococcus aureus infections. *New England Journal of medicine*, 339(8) 520-532.

28. Achinto, S. and Munirudin, A., (2009). The Analgestic and anti-inflammatory activities of the

extract of Albizia lebbeck in animal model. *Pakistan Journal of Pharmaceutical Sciences*, 22; 74-77.

29. Raccach, M., (1984). The Antimicrobial Activity of Phenolic Antioxidants in Foods. *Journal of Food Safety*. 6 (3); 141–170

30. Blaszyk, M., Holley, R. A., (1998). Interaction of monolaurin, eugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms. *International Journal of Food Microbiology.* 39; 175-183

Table 1: Physical properties of the 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones 1′-13′.

s/n	Molecular formula/ mass	reaction time(hr)	yield (%)	Colour	melting point (^{O}C)
1	$C_8H_8N_4O_4S$ (256.24)	6	90	White	> 330
2	$C_{15}H_{12}N_4O_5S$(360.34)	3	65	Yellow	249-250
3	$C_{15}H_{11}ClN_4O_4S$(378.79)	3	73	Yellow	241-243
4	$C_{15}H_{11}N_5O_6S$(389.34)	3	80	Yellow	250(decomposed)
5	$C_{15}H_{12}N_4O_4S$(344.35)	3	57	Yellow	274-276
6	$C_{16}H_{14}N_4O_5S$(374.37)	3	58	Yellow	240-242
7	$C_{16}H_{14}N_4O_5S$(374.37)	3	72	Yellow	262-263
8	$C_{15}H_{12}N_4O_5S$(360.34)	3	43	Yellow	238-240
9	$C_{15}H_{12}N_4O_5S$(360.34)	3	56	Yellow	>310

10	$C_{17}H_{17}N_5O_4S$(387.41)	3	70	Purple	270-272
11	$C_{16}H_{14}N_4O_6S$(390.37)	3	80	Yellow	233(decomposed)
12	$C_{16}H_{11}N_5O_5S$(385.35)	3	88	Red	273-274
13	$C_{16}H_{14}N_4O_4S$(358.37)	3	63	Yellow	290-292

Table 2: The Sensitivity Patterns Exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones (2-7) 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)**	**7 (2 mg/mL)**	**Strep (1 mg/mL)**	**Tet (1 mg/mL)**
Bacillus polymyxa (LIO)	22±0.29	18±0.29	22±0.29	24±1.00	20±1.00	24±1.00	15±0.50	20±0.56
B. cereus (NCIB 6349)	22±1.00	24±0.50	20±1.00	22±0.50	18±0.50	20±1.00	28±0.56	18±1.00

Corynebacterium pyogenes (LIO)	18±0.50	22±1.00	22±0.29	18±1.00	18±1.00	18±1.00	20±0.56	20±1.00
Clostridium sporogenes (NCIB 532)	20±0.29	22±0.50	22±1.00	18±0.50	18±1.00	24±0.50	25±0.56	20±1.00
B. stearotherphilus (NCIB 8222)	20±0.50	22±1.00	18±0.29	24±1.00	24±1.00	24±1.00	23±0.56	22±1.00
Streptococcus pneumoniae (LIO)	13±0.50	25±0.29	22±1.00	21±1.00	20±0.50	21±1.00	24±0.56	24±0.56
Streptococcus pneumoniae (PS)	21±1.00	25±1.00	24±1.00	19±1.00	26±1.00	20±1.00	26±0.50	15±0.56
B. subtilis (NCIB 3610)	22±1.00	20±0.50	22±0.29	20±1.00	24±1.00	20±0.56	20±1.00	22±0.56
Staphylococcus aureus (NCIB 8588)	20±0.58	20±1.00	18±1.00	19±0.50	10±0.50	14±0.56	20±1.00	15±0.56
Staphylococcus aureus (SW)	22±1.00	22±0.29	20±0.50	22±1.00	16±1.00	18±0.56	21±0.56	17±0.56
Enterococcus feacalis (NCIB 775)	18±0.29	18±0.56	16±0.29	18±0.29	30±1.00	18±0.56	23±0.56	28±0.50
Micrococcus luteus (NCIB 196)	22±1.00	22±0.50	18±0.29	20±1.00	16±1.00	20±0.50	21±0.50	22±1.00
Bacillus anthracis (LIO)	18±0.29	24±1.00	22±1.00	22±1.00	20±0.50	20±1.00	22±1.00	25±1.00
Escherichia coli (NCIB 86)	26±1.00	28±0.85	28±1.00	28±1.00	28±1.15	28±0.85	0±0.00	18±1.15
Citrobacter freundii (PS)	23±1.00	20±0.85	25±1.00	27±1.00	25±1.15	20±0.85	18±1.00	0±0.00
Pseudomonas fluorescence (NCIB 3756)	22±0.85	24±0.58	26±1.00	24±1.00	24±0.85	20±1.15	30±1.15	0±0.00
Klebsiella pneumoniae (418)	26±0.85	26±0.58	28±1.00	26±1.00	22±1.15	26±0.85	0±0.00	12±0.85
Pseudomonas aeruginosa (NCIB 950)	25±0.85	28±1.00	23±1.15	29±1.00	23±1.15	20±1.00	25±0.85	12±1.00
Pseudomonas aeruginosa (PS)	29±1.00	23±1.00	24±1.00	24±1.00	15±1.15	16±1.00	20±1.00	15±1.00
Pseudomonas aeruginosa (PS)	25±0.85	24±1.00	27±1.00	29±1.00	22±1.15	20±1.00	10±1.00	16±0.85
Pseudomonas aeruginosa (PS)	23±0.85	27±1.00	27±0.58	28±1.00	23±1.00	20±1.15	17±1.00	12±1.00

Table 2(contd.) The Sensitivity Patterns Exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones (2-7) Against Bacterial Strains (Continued)

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)**	**7 (2 mg/mL)**	**Strep (1 mg/mL)**	**Tet (1 mg/mL)**
Shigella species (LIO)	22±0.85	24±0.85	27±0.58	20±0.85	24±0.85	23±0.85	22±0.85	0±0.00
Proteus vulgaris (NCIB 67)	22±0.85	24±1.00	18±0.58	24±0.85	20±1.15	24±1.15	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 3: The Minimum Inhibitory Concentrations (MIC) exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones (2-7) against Susceptible Bacterial Strains

Bacterial Strains	Compounds (mg/mL)							
	2	**3**	**4**	**5**	**6**	**7**	**Strep**	**Tet**
Bacillus polymyxa (LIO)	0.0625	0.125	0.125	0.125	0.125	0.125	0.125	0.0625

B. cereus (NCIB 6349)	0.0625	0.0625	0.125	0.0625	0.125	0.125	0.0313	0.250
Corynebacterium pyogenes (LIO)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0313	0.0313
Clostridium sporogenes (NCIB 532)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0078	0.0313
B. stearotherphilus (NCIB 8222)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0625	0.125
Streptococcus pneumoniae (LIO)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0625	0.125
Streptococcus pneumoniae (PS)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0625	0.125
B. subtilis (NCIB 3610)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0625	0.250
Staphylococcus aureus (NCIB 8588)	0.0625	0.125	0.125	0.125	0.125	0.125	0.500	0.0313
Staphylococcus aureus (SW)	0.0625	0.125	0.125	0.125	0.125	0.125	0.0625	0.125
Enterococcus feacalis (NCIB 775)	0.0625	0.0625	0.0625	0.125	0.0625	0.125	0.0625	0.250
Micrococcus luteus (NCIB 196)	0.0313	0.125	0.125	0.125	0.125	0.0625	0.0625	0.250
Bacillus anthracis (LIO)	0.0625	0.125	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	0.125	ND	0.0313
Citrobacter freundii (PS)	0.0625	0.125	0.0625	0.125	0.0625	0.125	ND	0.0313
Pseudomonas fluorescence (NCIB 3756)	0.0625	0.125	0.125	0.250	0.125	0.125	0.250	ND
Klebsiella pneumoniae (418)	0.0625	0.125	0.0625	0.0625	0.0625	0.125	ND	0.50
Pseudomonas aeruginosa (NCIB 950)	0.0625	0.125	0.125	0.250	0.125	0.125	0.250	ND
Pseudomonas aeruginosa (PS)	0.0625	0.125	0.0625	0.0625	0.0625	0.125	0.250	0.50

Table 3 (contd.) The Minimum Inhibitory Concentrations (MIC) exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones (2-7) against Susceptible Bacterial Strains (Continued)

Bacterial Strains	**Compounds (mg/mL)**							
	2	**3**	**4**	**5**	**6**	**7**	**Strep**	**Tet**
Pseudomonas aeruginosa (PS)	0.0625	0.125	0.125	0.250	0.125	0.125	0.250	ND

Pseudomonas aeruginosa (PS)	0.0625	0.125	0.0625	0.0625	0.0625	0.125	0.250	0.50
Shigella species (LIO)	0.0625	0.125	0.125	0.250	0.125	0.125	0.250	ND
Proteus vulgaris (NCIB 67)	0.0625	0.125	0.125	0.125	0.125	0.125	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 4: The Minimum Bactericidal Concentrations Exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones 2-7 (2 mg/ml) against Susceptible Bacterial Strains

Bacterial Strains	**Compounds (mg/mL)**							
	2	**3**	**4**	**5**	**6**	**7**	**Strep**	**Tet**
Bacillus polymyxa (LIO)	0.125	0.250	0.250	0.250	0.250	0.250	0.250	0.125

B. cereus (NCIB 6349)	0.125	0.125	0.250	0.125	0.250	0.250	0.0625	0.250
Corynebacterium pyogenes (LIO)	0.125	0.250	0.250	0.250	0.250	0.250	0.0625	0.0625
Clostridium sporogenes (NCIB 532)	0.125	0.250	0.250	0.250	0.250	0.250	0.0313	0.0625
B. stearotherphilus (NCIB 8222)	0.125	0.250	0.250	0.250	0.250	0.250	0.125	0.250
Streptococcus pneumoniae (LIO)	0.125	0.250	0.250	0.250	0.250	0.250	0.125	0.250
Streptococcus pneumoniae (PS)	0.125	0.250	0.250	0.250	0.250	0.250	0.125	0.250
B. subtilis (NCIB 3610)	0.125	0.250	0.250	0.250	0.250	0.250	0.125	0.500
Staphylococcus aureus (NCIB 8588)	0.125	0.250	0.250	0.250	0.250	0.250	0.500	0.0313
Staphylococcus aureus (SW)	0.125	0.250	0.250	0.250	0.250	0.250	0.125	0.125
Enterococcus feacalis (NCIB 775)	0.125	0.125	0.125	0.250	0.125	0.250	0.125	0.250
Micrococcus luteus (NCIB 196)	0.0625	0.250	0.250	0.250	0.250	0.125	0.125	0.250
Bacillus anthracis (LIO)	0.125	0.250	0.250	0.250	0.250	0.250	0.500	0.500
Escherichia coli (NCIB 86)	0.125	0.250	0.125	0.250	0.125	0.250	ND	0.0625
Citrobacter freundii (PS)	0.125	0.250	0.125	0.250	0.125	0.250	ND	0.0625
Pseudomonas fluorescence (NCIB 3756)	0.125	0.250	0.250	0.500	0.250	0.250	0.500	ND
Klebsiella pneumoniae (418)	0.125	0.250	0.125	0.125	0.125	0.250	ND	1.00
Pseudomonas aeruginosa (NCIB 950)	0.125	0.250	0.250	0.500	0.250	0.250	0.500	ND
Pseudomonas aeruginosa (PS)	0.125	0.250	0.125	0.125	0.125	0.250	0.500	1.00

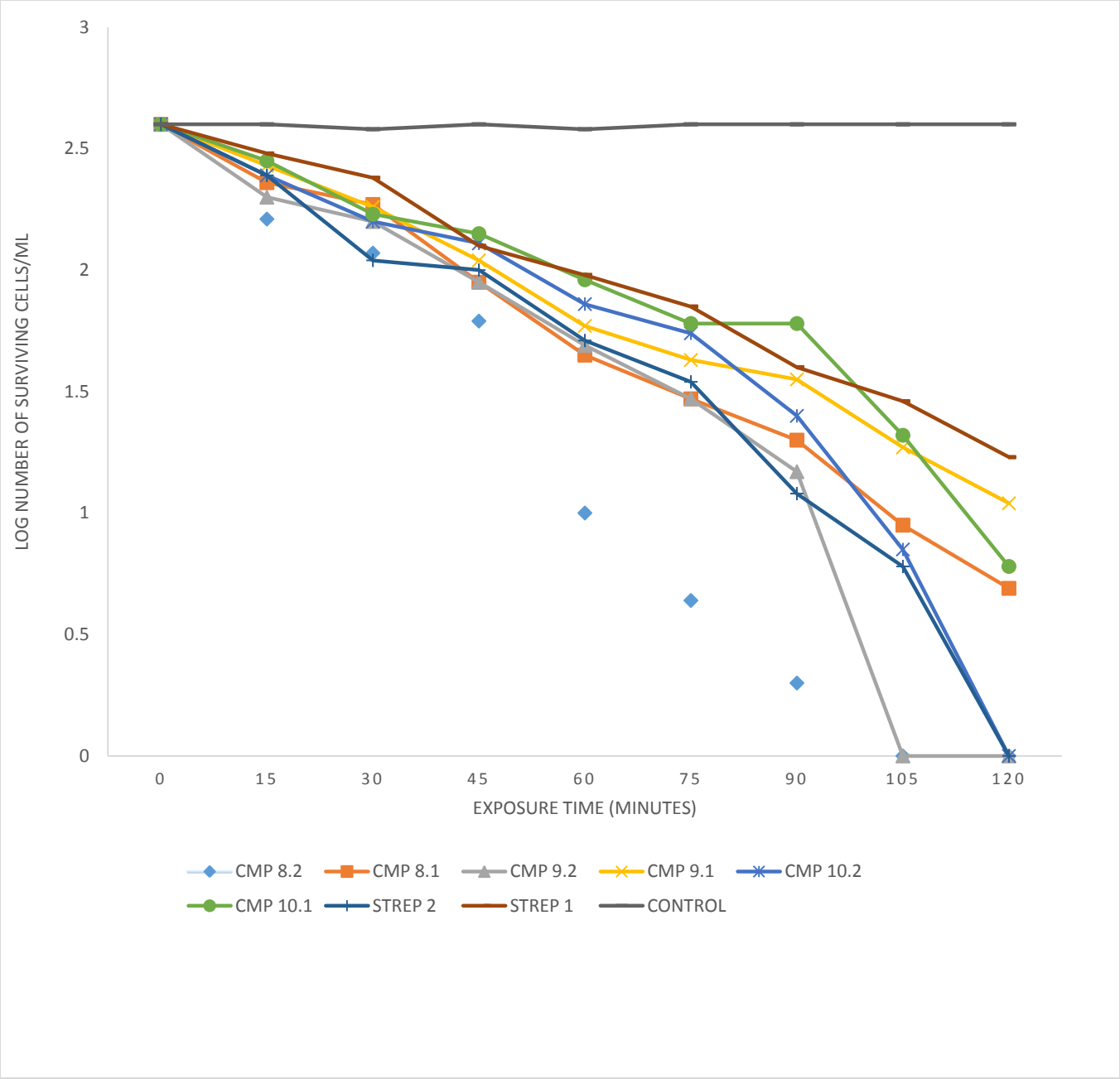
Table 4(contd.) The Minimum Bactericidal Concentrations Exhibited by 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl hydrazones 2-7 (2 mg/ml) against Susceptible Bacterial Strains (Continued)

Bacterial Strains	**Compounds (mg/mL)**							
	2	**3**	**4**	**5**	**6**	**7**	**Strep**	**Tet**

Pseudomonas aeruginosa (PS)	0.125	0.250	0.250	0.500	0.250	0.250	0.500	ND
Pseudomonas aeruginosa (PS)	0.125	0.250	0.250	0.125	0.125	0.250	0.500	0.50
Shigella species (LIO)	0.125	0.250	0.250	0.50	0.250	0.250	0.500	ND
Proteus vulgaris (NCIB 67)	0.125	0.250	0.250	0.250	0.250	0.250	0.500	1.00

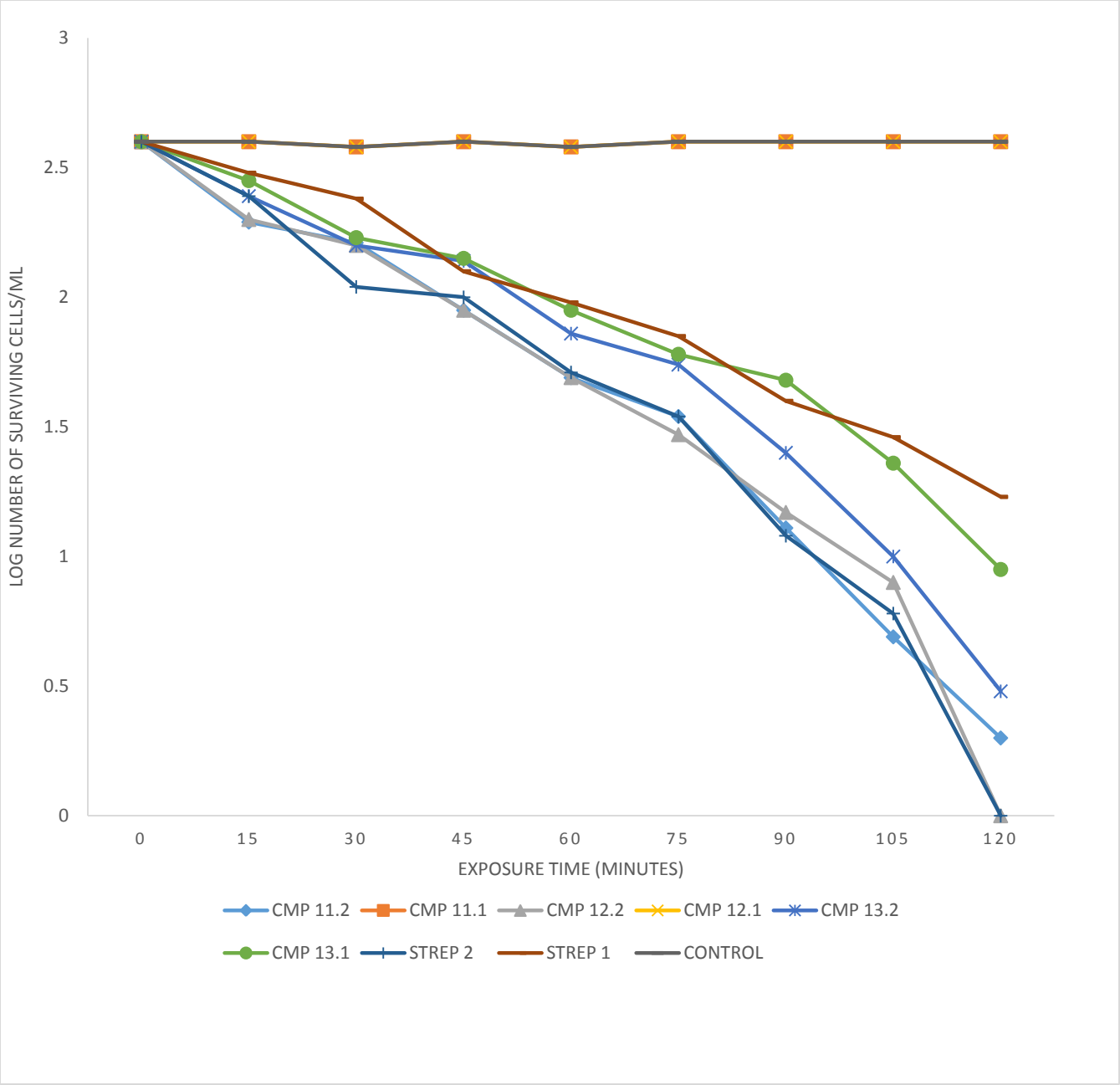
Key: NCIB = National Collection of Industrial Bacterial
LIO = Locally Isolated Organisms
PS = Pus Sample isolate
SW = Surgical wound isolate
Strep = Streptomycin
Tet = Tetracycline
ND = Not Done

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



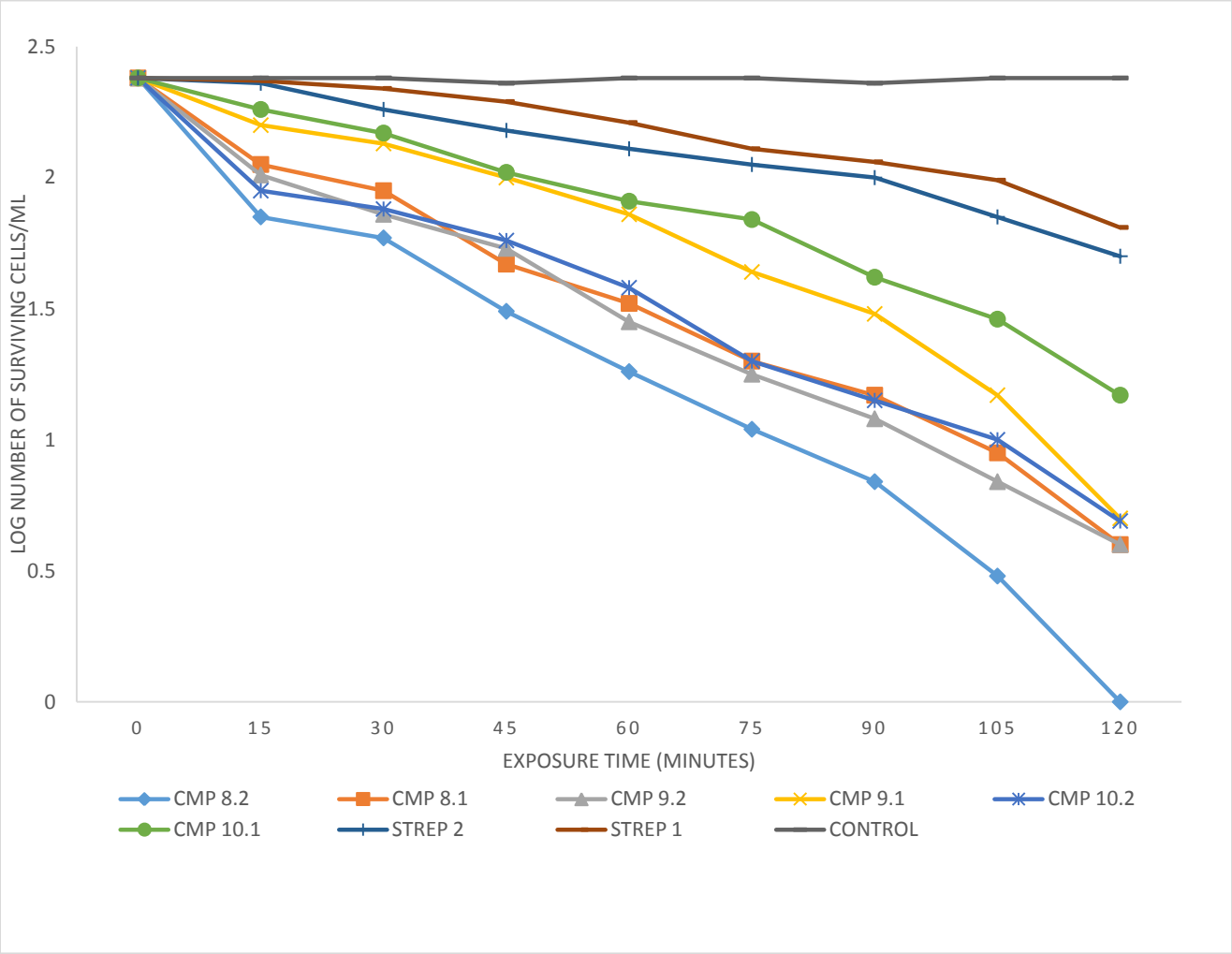
CMP 8.2 = Log of number of surviving cells for compound 2 at 2XMIC
CMP 8.1 = Log of number of surviving cells for compound 2 at 1XMIC
CMP 9.2 = Log of number of surviving cells for compound 3 at 2XMIC
CMP 9.1 = Log of number of surviving cells for compound 3 at 1XMIC
CMP 10.2 = Log of number of surviving cells for compound 4 at 2XMIC
CMP 10.1 = Log of number of surviving cells for compound 4 at 1XMIC

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



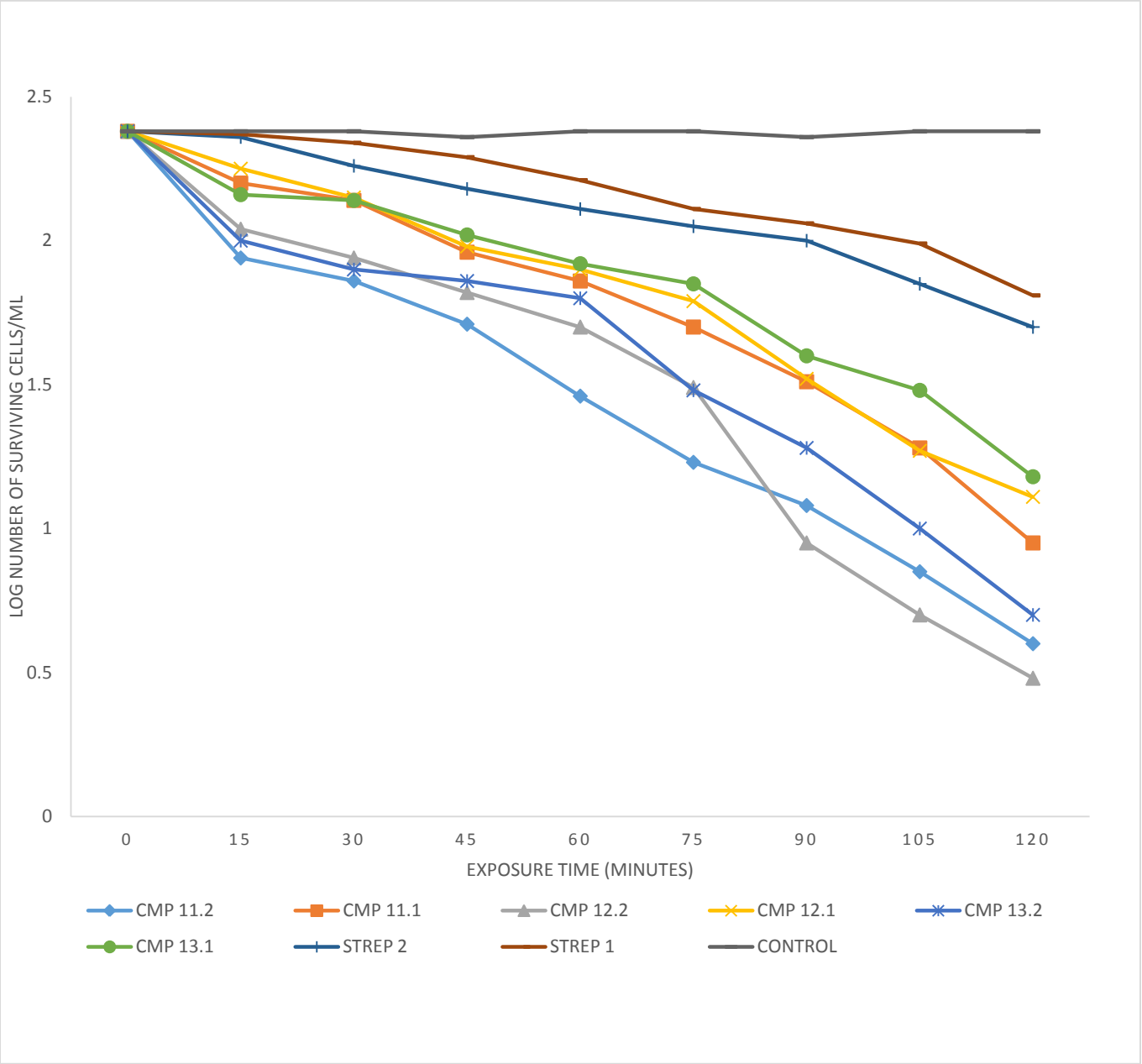
CMP 11.2 = Log of number of surviving cells for compound 5 at 2XMIC
CMP 11.1 = Log of number of surviving cells for compound 5 at 1XMIC
CMP 12.2 = Log of number of surviving cells for compound 6 at 2XMIC
CMP 12.1 = Log of number of surviving cells for compound 6 at 1XMIC
CMP 13.2 = Log of number of surviving cells for compound 7 at 2XMIC
CMP 13.1 = Log of number of surviving cells for compound 7 at 1XMIC

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



CMP 8.2 = Log of number of surviving cells for compound 2 at 2XMIC
CMP 8.1 = Log of number of surviving cells for compound 2 at 1XMIC
CMP 9.2 = Log of number of surviving cells for compound 3 at 2XMIC
CMP 9.1 = Log of number of surviving cells for compound 3 at 1XMIC
CMP 10.2 = Log of number of surviving cells for compound 4 at 2XMIC
CMP 10.1 = Log of number of surviving cells for compound 4 at 1XMIC

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



CMP 11.2 = Log of number of surviving cells for compound 5 at 2XMIC
CMP 11.1 = Log of number of surviving cells for compound 5 at 1XMIC
CMP 12.2 = Log of number of surviving cells for compound 6 at 2XMIC
CMP 12.1 = Log of number of surviving cells for compound 6 at 1XMIC
CMP 13.2 = Log of number of surviving cells for compound 7 at 2XMIC
CMP 13.1 = Log of number of surviving cells for compound 7 at 1XMIC