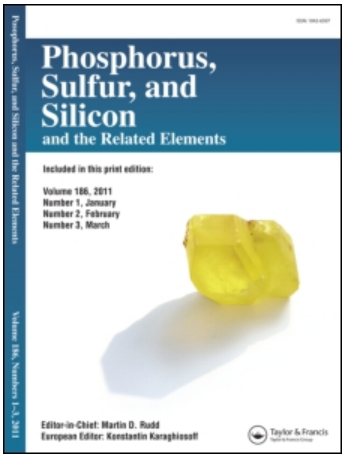

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Synthesis and Antimicrobial Activity of Some 2(1H)-quinoxalinone-6-sulfonyl Derivatives

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Synthesis and Antimicrobial Activity of Some 2(1H)-quinoxalinone-6-sulfonyl Derivatives

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*The synthesis of some quinoxalinesulfonyl derivatives is described. Two of the synthesized derivatives, 2-oxo-1,2-dihydroquinoxaline-6-sulfonyl azide (**3a**) and 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl azide (**3b**), were screened* in vitro *for their growth inhibitory activity against nine strains of Gram-positive and seven strains of Gram-negative bacteria, along with two fungal isolates. The two compounds showed broad spectrum (*in vitro*) activity against the bacterial strains.*

Keywords 2-Oxo-1,2-dihydroquinoxaline-6-sulfonyl azide; 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl azide; antimicrobial activity; synthesis

INTRODUCTION

Various organosulfonyl derivatives, $ArSO_2X$, (sulfonamides, sulfonohydrazides, sulfonates, thiosulfonyl compounds, etc.) have been shown to possess pharmacological activities, including antibacterial,[1,2] antifungal,[1] nematicidal,[3] diuretic,[4–5] hypoglycemic,[6] antitumor,[7] and protease inhibitory[8–10] activity.

Quinoxaline derivatives constitute an important class of pharmaceutical and agricultural chemicals:[11,22] They are used to impart a microbicidal finish on cotton fabrics,[11] and act as herbicides,[12–14] fungicides,[15] antagonists,[16] antibiotics,[17] and dyes,[18,19] amongst others. In addition, certain quinoxaline-2,3-diones and quinoxaline-2-ones have been reported to be highly potent NMDA receptor antagonists[20,21] and HIV-1 TAR-Tat inhibitors.[22]

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There is the need for continuous search for simple new compounds with more potent antimicrobial activities because of constant emergence of drug-resistant microorganisms.

In this article, we report the synthesis of some quinoxalinonesulfonyl derivatives and the antimicrobial evaluation of two sulfonyl azide derivatives.

RESULTS AND DISCUSSION

Chemistry

The quinoxalinesulfonyl derivatives **2** were prepared following the general route of chlorosulfonation of aromatic compounds, followed by the reaction of the formed organosulfonyl chlorides with the desired nucleophile.

1,2,3,4-Tetrahydroquinoxaline-2,3-dione (**1b**) was prepared via two routes: (i) by oxidation of quinoxalin-2(1H)-one (**1a**) with $KMnO_4$[23] and (ii) by the reaction of 1,2-diaminobenzene with oxalic acid under microwave irradiation.

1a was reacted with excess chlorosulfonic acid at about 110°C to give 2-oxo-1,2-dihydroquinoxaline-6-sulfonyl chloride (**2a**) as the major isomer. 2,3-Dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride (**2b**) was similarly prepared from **1b**.

Chlorination of **2a** and **2b** with thionyl chloride-DMF under reflux afforded 2-chloroquinoxaline-6-sulfonyl chloride (**4a**) and 2,3-dichloroquinoxaline-6-sulfonyl chloride (**4b**), respectively. Compound **4a** was also obtained from the reaction of **1a** with a mixture of chlorosulfonic acid and thionyl chloride, together with the formation of 2-chloroquinoxaline (**8**).

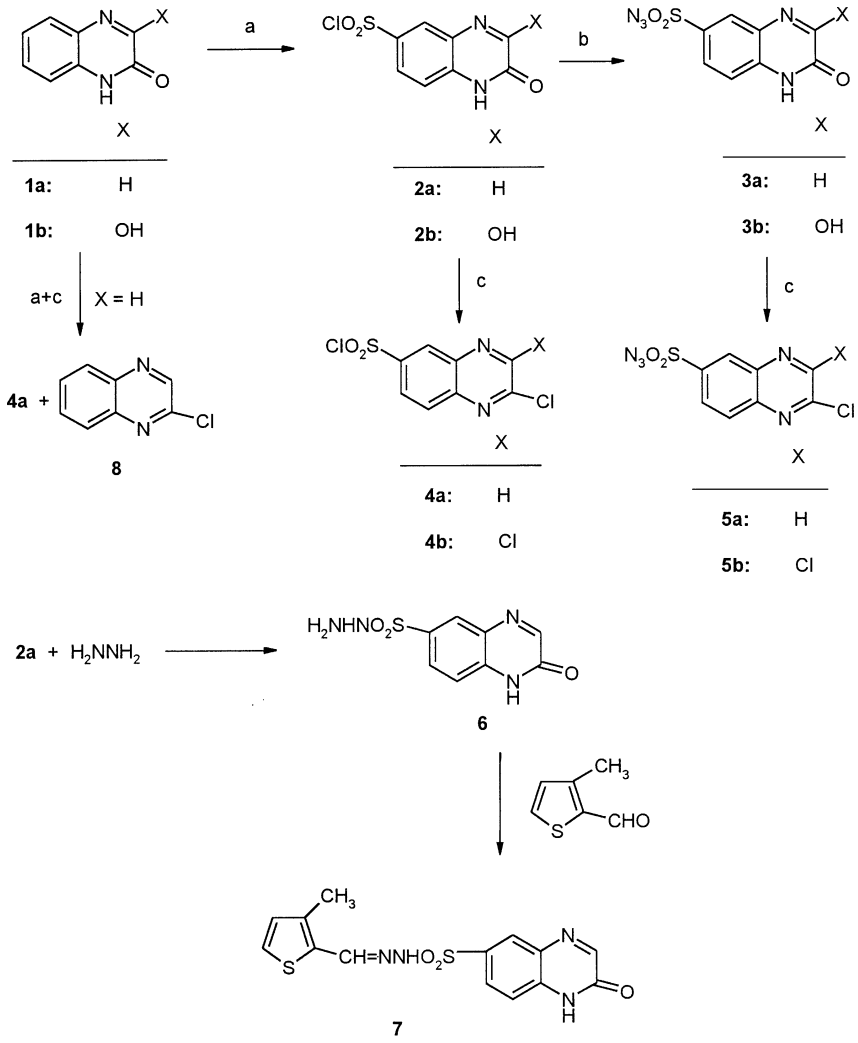
In addition, the reaction of **2a** and **2b** with sodium azide gave the corresponding sulfonyl azides: 2-oxo-1,2-dihydroquinoxaline-6-sulfonyl azide (**3a**) and 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl azide (**3b**).

Chlorination of **3a** and **3b** with thionyl chloride-DMF gave 2-chloroquinoxaline-6-sulfonyl azide (**5a**) and 2,3-dichloroquinoxaline-6-sulfonyl azide (**5b**), respectively.

Treatment of **2a** with hydrazine hydrate gave 2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide (**6**), which was further reacted with 3-methylthiophene-2-carbaldehyde to give 3-methylthiophene-2-carbaldehyde[2-oxo-1,2-dihydroquinoxalin-6-yl)sulfonyl]hydrazone (**7**).

These reactions are summarized in Scheme 1.

The yields, physical properties, and analytical data of the synthesized compounds are given in Table I. The structures of the compounds were



SCHEME 1 Synthetic route to quinoxaline derivatives **1–8**. (a) $CISO_3H$/heat, (b) NaN_3/DMF, and (c) $SOCl_2$-DMF.

assigned by their chemical analysis and infrared, mass, and ^{1}H-nmr spectra.

The ^{1}H-nmr data are listed in Table II. The infrared spectra of the compounds show absorptions due to the stretching vibrations of N_3, C=O, and SO_2 groups in the range that agree with literature data and are given in the experimental section.

TABLE I Analytical Data and Some Physical Properties of Quinoxaline 1–8

Compd. no	Yield (%)	MP (°E)	Formula	Calculated (%)			Found (%)		
				C	H	N	C	H	N
1b	99*	>330 (Lit[23] > 340)	$C_8H_6N_2O_2$						
2a	81	280 (dec.)	$C_8H_5ClN_2O_3S$	39.27	2.06	11.45	38.79	1.90	11.15
2b	88	330 (dec.) (lit.[24] 292)	$C_8H_5ClN_2O_4S$	36.86	1.93	10.75	36.59	2.00	10.95
3a	96	183–184(dec.)	$C_8H_5N_5O_3S$	38.25	2.01	27.88	38.16	2.15	27.71
3b	91	> 330	$C_8H_5N_5O_4S$	35.96	1.89	26.21	35.57	1.93	26.11
4a	88	123–125 (lit.[25] 125–127)	$C_8H_4Cl_2N_2O_2S$	36.52	1.53	10.65	36.50	1.60	10.50
4b	89	88–89 (lit.[26] 86–87)	$C_8H_3Cl_3N_2O_2S$	32.29	1.02	9.41	32.35	1.10	9.31
5a	92	130–131 (dec.)	$C_8H_4ClN_5O_2S$	35.63	1.50	25.97	35.38	1.66	25.58
5b	90	93–94 (dec.)	$C_8H_3Cl_2N_5O_2S$	31.59	0.99	23.03	31.45	0.98	22.90
6	66	>330	$C_8H_8N_4O_3S$	40.00	3.36	23.32	40.11	3.12	23.50
7	96	>340	$C_{14}H_{12}N_4O_3S$	48.26	3.47	16.08	48.11	3.30	16.22

*Microwave-assisted method.

TABLE II ^{1}H NMR Data of Quinoxaline Derivatives

Product	^{1}H NMR (DMSO-d_6 or $CDCl_3$, ppm)
1a*	12.40 (br s, 1H, NH, D_2O exchangeable), 8.20 (s, 1H, H-3), 7.81–7.30 (m, 4H, Ar).
1b*	11.91 (s, 2H, 2NH, D_2O exchangeable), 7.13–7.04 (m, 4H, Ar).
2a*	12.76 (br s, 1H, NH, D_2O exchangeable), 8.32 (s, 1H, H-3), 8.30 (d, 1H, J = 2.00 Hz, H-5), 8.12 (dd, 1H, H-7, J = 8.69 Hz, J = 2.04 Hz), 7.60 (d, 1H, H–8, J = 8.70 Hz).
2b*	12.30 (s, 1H, NH, D_2O exchangeable), 12.01 (s, 1H, NH, D_2O exchangeable), 7.84–7.81 (m, 2H, Ar), 7.15 (d, 1H, Ar).
3a*	12.94 (br s, 1H, NH, D_2O exchangeable), 8.34 (s, 1H, H-3), 8.32 (d, 1H, H-5 J = 1.98 Hz), 8.13 (dd, 1H, H-7, J = 8.67 Hz, J = 2.06 Hz), 7.56 (d, 1H, H-8, J = 8.71 Hz).
4a**	8.98 (s, 1H, H-3), 8.87 (d, 1H, H-5, J = 2.12 Hz), 8.36 (dd, 1H, H-7, J = 8.98 Hz, J = 2.15 Hz), 8.28 (d, 1H, H-8, J = 9.10).
4b**	8.85 (d, 1H, H-5, J = 2.30 Hz), 8.40 (dd, 1H, H-7, J = 8.90 Hz, J = 2.10 Hz), 8.30 (d, 1H, H-8, J = 9.15).
5a**	8.96 (s, 1H, H-3), 8.80 (t, 1H, J = 1.11 Hz), 8.26 (s, 1H), 8.25 (s, 1H).
6*	12.74 (br s, 1H, NH, D_2O exchangeable), 8.46 (br s, 1H, NH, D_2O exchangeable), 8.27 (s, 1H, H-3), 8.13 (d, 1H, H-5, J = 1.95), 7.90 (dd, 1H, H-7, J = 8.62 Hz, J = 2.00 Hz), 7.43 (d, 1H, H-8, J = 8.64 Hz), 4.17 (br s, 2H, NH_2, D_2O exchangeable).
7*	12.75 (s, 1H, NH, D_2O exchangeable), 11.35 (s, 1H, NH, D_2O exchangeable), 8.25 (s, 1H, H-3), 8.14 (s, 1H), 8.09 (s, 1H), 7.94 (d, 1H, J = 8.52 Hz), 7.48 −7.41 (m, 2H), 6.86 (d, 1H, J = 4.88), 2.19 (s, 3H, CH_3).
8**	8.97 (s, 1H, H-3), 8.14–8.00 (m, 2H, Ar), 7.98–7.84 (m, 2H, Ar).

*in DMSO-d_6 solvent.
**in $CDCl_3$ solvent.

Antimicrobial Activity

The two quinoxaline-6-sulfonyl azides **3a** and **3b** were screened *in vitro* for possible antibacterial activity. The sensitivity testings (inhibition zones (mm)) of **3a** and **3b** (at 2 mg mL^{-1}), streptomycin (a reference clinical antibiotic, at 1 mg mL^{-1}), and DMSO (solvent) against nine species of Gram-positive and seven Gram-negative bacteria, along with two fungal isolates, are reported in Table III.

The results of the minimum inhibitory concentration (MIC) (defined as the lowest concentration of drug that completely inhibited the growth of the organism) of the two compounds are shown in Table IV.

The results in Table III indicated that the two compounds showed broad spectrum activity against bacteria strains (a variety of Gram positive and Gram negative), comparable to the standard reference streptomycin. However, **3b** showed larger zones of inhibition than **3a** for most strains.

TABLE III Antimicrobial Activity of 3a, 3b, and Streptomycin (stm.) as Determined by Dilution Techniques

		Inhibition Zone (mm)			
Microorganism	Gram	**3a** 2 mg mL^{-1}	**3b** 2 mg mL^{-1}	**Stm** 1 mg mL^{-1}	DMSO
Clostridium sporogenes (NCIB 532)	–	22	0	25	0
Escherichia coli (NCIB 86)	–	17	32	0	0
Klebsiella pneumoniae (NCIB 418)	–	15	30	0	0
Proteus vulgaris (NCIB 67)	–	17	24	28	0
Pseudomonas fluorescens (NCIB 3756)	–	18	0	30	0
Serratia marcescens (NCIB 1377)	–	16	25	19	0
Shigella dysenteriae (LIO)	–	25	18	22	0
Bacillus anthracis (LIO)	+	0	24	18	0
Bacillus cereus (NCIB 6349)	+	20	16	28	0
Bacillus polymyxa (LIO)	+	0	17	15	0
Bacillus stearothermophilus (NCIB 8222)	+	18	31	23	0
Bacillus subtilis (NCIB 3610)	+	12	32	20	0
Corynebacterium pycogenes (LIO)	+	11	15	20	0
Micrococcus luteus (NCIB 196)	+	0	0	25	0
Staphylococcus aureus (NCIB 8588)	+	27	21	21	0
Streptococcus faecalis (NCIB 755)	+	14	13	23	0
Aspergillus flavus		18	15	ND	0
Candida albicaus		26	25	ND	0

NCIB = National Collection of Industrial Bacteria.
LIO = Locally Isolated Organism.
ND = Not done.

The MICS of **3a** varied between 31.3 μg mL^{-1} and 1000 μg mL^{-1} for all strains, and between 15.6 μg mL^{-1} and 500 μg mL^{-1} for compound **3b**. These results indicated that **3b** has a greater activity than **3a**.

The two quinoxalinesulfonyl azides were further characterized in the time-kill studies, using *Escherichia coli* as a Gram-negative representative and *Staphylococcus aureus* (NCIB 8588) as a representative of Gram-positive organism, and the results are shown in Figures 1 and 2, respectively.

As shown in Figure 1, at their MIC values (250 μg mL^{-1} for **3a** and 62.5 μg mL^{-1} for **3b**), compound **3a** exhibited a higher degree of killing, reaching 96% killing at 90 min compared to **3b** which reached only 52% killing at 90 min. At four times the MICs, the two compounds showed a similar trend, with **3a** reaching a bactericidal activity (100% killing) at 60 min, while 3b only reached 100% killing at 90 min.

TABLE IV Minimum Inhibitory Concentration (MIC) for 3a and 3b

Microorganism	**3a** (μg mL^{-1})	**3b** (μg mL^{-1})
Clostridium sporogenes (NCIB 532)	250	—
Escherichia coli (NCIB 86)	250	62.5
Klebsiella pneumoniae (NCIB 418)	31.3	31.3
Proteus vulgaris (NCIB 67)	31.3	250
Pseudomonas fluorescens (NCIB 3756)	250	—
Serratia marcescens (NCIB 1377)	250	15.6
Shigella dysenteriae (LIO)	250	15.6
Bacillus anthracis (LIO)	—	500
Bacillus cereus (NCIB 6349)	125	15.6
Bacillus polymyxa (LIO)	—	500
Bacillus stearothermophilus (NCIB 8222)	250	62.5
Bacillus subtilis (NCIB 3610)	125	15.6
Corynebacterium pyogenes (LIO)	500	62.5
Micrococcus luteus (NCIB 196)	—	—
Staphylococcus aureus (NCIB 8588)	1000	15.6
Streptococcus faecalis	500	250

NCIB = National Collection of Industrial Bacteria.
LIO = Locally Isolated Organism.

In the case of *S. aureus* as shown in Figure 2, at their MIC values (1000 μg mL^{-1} for **3a** and 15.6 μg mL^{-1} for **3b**), **3a** again showed a higher rate of killing and reaching 100% killing within 90 min, and **3b** reached only 90% killing. There was no significant effective change at two times the MIC of **3b**. Even at four times the MIC, compound **3b**

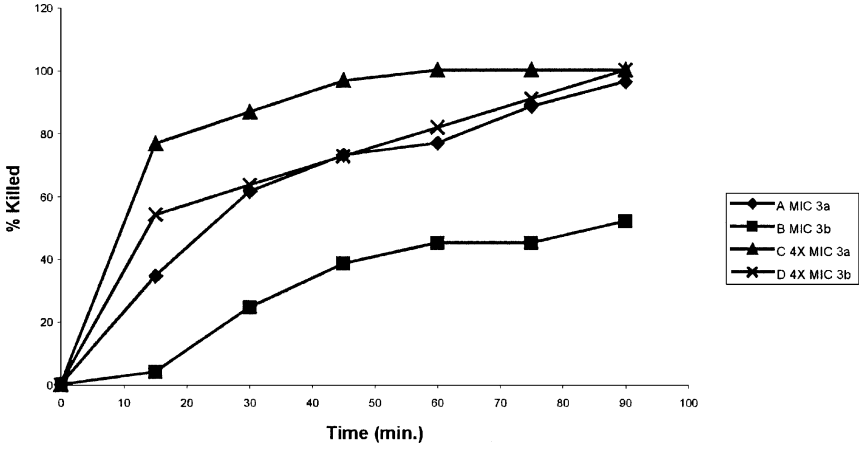


Figure 1 Rate of killing of *E. coli* (representing Gram-Organism) by 3a and 3b.

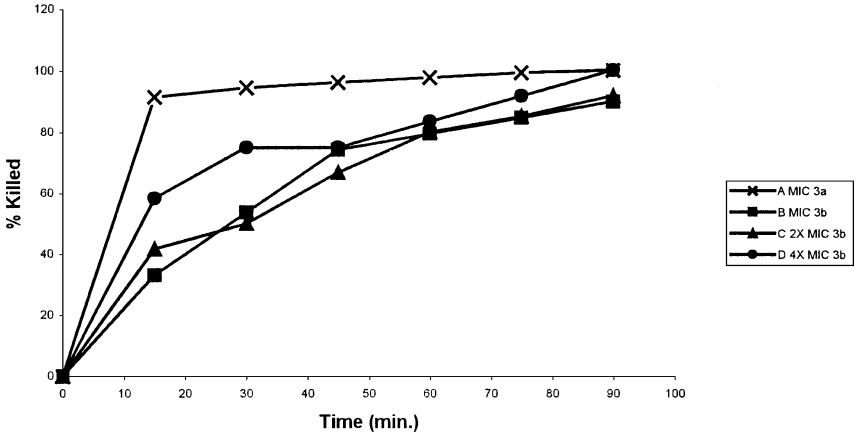


Figure 2 Rate of killing of *S. aureus* (representing Gram + Organism) by 3a and 3b.

still exhibited a lower rate of killing compared to the killing rate by **3a** at its MIC.

EXPERIMENTAL

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and no corrections. For TLC, precoated silica gel thin-layer sheets F 254 from MERCK was used. ^{1}H- and ^{13}C-NMR were recorded as $CDCl_3$ or DMSO-d_6 solutions on a Brucker-AC-250 and JOEL-JNMGX 400-MHZ spectrometer (δ in ppm relative to Me_4Si and H_3PO_4). Mass spectra (E1–MS) were recorded on a finnigan MAT 312 machine (in m/z (rel. %)) (all at the Fakultat fur Chemie, Universitat Konstanz, Konstanz, Germany). Infrared spectra were recorded on a BUCK Scientific spectrometer.

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

Method a. A solution of $KMnO_4$ (27.0 g, 171 mmol) in water (300 mL) was added dropwise to a boiling solution of quinoxalin-2(1H)-one (**1a**) (5.0 g, 34 mmol) and NaOH (1.4 g, 35 mmol) in water (300 mL) for a period of 2 h with vigorous stirring. The resulting mixture was refluxed for another 1 h and then cooled to about 60°. The MnO_2 was filtered off and washed with hot water twice (2 × 50 mL). The combined filterate was concentrated to 50 mL and then acidified with acetic acid. The resulting solid product was purified by column chromatography

(alumina-neutral). Elution with $CHCl_3/CH_3OH$ (9/1) gave unreacted **1a** (2.2 g), while acetic acid gave **1b** (2.9 g, 52%). mp > 320°C. MS: 162 (17, M^+), 134 (46, $[M\text{-}CO]^+$), 106 (100, $[M\text{-}2CO]^+$).

Method b: Microwave-assisted synthesis. A mixture of oxalic acid dihydrate (10.0 g, 79.4 mmol) and benzene-1,2-diamine (8.6 g, 79.6 mmol) was ground in a mortar and placed in an open beaker. Water (1 mL) was added and mixed thoroughly and then irradiated in a domestic MW oven at an emitted power of 400 W for 3 min. Water (100 mL) was added and irradiated for 1 min to give a clear solution and then left to stand at room temperature to give colorless needles of **1b** (12.8 g, 99%). The physical and spectroscopic data of **1b** were identical to those of authentic 1,2,3,4-tetrahydroquinoxaline-2,3-dione.

2-Oxo-1,2-dihydroquinoxaline-6-sulfonyl chloride (2a)

Quinoxalin-2(1H)-one (**1a**) (10.0 g, 68.5 mmol) was added in portions to chlorosulfonic acid (46 mL, 10 mol. Equiv.), after which the mixture was heated (under reflux) at 110° for 8 h. The reaction mixture was cooled in ice water before it was poured into crushed ice. The resulting solid was filtered and washed with cold water and dried. Recrystallization from toluene-acetone gave **2a** (13.5 g). Ir (cm^{-1}) 3300 (NH), 1680 (C=O), 1380, 1165 (SO_2). MS: 246 (7.8, $[M + 2]^+$), 244 (20.0 $[M^+]$, 209 (55.3, $[M\text{—}Cl]^+$), 145 (43.2, $[M\text{—}Cl\text{—}SO_2]^+$), 117 (100), 90 (25.4), 64 (18.7).

Compound **2b** was similarly obtained from **1b**. Ir (cm^{-1}): 3380 (NH), 1680 (C=O), 1355, 1140 (SO_2). MS: 262 (5.3, $[M + 2]^+$), 260 (14.0, $[M^+]$, 225 (48.1, $[M\text{—}Cl]^+$), 161 (100, $[M\text{—}Cl\text{—}SO_2]^+$), 133 (45.4), 105 (70.5), 78 (45.2), 51 (82.3).

2-Oxo-1,2-dihydroquinoxaline-6-sulfonyl azide (3a)

Sodium azide (7.0 g, 108 mmol) was added to a stirring solution of **2a** (13.2 g, 54 mmol in acetone (250 mL)), followed by addition of water (5.0 mL) and the mixture stirred at room temperature for 8 h. Removal of acetone followed by addition of water gave 2-oxo-1,2-dihydroquinoxaline-6-sulfonyl azide as light pink crystals. Recrystallization from ethanol gave pure **3a** (13.0 g). Ir (cm^{-1}): 3290 (w, NH), 2131 (N_3), 1664 (C=O), 1350, 1173 (SO_2). MS: 251 (28.0, M^+), 209 (59.3, $[M\text{—}N_3]^+$), 145 (77.2), 117 (100), 90 (12.5).

Compound **3b** was prepared in a similar manner from **2b**.

3b: Ir (cm^{-1}): 2159 (N_3), 1690 (C=O), 1365, 1160 (SO_2). MS: 267 (100, M^+), 239 (17.0, $[M\text{—}CO]^+$), 225 (18.3, $[M\text{—}N_3]^+$), 211 (6.0), 161 (10.0), 133 (5.0), 105 (22.1).

2-Chloroquinoxaline-6-sulfonyl chloride (4a)

A mixture of **2a** (2.0 g, 8.2 mmol), DMF (0.1 mL) and excess $SOCl_2$ (5.0 mL) was heated to reflux for 1.5 h with continuous stirring. The excess $SOCl_2$ was distilled off, with the last traces removed on a rotatory evaporator. The remaining solid residue was washed with cold water (3 × 10 mL), dried in air, and then recrystallized from petroleum ether (bp 30–80°) to give **4a** (1.9 g). Ir (cm^{-1}): 1380, 1180, 1160 (SO_2). MS: 264 (16.4, $[M + 2]^+$), 262 (22.4, M^+), 229 (25.1), 227 (71.0 $[M—Cl]^+$), 165 (32.2), 163 (100, $[M—Cl—SO_2]^+$), 127 (10.2). 101 (15.6), 75 (24.1).

Compounds **4b**, **5a**, and **5b** were prepared in a similar manner:

4b: Ir (cm^{-1}): 1370, 1170 (SO_2), MS: 300 (6.7 $[M + 4]^+$), 298 (18.4, $[M + 2]^+$), 296 (19.1, M^+), 263 (39.5), 261 (55.5, $[M—Cl]^+$), 199 (54.4), 197 (88.1, $[M—Cl—SO_2]^+$), 162 (14.4, $[M—Cl_2—SO_2]^+$), 136 (18.5), 101 (34.7), 75 (85).

5a: Ir (cm^{-1}): 2160 (N_3), 1380, 1160 (SO_2). MS: 271 (5.9, $[M + 2]^+$), 269 (14.2, M^+), 229 (26.3), 227 (72.8, $[M—N_3]^+$), 165 (35.8), 163 (100, $[M\text{-}N_3\text{-}SO_2]^+$), 127 (8.6, $[M\text{-}N_3\text{-}SO_2\text{-}HCl]^+$), 101 (13.7), 75 (20.4).

5b: Ir (cm^{-1}): 2120 (N_3), 1370, 1160 (SO_2), MS: 305 (11.7, $[M + 2]^+$), 303 (16.5, M^+), 263 (49.9), 261 (69.9, $[M\text{-}N_3]^+$), 199 (66.4), 197 (100, $[M\text{-}N_3\text{-}SO_2]^+$), 162 (15.8), 136 (21.0), 101 (33.8), 75 (84.1).

2-Oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide (6)

2-Oxo-1,2-dihydroquinoxaline-6-sulfonyl chloride (**2a**) (2.0 g, 8.2 mmol) was added in portions to hydrazine hydrate (2.0 g, 34 mmol) in alcohol, after which the reaction mixture was stirred at room temperature overnight. The mixture was poured into cold water to give a solid product. Recrystallization from ethanol gave the pure hydrazide **6** (1.3 g). Ir (cm^{-1}): 3320, 3300, 3210, 3200 (NH), 1670 (C=O), 1355, 1155 (SO_2). MS: 240 (2.0, M^+), 225 (10.0), 210 (11.3), 209 (2.0, $[M—NHNH_2]^+$), 193 (6.4), 178 (11.9), 161 (17.6), 146 (40.9), 145 (11.4), 117 (54.5), 106 (100).

3-Methylthiophene-2-carbaldehyde[2-oxo-1,2-dihydroquinoxaline-6-yl) sulfonyl]hydrazone (7)

A mixture of 2-oxo-1,2-dihydroquinoxaline-6-sulfonohydrazide (**6**) (1.0 g, 4.2 mmol) in ethanol and 3-methyl-thiophene-2-carbaldehyde (0.5 mL, 4.6 mmol) was refluxed for 1 h and then allowed to cool to room temperature to give analytically pure **7** (1.4 g). Ir (cm^{-1}): 1665 (C=O), 1610, 1369, 1165 (SO_2). MS: 348 (11.8, M^+), 248 (31.8), 221 (15.1), 210 (11.0), 193 (12.6), 178 (74.7), 150 (17.8), 146 (45.9), 140 (10.6), 139 (100),

126 (37.4), 125 (52.4), 123 (35.3), 122 (37.2), 118 (18.2), 117 (19.3), 112 (23.9), 110 (77.2), 98 (22.8), 97 (28.2), 69 (25.8).

Chlorosulfonation of Quinoxalin-2(1H)-one (1a) in the Presence of Thionyl Chloride

Quinoxalin-2(1H)-one (**1a**) (10.0 g, 68.5 mmol) was added in portions to chlorosulfonic acid (46 mL, 697 mmol), followed by addition of thionyl chloride (25 mL, 348 mmol) and the mixture heated at 110 for 11 h. Excess thionyl chloride was distilled off, the remaining mixture cooled in ice-water and then poured carefully into crushed ice with continuous stirring. The resulting solid was filtered, washed with cold water and dried in air. Repeated recrystallizations from petroleum either (bp 30–80°) gave pure **4a** (8.0 g, 44%). From the mother liquors was obtained 2-chloroquinoxaline (**8**) (8.5 g) (mp, mmp, ^{1}H—and mass spectra).

Antimicrobial Screening

Media Used

Nutrient broth (Oxoid Ltd.) and nutrient agar (Oxoid Ltd.) were used for subculturing the bacterial isolates, while diagnostic sensitivity test agar (Oxoid Ltd.) was used for sensitivity testing.

Microorganisms Used

The following microorganisms were used *Clostridium sporogenes* (NCIB 532), *Escherichia coli* (NCIB 86), *Klebsiella pneumoniae* (NCIB 418), *Proteus vulgaris* (NCIB 67), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Serratia marcescens* (NCIB 1377), *Shigella dyseuteriae* (LIO), *Bacillus anthracis* (LIO), *Bacillus cereus* (NCIB 6349), *Bacillus polymyxa* (LIO), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus subtilis* (NCIB 3610), *Corynebacterium pyogenes* (LIO), *Micrococcus luteus* (NCIB 196), *Staphylococcus aureus* (NCIB 8588), *Streptococcus faecalis* (NCIB 755), *Aspergillus flavus*, and *Candida albicans*.

Sensitivity Testing[27]

The sensitivity testing of the two synthetic compounds were determined using agar-well diffusion method.

The bacterial isolates were first grown in nutrient broth (Oxoid Ltd.) for 18 h before use. The inoculum suspensions were standardized and then tested against the effect of the two compounds at a concentration of 2 mg/mL each in diagnostic sensitivity test agar (Oxoid Ltd.). The plates were observed for zones of inhibition after 24 h incubation at 37°C. The effects were compared with

that of the streptomycin standard antibiotic at a concentration of 1 mg/mL.

Minimum Inhibitory Concentration (MIC)[28]

The MIC of different concentrations of the two compounds was determined using two-fold dilutions method. Different concentrations of the synthetic compound ranging between 0.0313 mg/mL and 1 mg/mL were prepared. Two millimetres of the concentrate from each dilution was added to 18 mL of molten sterile nutrient agar (Oxoid Ltd.) aseptically, and thoroughly mixed together in a sterile Petri dish. This was allowed to set. The surface of the nutrient agar was allowed to dry properly before streaking with the appropriate bacterial isolate. The plates were then incubated at 37°C for up to 72 h. The lowest concentration preventing the growth was taken as the minimum inhibitory concentration of the compound.

Determination of the Rate of Killing of the Compounds (Bactericidal Activity of the Compounds)

The bactericidal activity of different concentrations of the two compounds were determined by the extent of killing at time intervals 0, 15, 30, 45, 60, 75, and 90 min. Viable counts of the bacterial cells to be used were determined first. Two representatives of the bacterial isolates were used; these were *Staphylococcus aureus* representing Gram-positive bacteria and *Escherichia coli* representing Gram-negative bacteria. Serial dilutions of the test organism was done and about 1 mL of 10^{-5} dilution of the test organism was added to 4 mL of the test concentration of the compound. The suspension was thoroughly mixed and held at room temperature (28°C). Exactly 1 mL volume of each suspension was withdrawn at the appropriate intervals and transferred to 4.0 mL of nutrient broth (Oxoid Ltd.) recovery medium containing 3% Tween 80 to neutralize the effects of any antimicrobial compound carry-overs from the suspension. One mL of the final dilution was plated out and incubated at 37°C for 72 h. Depressions in the viable counts indicated killing by the compound.

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