SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME QUINOXALINONE DERIVATIVES

F.O. TAIWO[1], D.A. AKINPELU[2] and C.A. OBAFEMI[1,+]

1. Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.
2. Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

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Abstract

Some quinoxaline derivatives **1-8** were synthesized and screened in vitro for their growth inhibitory activity against nine strains of Gram-positive and four strains of Gram-negative bacteria. Some of the compounds exhibited broad spectrum (in vitro) activity against the bacterial strains.

One of the compounds, 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl azide (**6**), exhibited the highest activity with an MIC value of 7.8 ìgmL^{-1} against four Gram-positive bacterial strains.

Key words: Quinoxalinones, antibacterial, Gram-positive, Gram-negative.

1. Introduction

Quinoxalines (easily prepared from an aromatic-1,2-diamine and 1,2-dicarbonyl derivatives under different reaction conditions) have been reported to show various biological properties. The quinoxaline ring system has been described as bioisoster of aromatic rings, including quinoline, naphthalene, benzothiophene, and others (Lima *et al.*, 2005).

Many of the quinoxaline derivatives have been shown to posses antibacterial (El-Gendy *et al.*, 1995; Badran *et al.*, 2003, Refaat *et al.*, 2004), antifungal (Loriga *et al.*, 1997), anticancer (Loriga *et al.*, 1997; El-Hawash *et al.*, 2006), antidepressant (Sarges *et al.*, 1990), anti-tubercular (Waring *et al.*, 2002), antidiabetic (Bahekar *et al.*, 2007), anti-HIV (Loriga *et al.*, 1997; El-Hawash *et al.*, 2006) and anti-malarial (Zarranz *et al.*, 2005) activities.

Of particular interest are the mono- and di-N-oxides of various quinoxalines which have been shown to exhibit wider range of biological properties, including antibacterial activity (Badran *et al.*, 2003; Takatake *et al.*,1996), anticancer and hypoxia-selctive cytotoxins agents (Amin *et al.*, 2006), antimycobacterial and protozoal activities(Villar *et al.*, 2008; Zarranz *et al.*, 2006).

We have recently reported the synthesis, antimicrobial and neuropharmacological activities of some quinoxalinone derivatives (Obafemi *et al.*, 2005; Olayiwola *et al.*, 2007). In continuation of our studies on the quinoxaline system, we have evaluated eight simple 2-quinoxalinones and 2,3-quinoxalinediones for their antibacterial property.

2. Experimental

Chemistry

Melting points were determined in open capillary tubes on a Gallenkamp (variable heater) melting point apparatus and are uncorrected. Infrared spectra were recorded (in KBr) on a Buck Scientific Spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker 400 MHz spectrometer at the Chemistry Department, University of Botswana, Botswana and on a Varian 200 MHz spectrometer at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife and mass spectra of the compounds were obtained using Finnigan MAT 312 machine. The purity of the synthesized compounds was checked by thin layer chromatography on silica gel plate, using $CHCl_3$: CH_3OH (9:1, v/v).

1,2,3,4-Tetrahydroquinoxaline-2, 3-dione **(1)**

A powdered mixture of oxalic acid dihydrate (5.0 g, 39.7 mmol) and 1, 2-diaminobenzene (4.3 g, 39.8 mmol) was put in an open beaker and 1 ml of water added and mixed thoroughly. The mixture was irradiated in a domestic microwave (MW) oven at an emitted power of 400 W for 3 min. 100 ml of water was added, followed by further irradiation for 1 min. to give a clear solution and then left to stand at room temperature to afford colorless needles of **1** (6.4 g, 99 %). mp > 340 °C.

+ *corresponding author* (email: adeyemi01@yahoo.com)

6-Chloro-1, 4-dihydroquinoxaline-2,3-dione **(2)**

A powdered mixture of oxalic acid dihydrate (5.0 g, 39.7 mmol) and 4-chloro-1,2-diaminobenzene (5.62 g, 39.7 mmol) was put in an open beaker and 1 ml of water added and mixed thoroughly. The mixture was irradiated in a domestic microwave (MW) oven at an emitted power of 400 W for 3 min. 100 ml of water was added, followed by further irradiation for 1 min. to give a clear solution and then left to stand at room temperature to afford **2** as ash-gray colored crystals. mp > 320 °C, (5.6 g, 98 %). IR: 3145(NH), 3046(NH), 1695(C=O), 1390; ^{1}H NMR (DMSO-d_6): 7.08 (s, 3H, ArH), 11.95 (s, br, 2H, 2NH, exchangeable with D_2O). MS: in m/z (rel. %): 196 (90), 168 (69), 140 (18), 113 (20), 105 (100), 78 (28).

6-Methyl-1, 4-dihydroquinoxaline-2, 3-dione **(3)**

A mixture of oxalic acid dihydrate (5.0 g, 39.7 mmol) and 4-methyl-1,2-diaminobenzene(4.80 g, 39.7 mmol) was put in an open beaker and 1ml of water added and mixed thoroughly. The mixture was irradiated in a domestic microwave (MW) oven at an emitted power of 400 W for 3 min. 100 ml of water was added, followed by further irradiation for 1min to give a clear solution and then left to stand at room temperature to afford **3** as white crystals mp > 300 ÚC, (6.07 g, 98 %). IR: 3180 (NH), 2985, 1695 (C=O). ^{1}H NMR (DMSO-d_6): 2.26 (s, 3H, CH_3), 6.89 (d, 1H, ArH), 6.94 (s, 1H, ArH), 7.03 (d, 1H, ArH), 11.88 (s, 1H, NH exchangeable with D_2O), 11.92 (s, 1H, NH, D_2O exchangeable). MS: in m/z (rel. %): 176 (100), 148 (72), 120 (50), 105 (47), 93 (47), 77 (46).

6-Nitro-1, 4- dihydroquinoxaline-2, 3-dione **(4)**

A mixture of oxalic acid dihydrate (5.0 g, 39.7 mmol) and 4-nitro-1, 2-diaminobenzene (6.03 g, 39.7 mmol) was put in an open beaker and 1ml of water added and mixed thoroughly. The mixture was irradiated in a microwave (MW) oven at an emitted power of 400 W for 3 min. 100 ml of water was added, followed by further irradiation for 1 min. to give a clear solution and then left to stand at room temperature to give **4** as dark-brown crystals mp > 320 °C (6.5 g, 97 %). IR: 3442 (NH), 3050 (NH), 1690 (C=O), 1535, 1330. ^{1}H NMR (DMSO-d_6): 7.15 (d, 1H, C (8)-H), 7.82 – 7.89 (m, 2H, ArH), 12.15 (s, 1H, NH D_2O exchangeable), 12.36 (s, 1H, NH, D_2O exchangeable).

^{13}C NMR: 155.0 (C = O), 154.6 (C = O), 141.9, 131.4, 125.8, 118.4, 115.3, 110.2 MS: 207 (M^+). Anal. Calc. for $C_8H_5N_3O_4$ (207.1): C 46.39, H 2.43, and N 20.29; found: C 46.21, H 2.50, and N 20.13.

2, 3-Dioxo-1, 2, 3, 4-tetrahydroquinoxaline-6-sulfonyl chloride **(5a)**

Pure and dry **1'** (5.0 g, 30.9 mmol) was added in small portions to chlorosulfonic acid (21 ml, 10 mmol equiv.) at room temperature, after which the resulting mixture was heated at 110 °C for 8 h. The reaction mixture was cooled in ice and then poured into crushed ice to give white solid. The product was filtered and washed three times with cold water and dried. The solid was recrystallised from dry toluene-acetone mixture to give white crystals of 2, 3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride **5a** mp 330 °C (dec), (6.6 g, 88 %). IR: 3380(NH), 1680(C=O), 1355, 1140. MS: in m/z (rel. %): 262 (5.3), 260 (14.0), 225 (48.1), 161 (100), 133 (45.4), 105 (70.5), 78 (45.2), 51 (82.3). Anal. Calc. for $C_8H_5ClN_2O_4S$ (260.55): C 36.86, H 1.93, and N 10.75; found: C 36.59, H 2.01, and N 10.90.

N, N-dibenzyl-2, 3-dioxo-1, 2, 3, 4-tetra hydroquinoxaline-6-sulfonamide **(5)**

2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride (10.0 g, 38.4 mmol) was dissolved in dry dimethylformamide (DMF) (200 ml), followed by addition of dibenzylamine (15 ml, 78 mmol) and the resulting mixture was kept under stirring at room temperature for 10 h. The reaction mixture was then poured into water (500 ml) to give a foamy white precipitate. Recrystallization from aqueous ethanol gave white crystals of **5** (12.1 g, 75 %) (mp > 300 °C). IR (cm^{-1}). 3330, 1682, 1601, 1350, 1175.

2,3-Dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl azide **(6)**

2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride **5'** (2.0 g, 7.8 mmol) was dissolved in acetone (50 ml) and sodium azide (1.0 g, 15.4 mmol) in minimum amount of water was added in drops with continuous stirring. The mixture was stirred at room temperature for 8 h. Acetone was removed under reduced pressure followed by addition of water to give crude crystals of **6.** Recrystallization from ethanol gave pure crystals of **6** (mp > 330 °C (dec.), 92 %. IR (cm^{-1}): 3320, 2150, 1690, 1360, 1165. ^{1}H NMR (DMSO-d_6). 7.95 – 7.19 (m, 3H, ArH), 12.74 (s, 1H, NH, D_2O exchangeable), 12.92 (s, 1H, NH, D_2O exchangeable). MS: in m/z (rel. %): 267 (100), 239 (17.0), 225 (18.3), 211 (6.0), 161 (10.0), 133 (5.0), 105 (22.1). Anal. Calc. for $C_8H_5N_5O_4S$ (267.22): C 35.96, H 1.89, N 26.21 found: C 35.65, H 1.91, N 26.13.

1-acetyl-1H-indole-2, 3-Dione **(7a)**

Isatin (5.0 g, 34 mmol) was added to acetic anhydride (80 ml) and the mixture heated with continuous stirring at 90-100 °C for 3 h. The reaction mixture was allowed to cool and then left in a fridge to give fine yellow crystals of 1-acetylisatin, **7a** The product was filtered and the solvent was reduced to half to get more of the product, mp 143 – 144 °C (5.3 g, 82 %). IR (cm^{-1}), 3300 (NH), 2910, 1697 (C=O), 1365, 1120.

N [2-(3-oxo-3, 4-dihydroquinoxalin-2-yl) phenyl] acetamide **(7)**

1-acetylisatin **7a** 5.0 g, 26.4 mmol) was dissolved in ethanol (50 ml) in an open beaker. The solution was then irradiated (pulsed) in a microwave (MW) oven

for 2 min. Ortho-phenylenediamine (2.9 g, 26.8 mmol) in ethanol (30 ml) was added and the resulting mixture again irradiation in a MW oven (400 W) for 2 min (at 30 s intervals) and the solution allowed to cool at room temperature, to give white crystals of **7**, mp 291-292 °C, (6.9 g, 93 %). IR (cm^{-1}): 3260, 1675, 1642, 1588, 1545, 1320. ^1H NMR (DMSO-d_6): 1.95 (s, 3H, CH_3), 7.10 – 7.83 (m, 8H, Ar-H), 9.78 (s, 1H, NH, D_2O exchangeable), 12.50 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR 168.7 (C = 0), 157.6 (C = 0), 155, 137.5 , 133.0 , 132.7 , 131.4 , 130.9 130.2 , 129.2 , 128.4 , 123.9 , 123.7 , 115.8 , 24.4.

3-(2-aminophenyl) quinoxalin-2(1H)-one **(8)**

Compound **7** (5.0 g, 17.9 mmol) was dissolved in 50 % aqueous ethanol (50 ml) and KOH (5.0 g) was added and the reaction mixture was heated to reflux with stirring for 4 h. The resulting solution was concentrated, using a rotatory evaporator under vacuum, to half its volume and then acidified with acetic acid. The solution obtained was left to stand to give yellow crystals of **8** 3.1 g, 72.87 % (mp 258–260 °C) IR (cm^{-1}): 3400 (NH), 3256 (NH) 1698 (C=O) 1385, 1194.

Antibacterial Activity

Microorganisms

The following standard bacteria of National Collection for Industrial Bacteria (NCIB) and Locally Isolated Organisms (LIO) used in this research work were obtained from the culture of Dr. D.A Akinpelu of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.

Bacillus cereous(NCIB 6349), *Bacillus polymyxa* (LIO), *Bacillus stearothermophilus* (NCIB 8222), *Clostridium sporogenes* (LIO), *Bacillus substilis* (NCIB3610), *Corynebacterium pyogenes* (LIO, *Escherichia coli* (NCIB86), *Klebsiella pneumonias* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescence* (NCIB 3756), *Staphylococcus faecalis* (NCIB 775), *Staphylococcus aureus* (NCIB 8588).

Antibacterial sensitivity testing of the Compounds **1-8**

All the synthesized compounds were screened for antibacterial activity using the agar well diffusion method as described by Akinpelu (1999). The medium employed was diagnostic sensitivity test agar (Biotech .Ltd)

With the aid of a sterile 1 ml pipette, about 0.2 ml of the broth culture of test organism was added to 18 ml sterile molten diagonist sensitivity test agar (Biotech Ltd) which had already cooled down to 45 °C. This was well mixed and poured into previously sterilized Petri dishes, which have been properly labeled according to the test organism. 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 of about 5 mm to the edge of the plate. The wells were filled up aseptically with the solution of the compound using Pasteur pipettes. Streptomycin phosphate was 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 as then incubated uprightly at

37 °C for 24 hours. Care was taken not to stockpile the plates. Clear zones of inhibition indicated the relative susceptivity of the bacteria to the compounds. These were recorded in millimeters.

Determination of Minimum Inhibition Concentration (MIC)

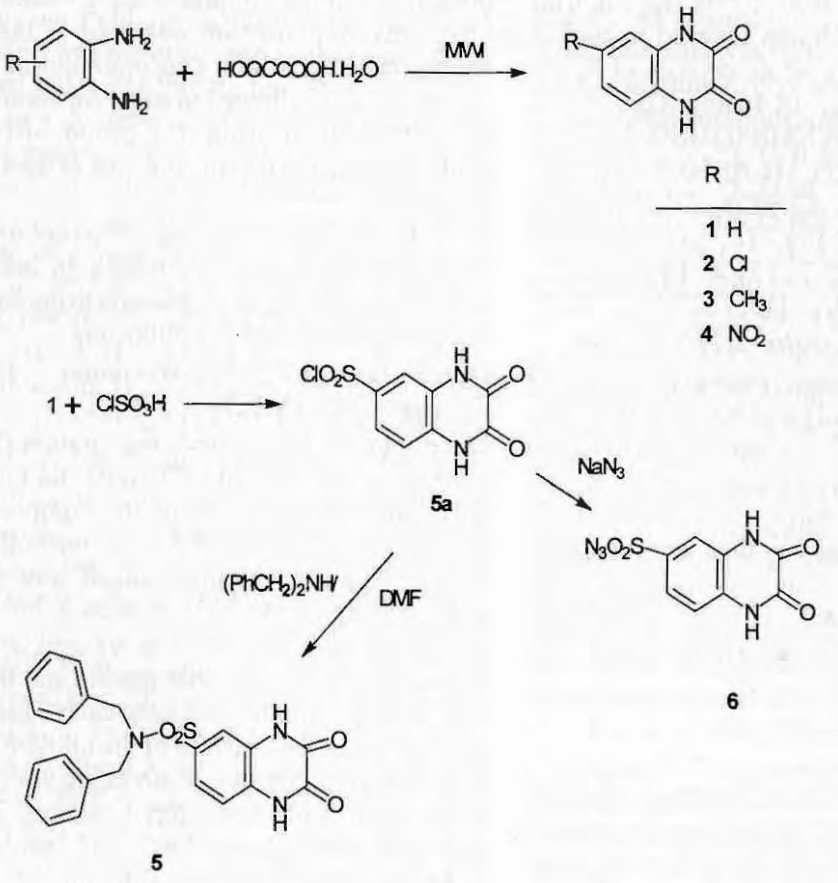
The minimum Inhibition Concentration (MIC) was done using the method of Russell and Furr (1977). Different concentrations of the compounds were prepared using a two-fold dilution method in DMSO solvent. The concentration ranged between 0.0078 and 1.000 mg/ml. About 2 ml of the solution of each compound from each dilution was put into a sterile plate with the aid of sterile pipette and then mixed with 18 ml of molten Nutrient agar. This was then allowed to set. The surface of the nutrient agar plate was allowed to dry before streaking with overnight broth cultures of the bacterial isolates. The plates were then labeled accordingly and incubated at 37 °C for 72 hours. They were subsequently examined for the presence or absence of growth. The lowest concentration preventing growth was taken as the minimum inhibitory concentration of the compound. This procedure was likewise both repeated for every of all other compounds.

3. Results and Discussion

Chemistry

The 2, 3-quinoxalinediones **1- 4** were synthesized by the reaction of appropriate benzene-1, 2-diamines with oxalic acid dihydrate, under microwave irradiation. N-N-Dibenzyl-2, 3-dioxo-1, 2, 3, 4-tetrahydroquinoxaline-6-sulfonamide, **5,** was prepared starting from the reaction of 1, 2, 3, 4-tetrahydroquinoxaline-2, 3-dione, **1** with chlorosulfonic acid to obtain the corresponding quinoxaline-6-sulfonyl chloride, **5a** which was then reacted with dibenzylamine in DMF. The reaction of **5a** with sodium azide gave the expected 2, 3-dioxoquinoxaline-6-sulfonyl azide, **6**. The sequences of reactions are shown in Scheme 1. 1-acetyl-1H-indole-2, 3-dione **7a** was prepared as shown in Scheme 2 by the reaction of isatin with acetic anhydride. N-(2-(3-oxo-3,4-dihydroquinoxaline-2-yl)phenyl) acetamide **7** was prepared from the reaction of **7a** with benzene-1,2-diamine in ethanol under microwave irradiation. 3-(2-Aminophenyl)

SCHEME 1: Synthetic route to compounds **1-6**



SCHEME 2: Synthetic route to compounds **7** and **8**

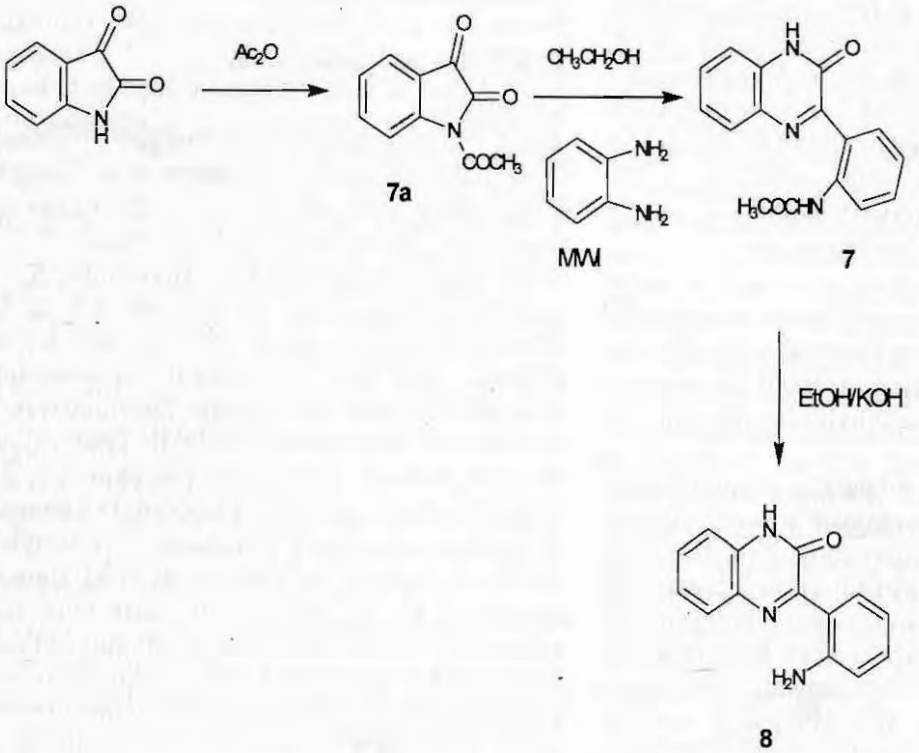


Table 1: Results of the antimicrobial screening (sensitivity testing) of the quinoxalinone derivatives on Gram-positive bacteria with the zone of inhibition in mm

Compound No? Microorganisms?	1	2	3	4	5	6	7	8	STREP
Staphylococcus epidermidis	10	21	25	25	20	25	18	26	18
Bacillus polymyxa (LIO)	13	20	20	17	15	21	15	18	15
Bacillus cerious	11	20	20	21	15	20	10	15	28
Streptococcus faecalis	10	16	18	0	0	20	0	0	23
Corynobacterium pyrogenin (LIO)	0	12	10	0	8	15	15	10	20
Clostridium sporogen	0	12	15	15	0	21	25	15	25
Bacillus stearothermophillus	18	25	30	15	15	15	17	15	23
Bacillus subtilis	20	25	25	22	20	21	21	24	20
Staphylococcus aureus	0	0	17	20	0	22	18	10	21

* STREP = Streptomycin

Table 2: Result of the antimicrobial screening (sensitivity testing) of the quinoxalinone derivatives on Gram-negative bacteria with the zone of inhibition in mm

Compound No ? microorganismss?	1	2	3	4	5	6	7	8	*Strep
Escherichia coli	17	17	8	0	17	17	17	17	0
Pseudomonas fluorescence	0	0	0	0	0	0	0	0	30
Klebsiella pneumonia	10	12	17	16	15	25	19	10	0
Pseudomonas auriginosa	0	8	12	10	10	10	15	20	ND

* STREP = Streptomycin

Table 3: Minimum Inhibitory Concentration (MIC) for some selected compounds in (mg/ml) on various Gram-positive bacteria

COMPOUND NO? microorganisms?	3	6	7	8	*STREP
Staphylococcus epidermidis	0.0313	0.0078	0.0313	0.0625	0.0313
Bacillus polymyxa(LIO)	0.500	0.0313	0.125	0.500	0.125
Bacillus cerius	0.0078	0.0078	0.0078	0.0078	0.0313
Streptococcus faecalis	0.500	0.500	1.000	1.000	0.0625
Corynebacterium pyogenes(LIO)	1.000	0.0625	0.500	1.000	0.0313
Clostridium sporogenes	0.0078	0.0078	0.500	0.500	0.0078
Bacillus stearothermophilus	0.125	0.0078	0.0078	0.0313	0.0625
Bacillus subtilis	0.500	0.0313	0.125	0.500	0.0625
Staphylococcus aureus	0.500	1.000	1.000	1.000	0.500

* STREP = Streptomycin

Table 4: Minimum inhibitory Concentration for some selected compounds in (mg/ml) on various Gram-negative bacteria

Compound No? microoganisms?	3	6	7	8	STREP
Escherichia coli	0.500	1.000	1.000	1.000	-
Pseudomonas aureginosa	1.000	1.000	1.000	1.000	0.2500
Klebsilla pneumonia	0.250	0.0313	0.125	0.250	-

* STREP = Streptomycin

quinoxaline-2-one, **8** was prepared from the hydrolysis of **7** with potassium hydroxide under reflux.

The infra-red spectra of the compounds show absorptions due to the stretching vibrations of N-H, C=O, C=N, C-SO_2 and C=C groups. The bands due to C=O stretching vibrations in compounds **1-8** showed in the region 1675 - 1698 cm^{-1}. The ^{1}H-NMR spectra of the quinoxalinones (**2-7**) exhibited two D_2O exchangeable protons at ä 12.92 - 9.78 ppm, due to 2 CONH groups. The signal corresponding to resonances of aromatic protons showed at ä 7.95 - 6.89 ppm.

The ^{13}C-NMR spectra showed signals that correspond to resonances of carbonyl (C=O) carbons at ä 168.7 -154.6 ppm while the aromatic carbon signals showed at the expected regions.

Antimicrobial activity

The synthesized compounds, **1-8** were screened in-vitro for possible antimicrobial activity. The sensitivity testing (with inhibition zones in mm) of **1-8** (at 2 mg/ml), streptomycin (a reference clinical antibiotic at 1 mg/ml) and DMSO (solvent) against nine species of Gram positive and four Gram negative bacteria are reported in Tables 1 and 2. In general, the results showed that all the synthesized compounds, **1-8** exhibited broad spectrum activity against the bacterial strains. Compounds **3** and **6** showed activity against all the nine Gram positive bacterial strains, just like the standard streptomycin. However, compounds **2, 3, 4, 6** and **8** showed larger zones of inhibition than streptomycin for *Staphylococcus epidermidis*, *Bacillus polymyxa, and Bacillus subtilis*, while compounds **2** and **3** showed larger inhibition zones than streptomycin for *Bacillus stearothermophilus.* Only five of the compounds showed varying zones of inhibition (10-22 mm) against *Staphylococcus aureus.*

On the other hand, for the Gram negative bacterial strains, all the compounds showed activity against *Escherichia coli,* except compound **4,** with zones of inhibition ranging from 8-17 mm. Compound **3** showed the smallest zone of inhibition. Seven compounds (**2- 8**) showed activity against *Klebsiella pneumonia* and *Pseudomonas aureginosa* with zones of inhibition ranging from 8-25 mm. Compound **6** showed the largest zone of inhibition against *Klebsiella pneumoniae*. It is noteworthy that streptomycin showed no activity against the bacterial strain. All the compounds showed no activity against *Pseudomonas fluorescence.* The lowest concentrations of drug that completely inhibited the growth of organism, (MIC values) for some selected compounds are shown in Tables 3 and 4. The compounds were selected based on their large zones of inhibition and broad spectrum of activity.

The MICs of **3, 6, 7,** and **8** varied between 7.8 ìg/ml and 1000 ìg/ml. The MIC values for streptomycin varied between 7.8 ìg/ml and 500 ìg/ml. The result indicated that compound **6** showed the highest activity, with higher activity than the standard streptomycin on Gram positive organisms; while all the compounds showed activity on two Gram negative strains, *Escherichia coli* and *Klebsiella pneumonia,* which are resistant to the standard streptomycin.

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