

MICROWAVE-ASSISTED SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME PYRAZOL-1-YLQUINOXALIN- 2(1H)-ONE DERIVATIVES

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3-Hydrazinoquinoxalin-2(1H)-one was prepared from quinoxaline-2,3-dione and subsequently used for the synthesis of some potentially biologically active 3-(pyrazol-1-yl)quinoxalin-2(1H)-one derivatives. While 3-(3,5-dimethylpyrazol-1-yl)quinoxalin-2(1H)-one showed a comparative effect with streptomycin, 3-(5-oxo-3-phenyl-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one was found to be the most active with an MIC value of 7.8 µg/ml.

Keywords: 3-hydrazinoquinoxalin-2(1H)-one, imines, Gram-positive bacteria.

Over the years it has been established that quinoxalines are, in general, relatively easy to prepare [1-3], and many derivatives have been synthesized with the aim of obtaining biologically active materials [4, 5]. Some quinoxaline and quinoxalinone derivatives have been reported to show antimicrobial [6, 7], antiinflammatory [8], antifungal [9], anticancer [10], antiviral [11], antimalarial [12], anticonvulsant [13], antidepressant [14], antitubercular [15], antibacterial [16], and antithrombotic [17] activities.

Thermal and chemically stable polyquinoxalines (PQs) find potential applications as films, coating adhesives [18], ultrafiltering materials, and composite matrices that demand stability in harsh environment [19]. In a similar manner the synthesis of novel pyrazole derivatives [20] and evaluation of their chemical behaviors have gained more importance in recent decades for biological [21–23], medicinal [24], and agricultural [25] purposes. Although numerous methods are available for construction of pyrazoles [26, 27], only little attention has been given to the pyrazolysis of quinoxalinone derivatives [28]. For instance, 1,3-dipolar cycloadditions of azomethine imines, available by acid catalyzed treatment of 3-pyrazolidinone with acetone and butyraldehyde, respectively, were studied [29]. The photoluminescence and electroluminescence of some new 1H-pyrazolo-

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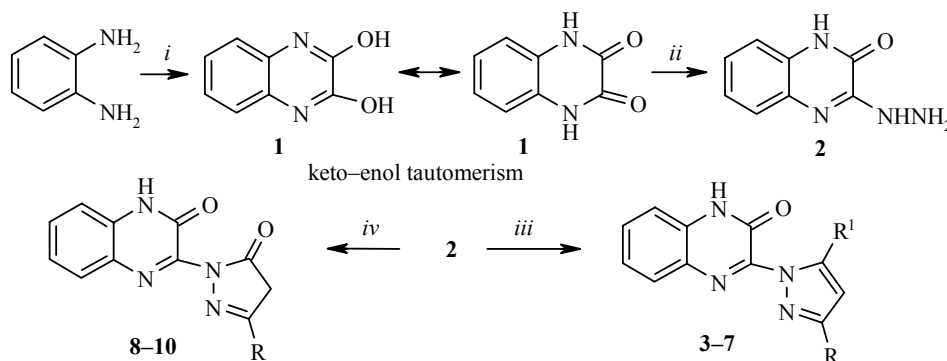
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[3,4-*b*]quinoxaline derivatives have been investigated [30], while the biological properties of such moieties have been understudied [31]. From this point of view it is of great interest to synthesize some pyrazolyquinoxalinone derivatives with the aim of investigating their antibacterial properties.

3-Hydrazinoquinoxalin-2(1H)-one (**2**) was prepared from hydrazinolysis of 1,2,3,4-tetrahydroquinoxaline-2,3-dione (**1**), which existed as a keto-enol tautomer (Scheme 1). The starting material **1** was prepared by the modified Obafemi and Pfeleiderer procedure [32]. To a heated (100°C) solution of oxalic acid dihydrate in water, acidified *o*-phenylenediamine was added cautiously with vigorous stirring at 100°C for 20 min. The resulting mixture was cooled and then filtered off. The crude solid obtained was purified by crystallization from water to afford colorless needles of compound **1**. 3-Hydrazinoquinoxalin-2(1H)-one **2** underwent condensation reaction with various β -diketones to afford (3,5-disubstituted pyrazol-1-yl)quinoxalin-2(1H)-one derivatives **3–7** in excellent yields (Scheme 1). For instance, the reaction of compound **2** with 2-thenoyltrifluoroacetone gave 97% of 2-trifluoromethyl-substituted pyrazole **7** as a single product. This is because the carbonyl next to CF₃ is more reactive than the carbonyl next to thiophene due to the high electron withdrawing ability of the trifluoromethyl side chain. Therefore, the nucleophilic attack by NH₂ of hydrazine was initiated first on the carbonyl next to CF₃.

Furthermore, condensation of compound **2** with α -keto esters followed by thermal cyclization of the reaction intermediate in the presence of ethanol solvent gave the target structure of (3-alkyl(aryl)-5-oxo-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one derivatives **8, 9**. The carbonyl of ketone is attacked first since it is more reactive than the carbonyl of ester. Finally, upon treatment of compound **2** with ethyl cyanoacetate the main product obtained was 3-(3-amino-5-oxo-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one (**10**) in moderate yield (Scheme). Melting points of all compounds were on the high side (177 to > 360°C) as a result of the presence of amide bonds and the probable existence of intramolecular hydrogen bonding.

Scheme 1



i: oxalic acid/HCl/H₂O; *ii*: H₂NNH₂·H₂O; *iii*: pentane- or hexane-, or heptane-2,4-dione, or heptane-3,5-dione, or 2-thenoyltrifluoroacetone; *iv*: ethyl acetoacetate or ethyl benzoylacetate, or ethyl cyanoacetate; **3** R = R¹ = Me, **4** R = Me, R¹ = Et; **5** R = Me, R¹ = Pr; **6** R = R¹ = Et; **7** R = CF₃, R¹ = Th; **8** R = Me, **9** R = Ph, **10** R = NH₂

The IR spectra of compounds **1–10** showed absorption bands due to the stretching vibrations of N–H, C=O, C=C, and C=N at 3470-3132, 1705-1648, 1620-1600, and 1580-1509 cm⁻¹, respectively. The IR spectrum of compound **9** showed a broad band at 3302 cm⁻¹ due to the stretching vibration of N–H, while its two carbonyl stretching vibration appeared at 1703 and 1648 cm⁻¹, respectively. The IR absorption band at 1600 cm⁻¹ depicted the presence of aromatic C=C, while C=N band was observed at 1509 cm⁻¹. The UV-visible spectrum of compound **9** gave rise to the wavelength (λ_{max}) at 208 and 352 nm, while a shoulder was observed at 244 nm. The wavelength at 208 nm is a result of $\pi \rightarrow \pi^*$ transition of the phenyl ring, while 352 nm is a result of contribution from the pyrazolyl ring. The electronic transition in the UV-visible spectra gave rise to the wavelength

TABLE 1. Antibacterial screening (sensitivity testing) on bacteria with inhibition zones

Bacteria	Antibacterial activity*										Str.*2	
	1	2	3	4	5	6	7	8	9	10		
<i>Corynebacterium pyogenes</i> (LJO)	+	R	R	R	R	R	R	R	R	R	R	+++
<i>Bacillus polymyxa</i> (LJO)	++	R	R	R	R	R	R	R	++	R	R	++
<i>Bacillus stearothermophilus</i> (NCIB 8222)	+++	++	++	++	++	++	++	++	++	++	+++	++
<i>Bacillus subtilis</i> (NCIB 3610)	+++	+++	++	+	+	+	+	+	+++	+++	+++	+++
<i>Bacillus anthracis</i> (LJO)	+++	+++	++	++	++	+	+	+	+++	+++	+	++
<i>Bacillus cereus</i> (NCIB 6349)	+++	++	++	++	++	+	+	+	+++	+++	+++	+++
<i>Streptococcus faecalis</i> (NCIB775)	+++	++	+++	++	+	+	++	+	+++	+++	R	+++
<i>Staphylococcus aureus</i> (NCIB 85888)	+++	R	R	R	R	R	+	+	R	++	++	+++
<i>Clostridium sporogenes</i> (LJO)	+++	+	+	R	R	R	R	+	+	+	R	+++
<i>Escherichia coli</i> (NCIB 86)	+++	+++	+++	+++	+++	+++	++	+++	++	++	+++	R
<i>Pseudomonas fluorescens</i> (NCIB 3756)	R	R	R	R	R	R	R	++	R	++	R	+++
<i>Klebsiella pneumoniae</i> (NCIB 418)	++	+	++	++	+	+	+++	++	+	+	+	R
<i>Shigella dysenteriae</i> (LJO)	+++	+++	++	+	++	++	++	++	++	++	R	+++
<i>Pseudomonas aeruginosa</i> (NCIB 950)	+	++	++	++	+	++	R	++	++	++	R	+++

* R – resistance, + – less active (0.5–1.2 mm), ++ – moderately active (1.3–1.9 mm), +++ – highly active (2.0–3.1 mm).

*2 Str. – streptomycin.

TABLE 2. Minimum inhibitory concentration (MIC) test of the compounds on some selected bacteria

Bacteria	MIC, µg/ml										Str.	
	1	2	3	4	5	6	7	8	9	10		
<i>B. stearothermophilus</i> (NCIB 8222)	7.8	15.6	15.6	31.3	31.3	15.6	15.6	62.5	15.6	31.3	31.3	31.3
<i>Bacillus subtilis</i> (NCIB 3610)	62.5	15.6	15.6	250.0	250.0	31.3	62.5	62.5	7.8	7.8	7.8	7.8
<i>Bacillus anthracis</i> (LJO)	31.3	500.0	31.3	31.3	15.6	250.0	31.3	7.8	7.8	250.0	250.0	31.3
<i>Streptococcus faecalis</i> (NCIB 775)	7.8	250.0	7.8	15.6	250.0	250.0	7.8	31.5	7.8	7.8	R	15.6
<i>Bacillus cereus</i> (NCIB 6349)	7.8	15.6	15.6	15.6	31.3	62.5	15.6	7.8	7.8	62.5	62.5	31.3
<i>Escherichia coli</i> (NCIB 86)	62.5	7.8	7.8	15.6	15.6	15.6	62.5	7.8	15.6	7.8	7.8	R
<i>Pseudomonas fluorescens</i> (NCIB3756)	R	R	R	R	R	R	R	250.0	R	R	R	7.8
<i>Klebsiella pneumoniae</i> (NCIB 418)	7.8	250.0	7.8	31.3	62.5	31.3	15.6	62.5	7.8	250.0	250.0	R
<i>Shigella dysenteriae</i> (LJO)	15.6	31.3	250.0	62.5	62.5	31.3	31.3	31.3	62.5	62.5	R	62.5
<i>Pseudomonas aeruginosa</i> (NCIB 950)	7.8	15.6	15.6	15.6	250.0	31.3	R	250.0	31.3	31.3	R	R

(λ_{\max}) ranging from 205 to 372 nm. Bathochromic shift was observed in compounds **3-10** compared with the precursor **2**. This might be as a result of extensive conjugation of π -electrons from the pyrazolyl ring and the σ -donating character of alkyl groups experienced in the former. The chemical shifts and multiplicity patterns in the NMR spectra correlated well with the proposed structures. For instance, the ^1H NMR of compound **9** showed a broad singlet corresponding to the resonance of N–H of amide at δ 8.00 ppm, and it was exchangeable with D_2O . The multiplet at δ 7.52-7.94 ppm confirmed the presence of five aromatic protons of phenyl attached to the azomethine carbon in position 3 of the pyrazolyl ring, while the multiplet at δ 7.08-8.27 ppm indicated the presence of four aromatic protons of the benzo-fused part of the quinoxaline nucleus of compound **9**. The position of the pyrazole double bond was confirmed to be between nitrogen and carbon as a result of a singlet at δ 2.20 ppm, which was due to the presence of two methylene protons (pyrazole CH_2). The ^{13}C NMR of compound **9** revealed 17 carbon atoms with two $\text{C}=\text{O}$ having the highest signals at 165.0 and 158.0 ppm, while 14 sp^2 -hybridized carbon atoms appeared at 157.6-115.2 ppm. The one CH_2 carbon atom appeared to have the least signal at δ 35.1 ppm.

In addition, the mass spectrum of compound **9** showed a molecular ion peak at m/z 304 [M^+] with a relative intensity of 54.3%, its mass spectrum was also characterized by the occurrence of a base peak at m/z 227 [$\text{M}^+ - 77(\text{Ph})$], which was the result of loss of the phenyl radical. Out of the three pyrazolone derivatives **8-10** only compound **9** had the highest steric effect due to the presence of a bulky substituent, which is a weakly activating group in the C-3 position of pyrazolone ring, while compounds **8** and **10** had a low to moderate steric interaction due to the presence of smaller groups (CH_3 and NH_2).

Ten compounds **1-10** were screened *in vitro* for possible antibacterial activities using the agar well diffusion method [33], while the minimum inhibitory concentration test was carried out using the Russell and Furr method [34]. The sensitivity testing (inhibition zones, mm) of the compounds and streptomycin (a reference clinical antibiotic) in DMSO (solvent) at 1000 $\mu\text{g/ml}$ against nine Gram-positive and five Gram-negative bacterial strains is reported in Table 1. The results indicated that starting materials **1-2** and the reaction products **3-10** as well as streptomycin showed a broad spectrum against the bacterial strains.

Compound **3** has been synthesized before by the conventional heating method [35]; however, a microwave-assisted approach (MWI) has not been used for compound **3** to the best of our knowledge; also, its antibacterial activity has not been investigated. *Escherichia coli* and *Klebsiella pneumoniae* developed a resistance against streptomycin, whereas all the compounds were active on these two bacteria. Most of the compounds were not active on *Corynebacterium pyogenes*, *Bacillus polymyxa*, and *Pseudomonas fluorescens*, whereas streptomycin was active on them.

Considering the pyrazolyl derivatives **3-7**, compound **3** with a lower steric hindrance due to the presence of a smaller side chain (CH_3) was observed to have a larger inhibition zone and a lower MIC value of 7.8 $\mu\text{g/ml}$ as compared to compounds **4-7**. So, it was discovered that the occurrence of steric hindrance created by the substituents on the pyrazolyl ring might have a probable effect on the biological activity of such moieties. On the other hand, considering the pyrazolone ring of compounds **8-10**, only compound **9** experienced extensive conjugation due to the presence of the phenyl group in C-3 position. Thus, compound **9** had better activity as compared to compounds **8** and **10**. In fact, compound **9** was the most active on the *Bacillus* species at on MIC value of 7.8 $\mu\text{g/ml}$ except for *Bacillus anthracis*, where the inhibition was at a concentration of 15.6 $\mu\text{g/ml}$.

Based on the size of inhibition zones and the resistance degree observed, the minimum inhibitory concentration (MIC) test was selectively carried out on five Gram-positive and five Gram-negative bacterial isolates (Table 2). The MIC of compounds **1, 2** varied between 7.8 and 500 $\mu\text{g/ml}$, it was between 7.8 and 250 $\mu\text{g/ml}$ for compounds **3, 8, 10**, and between 15.6 and 250.0 $\mu\text{g/ml}$ for compounds **4-6**. Finally, compounds **7, 9** inhibited the bacterial growth at MIC between 7.8 and 62.5 $\mu\text{g/ml}$. Overall result indicated that out of all the compounds prepared, compound **9** had the highest activity at 7.8 $\mu\text{g/ml}$, while compounds **1, 3** appeared to compete favorably with streptomycin at 15.6 and 7.8 $\mu\text{g/ml}$ on both the Gram-positive and Gram-negative bacteria. In particular, the π - π interaction experienced in the phenyl side chain in compound **9** might also be a contributing factor to the reasonably high activity observed in compound **9**.

In summary, we constructed a workable pathway and synthesized a series of (3,5-alkyl(aryl)pyrazol-1-yl)quinoxalin-2(1H)-ones **3–7**, 3-alkyl(aryl)-5-oxo-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one **8, 9**, and 3-(3-amino-5-oxo-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one **10**, which were structurally confirmed by IR, UV, ¹H NMR, ¹³C NMR, and MS spectral analyses and evaluated for antibacterial activity by the growth inhibition of some gram-positive and gram-negative bacterial strains. By visualizing the antimicrobial data it could be observed that some of the compounds possess a significant activity. In fact, the results show that the compounds exhibited a high potency as antibacterial agents. The most active compound was 3-(5-oxo-3-phenyl-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one (**9**) with an MIC value of 7.8 µg/ml. Thus, the pyrazol-1-ylquinoxalin-2(1H)-one derivatives synthesized as well as the starting material, be promising for further activity optimization studies.

EXPERIMENTAL

Melting points were determined with an open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. IR spectra were recorded as KBr pellets on a Buck spectrometer, while UV-visible spectra were recorded on a Unicam spectrophotometer using methanol solvent. ¹H and ¹³C NMR were run on a Bruker AC-50 and JEOL-JNM-GX 400-MHz spectrometer in MeOH-d₄ (compounds **2-9**) and DMSO-d₆ (compound **10**) (δ in ppm relative to Me₄Si). Mass spectra were registered on a Finnigan MAT 312 machine. All compounds were routinely checked by TLC on silica gel G plates using the CHCl₃:MeOH (9:1, v/v) solvent system and the developed plates were visualized by UV light. The elemental analyses (C, H, N) of compounds were performed using a Carlo Erba-1108 elemental analyzer. The solvents used were of reagent grade and, when necessary, were purified and dried by standard methods. The microwave-assisted syntheses were carried out in a domestic oven, Midea PJ21B-A 400W.

1,2,3,4-Tetrahydroquinoxaline-2,3-dione (1). To a heated (to 100°C) and stirred solution of oxalic acid dihydrate (30.0 g, 238.0 mmol) in water (200 ml), concentrated HCl (45 ml) was added, followed by *o*-phenylenediamine (22.0 g, 204.0 mmol), with continuous stirring at 100°C for 20 min. The resulting mixture was cooled by addition of crushed ice (100 g) to give silvery white needles, which were collected by filtration, washed with water, and oven dried to afford compound **1** (32.3 g, 98.0%) as colorless needles; mp > 340°C (EtOH) (Lit. mp > 340°C [32, 36]). Other physical and spectroscopic data were identical to those of the authentic sample.

3-Hydrazinoquinoxalin-2(1H)-one (2). To a mixture of compound **1** (20.1 g, 124.0 mmol) and hydrazine hydrate (100.0 ml, 2.2 mol), water (50 ml) was added. and the resulting mixture was refluxed for 3 h. The mixture was allowed to cool and the precipitate formed was filtered off and recrystallized from ethanol to give compound **2** (19.8 g, 90%) as a yellow solid; mp > 360°C. IR spectrum, ν_{\max} , cm⁻¹: 3412 (N–H), 3280 (N–H), 3175 (N–H), 1679 (C=O), 1620 (C=C). UV spectrum, λ_{\max} (log ϵ_{\max}): 216 (4.34), 247 (3.75 s), 327 (3.61 s). ¹H NMR spectrum, δ , ppm: 5.81 (2H, br. s, NH₂, D₂O exchangeable); 7.09–8.26 (4H, m, H Ar); 8.14 (1H, s, NH, D₂O exchangeable); 12.55 (1H, s, NH, D₂O exchangeable). ¹³C NMR spectrum, δ , ppm: 158.0 (C=O), 157.6, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2. Mass spectrum, m/z (I_{rel} , %): 176 [M⁺] (55.5), 161 (92.3), 146 (85.5), 118 (100), 106 (80.1), 78 (40.5). Found, %: C 54.52; H 4.57; N 31.83. C₈H₈N₄O. Calculated, %: C 54.55; H 4.55; N 31.82.

3,5-Disubstituted pyrazolyl Derivatives 3-7 (General Method). To a solution of compound **2** (1.0 g, 5.7 mmol) in β -diketone (5.7 mmol), ethanol (10 ml) was added, and the mixture was irradiated in a domestic microwave oven (MW) at an emitted power of 400 W for the appropriate period. The clear solution formed was left to stand at room temperature to crystallize. The solid crude product was recrystallized from the appropriate solvent to afford 3,5-disubstituted (pyrazol-1-yl)quinoxalin-2(1H)-one.

3-(3,5-Dimethylpyrazol-1-yl)quinoxalin-2(1H)-one (3). Reagents: compound **2** (1.0 g, 5.7 mmol), acetyl acetone (1.0 ml, 5.7 mmol), ethanol (10 ml). Conditions: 3 min, 400 W, MWI. Purification: recrystallization. Yield (1.3 g, 99.0%) as a brown solid; mp 177–179°C (EtOH). IR spectrum, ν_{\max} , cm^{-1} : 3436 (N–H), 1679 (C=O), 1618 (C=C). UV spectrum, λ_{\max} (log ϵ_{\max}): 208 (4.28), 239 (3.97 s), 325 (3.66 s), 352 (3.73). ^1H NMR spectrum, δ , ppm: 2.30 (3H, s, CH_3); 2.48 (3H, s, CH_3); 6.18 (1H, s, $-\text{CH}=\text{}$); 7.09–8.27 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 157.6 (C=O), 152.3, 149.4, 143.2, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 110.2, 13.5 (CH_3), 13.2 (CH_3). Mass spectrum, m/z (I_{rel} , %): 240 [M^+] (100), 226 (68.3), 212 (70.5), 146 (88.3), 106 (45.1), 78 (55.0). Found, %: C 65.02; H 5.01; N 23.36. $\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}$. Calculated, %: C 65.00; H 5.00; N 23.33.

3-(5-Ethyl-3-methylpyrazol-1-yl)quinoxalin-2(1H)-one (4). Reagents: Compound **2** (1.0 g, 5.7 mmol), hexane-2,4-dione (0.7 ml, 5.7 mmol), ethanol (10 ml). Conditions: 5 min, 400 W, MWI. Purification: recrystallization. Yield 1.35 g (93.1%) as a colorless solid; mp 183–185°C (EtOH). IR spectrum, ν_{\max} , cm^{-1} : 3430 (N–H), 1675 (C=O), 1620 (C=C). UV spectrum, λ_{\max} (log ϵ_{\max}): 210 (4.12), 240 (3.95 s), 330 (3.85 s), 350 (3.51). ^1H NMR spectrum, δ , ppm (J , Hz): 1.25 (3H, t, $J = 7.2$, CH_3); 2.30 (3H, s, CH_3); 3.07 (2H, q, $J = 7.2$, CH_2); 6.20 (1H, s, $-\text{CH}=\text{}$); 7.08–8.27 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 157.6 (C=O), 152.3, 149.4, 144.5, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 104.9, 20.8 (CH_2), 13.2 (CH_3), 12.7 (CH_3). Mass spectrum, m/z (I_{rel} , %): 254 [M^+] (89.1), 224 (66.3), 110 (100), 66 (57.5). Found, %: C 66.09; H 5.48; N 22.00. $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}$. Calculated, %: C 66.14; H 5.51; N 22.05.

3-(3-Methyl-5-propylpyrazol-1-yl)quinoxalin-2(1H)-one (5). Reagents: Compound **2** (1.0 g, 5.7 mmol), heptane-2,4-dione (0.8 ml, 5.7 mmol), ethanol (10 ml). Conditions: 5 min, 400 W, MWI. Purification: recrystallization. Yield 1.38 g (90.2%) as a colourless solid; mp 204–205°C (EtOH). IR spectrum, ν_{\max} , cm^{-1} : 3433 (N–H), 1685 (C=O), 1615 (C=C), 1579 (C=N). UV spectrum, λ_{\max} (log ϵ_{\max}): 208 (4.05), 244 (3.88), 338 (3.63 s), 370 (4.13). ^1H NMR spectrum, δ , ppm (J , Hz): 0.90 (3H, t, $J = 7.3$, CH_3); 1.65 (2H, sextet, $J = 7.3$, CH_2); 2.30 (3H, s, CH_3); 2.44 (2H, t, $J = 7.3$, CH_2); 6.19 (1H, s, $-\text{CH}=\text{}$); 7.09–8.27 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 157.6 (C=O), 152.3, 149.4, 144.5, 142.7, 131.7, 129.1, 125.9, 123.5, 115.3, 104.9, 31.1, 22.6, 13.7 (CH_3), 13.2 (CH_3). Mass spectrum, m/z (I_{rel} , %): 268 [M^+] (70.1), 240 (65.2), 146 (85.3), 124 (52.1), 118 (100), 96 (92.3). Found, %: C 67.18; H 5.99; N 20.87. $\text{C}_{15}\text{H}_{16}\text{N}_4\text{O}$. Calculated, %: C 67.16; H 5.97; N 20.90.

3-(3,5-Diethylpyrazol-1-yl)quinoxalin-2(1H)-one (6). Reagents: Compound **2** (1.0 g, 5.7 mmol), heptane-3,5-dione (0.8 ml, 5.7 mmol), ethanol (10 ml). Conditions: 5 min, 400 W, MWI. Purification: recrystallization. Yield 1.41 g (92.2%) as a colorless solid: mp 192–193°C (EtOH/DMF). IR spectrum, ν_{\max} , cm^{-1} : 3430 (N–H), 1690 (C=O), 1605 (C=C). UV spectrum, λ_{\max} (log ϵ_{\max}): 209 (4.05), 245 (3.91), 372 (3.61). ^1H NMR spectrum, δ , ppm (J , Hz): 1.25 (6H, t, $J = 7.2$, 2CH_3); 3.07 (4H, q, $J = 7.2$, 2CH_2); 6.22 (1H, s, $-\text{CH}=\text{}$); 7.10–8.28 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 157.6 (C=O), 149.4, 146.8, 144.5, 142.7, 131.7, 129.1, 125.9, 123.5, 115.2, 104.9, 20.8 (CH_2), 19.5 (CH_2), 13.2 (CH_3), 12.7 (CH_3). Mass spectrum, m/z (I_{rel} , %): 268 [M^+] (72.5), 240 (57.4), 212 (68.2), 146 (80.8). Found, %: C 67.13; H 5.92; N 20.92. $\text{C}_{15}\text{H}_{16}\text{N}_4\text{O}$. Calculated, %: C 67.16; H 5.97; N 20.90.

3-[5-Thien-2-yl-3-(trifluoromethyl)pyrazol-1-yl]quinoxalin-2(1H)-one (7). Reagents: Compound **2** (1.0 g, 5.7 mmol), 2-thenoyltrifluoroacetone (1.3 g, 5.7 mmol), 30 ml of DMF–ethanol, 1:5. Conditions: 2 min, 400 W, MWI. Purification: recrystallization. Yield 2.0 g (97.0%) as an orange solid; mp 328–330°C (EtOH). IR spectrum, ν_{\max} , cm^{-1} : 3241 (N–H), 1675 (C=O), 1620 (C=C), 1575 (C=N). UV spectrum, λ_{\max} (log ϵ_{\max}): 205 (3.38), 225 (3.00), 237 (2.51), 273 (3.10), 298 (3.70). ^1H NMR spectrum, δ , ppm: 6.02 (1H, s, pyrazol $-\text{CH}=\text{}$); 7.69–7.17 (3H, m, H Th); 7.09–8.27 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 157.6 (C=O), 149.4, 142.7, 139.9, 136.4, 131.7, 131.2, 129.1, 128.6, 128.0, 127.6, 125.9, 123.5, 121.4, 115.2, 105.9. Mass spectrum, m/z (I_{rel} , %): 362 [M^+] (68.3), 294 (100), 280 (31.5), 212 (70.4), 146 (85.1). Found, %: C 53.06; H 2.50; N 15.49. $\text{C}_{16}\text{H}_9\text{F}_3\text{N}_4\text{OS}$. Calculated, %: C 53.04; H 2.49; N 15.47.

3-(5-Oxo-3-R-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-ones 8, 9 (General Method). To anhydrous compound **2** (1.0 g, 5.7 mmol) a homogeneous mixture of β -keto ester (28.5 mmol) and ethanol (20 ml) was added dropwise at room temperature. The mixture was irradiated in a microwave oven at 15-s interval for the appropriate period to give a clear solution, which was left to stand at room temperature. The solid crude product was recrystallized from the appropriate solvent to afford compounds **8, 9**.

3-(3-Methyl-5-oxo-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one (8). Reagents: Compound **2** (1.0 g, 5.7 mmol), ethyl acetoacetate (3.6 ml, 28.5 mmol), ethanol (20 ml). Conditions: 3 min, 400 W, MWI. Purification: recrystallization. Yield 1.3 g (94.2%) as a yellow solid; mp 316–318°C (EtOH). IR spectrum, ν_{\max} , cm^{-1} : 3448 (N–H), 1705 (C=O), 1667 (C=O), 1610 (C=C). UV spectrum, λ_{\max} ($\log \epsilon_{\max}$): 220 (3.13), 305 (5.10), 320 (5.43 s). ^1H NMR spectrum, δ , ppm: 1.94 (3H, s, CH_3); 2.20 (2H, s, CH_2 pyrazol); 7.09–8.29 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 165.0 (C=O), 159.5 (C=O), 158.2, 157.3, 142.4, 131.8, 129.0, 125.8, 123.6, 115.1, 43.2, 16.4 (CH_3). Mass spectrum, m/z (I_{rel} , %): 242 [M^+] (54.3), 228 (83.4), 214 (100), 200 (75.4), 146 (86.1), 106 (24.5). Found, %: C 59.52; H 4.14; N 23.16. $\text{C}_{12}\text{H}_{10}\text{N}_4\text{O}_2$. Calculated, %: C 59.50; H 4.13; N 23.14.

3-(5-Oxo-3-phenyl-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one (9). Reagents: Compound **2** (1.0 g, 5.7 mmol), ethyl benzoylacetate (4.9 ml, 28.3 mmol), ethanol (20 ml). Conditions: 2 min, 400 W, MWI. Purification: recrystallization. Yield 1.7 g (99.0%) as a yellow solid; mp 287–288°C (EtOH). IR spectrum, ν_{\max} , cm^{-1} : 3302 (NH), 1703 (C=O), 1648 (C=O), 1600 (C=C), 1509 (C=N). UV spectrum, λ_{\max} ($\log \epsilon_{\max}$): 208 (4.29), 244 (4.09 s), 352 (3.76). ^1H NMR spectrum, δ , ppm: 2.20 (2H, s, CH_2 pyrazol); 7.09–8.27 (4H, m, H Ar); 7.52–7.94 (5H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 165.0 (C=O), 158.0 (C=O), 157.6, 155.6, 142.7, 134.0, 131.7, 131.0, 129.1, 128.8, 128.2, 125.9, 123.5, 115.2, 35.1 (CH_2). Mass spectrum, m/z (I_{rel} , %): 304 [M^+] (54.3), 227 (100). Found, %: C 67.09; H 3.94; N 18.40. $\text{C}_{17}\text{H}_{12}\text{N}_4\text{O}_2$. Calculated, %: C 67.11; H 3.95; N 18.42.

3-(3-Amino-5-oxo-4,5-dihydropyrazol-1-yl)quinoxalin-2(1H)-one (10). To anhydrous compound **2** (1.0 g, 5.7 mmol) dissolved in ethyl cyanoacetate (0.6 ml, 5.7 mmol) ethanol (30 ml) was added in an open beaker and the mixture was swirled thoroughly, then irradiated in a microwave oven at 15-s interval for 3 min to give a clear solution, which was left to stand at room temperature. The solid crude product obtained was recrystallized from ethanol to afford compound **10** (0.7 g, 50.0 %) as an orange solid; mp 335–338°C. IR spectrum, ν_{\max} , cm^{-1} : 3470 (NH), 3448–3132 (NH), 1667 (C=O), 1605 (C=C), 1580 (C=N). UV spectrum, λ_{\max} ($\log \epsilon_{\max}$): 216 (6.37), 304 (5.82), 330 (5.68 s), 336 (5.32), 348 (5.39). ^1H NMR spectrum, δ , ppm: 2.20 (2H, s, CH_2 pyrazol); 7.12–8.29 (4H, m, H Ar); 8.00 (1H, s, NH, D_2O exchangeable); 8.51 (2H, s, NH_2 , D_2O exchangeable). ^{13}C NMR spectrum, δ , ppm: 165.1 (C=O), 160.1 (C=O), 158.2, 157.8, 142.9, 132.1, 129.3, 125.9, 123.7, 115.0, 71.8. Mass spectrum, m/z (I_{rel} , %): 243 [M^+] (75). Found, %: C 54.33; H 3.72; N 28.83. $\text{C}_{11}\text{H}_9\text{N}_5\text{O}_2$. Calculated, %: C 54.32; H 3.70; N 28.81.

Antibacterial activity assays

Most of the organisms used were standard bacteria of the National Collection for Industrial Bacteria (NCIB), while a few others were locally isolated organisms (LIO). The Gram-positive bacteria were *Bacillus cereus* (NCIB 6349), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus subtilis* (NCIB 3610), *Bacillus anthracis* (LIO), *Bacillus polymyxa* (LIO), *Corynebacterium pyogenes* (LIO), *Streptococcus faecalis* (NCIB775), *Staphylococcus aureus* (NCIB 8588), and *Clostridium sporogenes* (LIO), while the Gram-negative ones were *Escherichia coli* (NCIB 86), *Pseudomonas fluorescens* (NCIB 3756), *Klebsiella pneumoniae* (NCIB 418), *Shigella dysenteriae* (LIO), and *Pseudomonas aeruginosa* (NCIB 950).

All the synthesized compounds **1–10** and streptomycin were screened for antibacterial activity on nine Gram-positive and five Gram-negative bacterial strains using the agar well diffusion method [33].

With the aid of a sterile 1-ml pipette, about 0.2 ml of the broth culture of the test organism was added to an 18-ml sterile molten diagnostic sensitivity test agar (Biotech, Ltd.), which was already cooled down to 45°C. This was well mixed and poured into previously sterilized Petri dishes, which were properly labelled according to the test organisms. The medium was then allowed to set. With the aid of a sterile cork borer the required number of holes was bored into the medium. The wells were made of about 5 mm to the edge of the plate. The wells were then filled up aseptically with the solution of the compound in DMSO using Pasteur pipettes. Streptomycin was used as a standard antibacterial agent at a concentration of 1000 µg/ml. The plates were allowed to stand for about 1 h on the bench for proper diffusion of the antibacterial agents into the medium and then incubated uprightly at 37°C for 24 h. Care was taken not to stockpile the plates. Clear inhibition zones, mm, indicated the relative susceptibility of the bacteria to compounds **1-10** and streptomycin standard.

The minimum inhibitory concentration (MIC) test was detected using the method of Russell and Furr [34]. Based on the level of resistance of some organisms and large inhibition zones experienced in others, MIC test was selectively done for five Gram-positive and five Gram-negative bacterial strains. Different concentrations (7.8 and 1000.0 µg/ml) of the compounds and standards were prepared using a twofold dilution, which was prepared in a sterile plate with a 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 strains. The plates were then labelled accordingly and incubated at 37°C for up to 72 h. They were subsequently examined for the presence or absence of growth. The lowest concentration preventing bacteria growth was taken as the MIC of the compounds. This procedure was likewise repeated for streptomycin.

REFERENCES

1. L. Zhenjiang, L. Weisi, S. Yingjie, and H. He, O. Pingkai, *J. Heterocycl. Chem.*, **45**, 285 (2008).
2. C. A. Obafemi, G. Olayiwola, and F. O. Taiwo, *African J. Biotech.*, **6**, 777 (2007).
3. G. S. Welmaker, J. A. Nelson, J. E. Sabalski, A. L. Sabb, J. R. Potoski, D. Graziano, M. Kagan, J. Coupet, J. Dunlop, H. Mazandarani, S. Rosenzweig-Lipson, S. Sukoff, and Y. Zhang, *Bioorg. Med. Chem. Lett.*, **10**, 1991 (2000).
4. M. A. J. Al-Mossawi, A. A. Salem, M. Salam, and A. Arani, *Environ. Int.*, **5**, 141 (1981).
5. H. M. Refaat, A. A. Moneer, and O. M. Khalil, *Arch. Pharm. Res.*, **27**, 1093 (2004).
6. M. M. Ali, M. M. F. Ismail, M. S. A. El-Gaby, M. A. Zahran, and Y. A. Ammar, *Molecules*, **5**, 864 (2000).
7. C. A. Obafemi and D. A. Akinpelu, *Phosphorus, Sulfur, Silicon, Relat. Elem.*, **180**, 1795 (2005).
8. Ch. Sridevi, K. Balaji, A. Naidu, S. Kavimani, D. Vankappayya, R. Suthakaran, and S. Parimala, *Int. J. Pharmatech. Res.*, **1**, 816 (2009).
9. A. Carta, P. Sanna, U. D. Gherardini, and S. Zanetti, *Farmaco*, **56**, 933 (2001).
10. P. Sanna, A. Carta, M. Loriga, S. Zanetti, and L. Sechi, *Farmaco*, **54**, 161 (1999).
11. M. A. H. Hamida, *Carbohydr. Res.*, **338**, 2301 (2003).
12. J. B. Rangisetty, C. N. V. H. B. Gupta, A. L. Prasad, P. Srinivas, N. Sridhar, P. Parimoo, and A. Veeranjanyulu, *J. Pharm. Pharmacol.*, **53**, 1409 (2001).
13. M. Kodama, N. Yamada, K. Sato, Y. Kitamura, F. Koyama, T. Sato, K. Morimoto, and S. Kuroda, *Eur. J. Pharmacol.*, **374**, 11 (1999).
14. R. Sarge, H. R. Howard, R. G. Browne, L. A. Lebel, P. A. Seymour, and B. K. Kve, *J. Med. Chem.*, **33**, 2240 (1990).
15. A. Jaso, B. Zarranz, I. Aldana, and A. Mongye, *Eur. J. Med. Chem.*, **38**, 791 (2003).
16. M. M. Badran, K. A. M. Abouzid, and M. H. M. Hussein, *Arch. Pharm. Res.*, **26**, 107 (2003).

17. U. J. Ries, H. W. Priekpe, N. H. Havel, S. Haandschuh, G. Mihm, J. M. Stassen, W. Wienen, and H. Nar, *Bioorg. Med. Chem. Lett.*, **13**, 2297 (2003).
18. L. Fengcai, W. Baigeng, and C. Jinbao, *Polym. Sci. Tech.*, **26**, 261 (1984).
19. J. P. Critchley, (editor), *Polymers: thermally stable quinoxaline polymers*, in: *Encyclopedia of Physical Science and Technology*, 3rd ed., Acad. Press, 2002, p. 775.
20. A. F. R. Sherif, A. S. Manal, and A. E.-D. Maha, *Eur. J. Med. Chem.*, **38**, 959 (2003).
21. A. B. Adnan, M. A. A. Hayam, S. A. G. Yasser, E.-D. A. B. Alan, and B. Azza, *Eur. J. Med. Chem.*, **43**, 456 (2008).
22. W. W. Wagnat and A. L. Nadia, *J. Chilean Chem. Soc.*, **52**, 1145 (2007).
23. D. Zainaba, L. Meryem, S. Abdelfatah, B. Abdelmejid, H. Mohammed, K. Said, and B. Mohammed, *Arch. Pharm.*, **339**, 291 (2006).
24. I. Bouabdallah, L. A. M'Barek, A. Ziad, A. Ramdani, I. Zidane, and A. Melhaoui, *Nat. Prod. Res.*, **20**, 1024 (2006).
25. A. Şener, E. Akbas, and M. K. Şener, *Turkey J. Chem.*, **28**, 271 (2004).
26. H. E. El Sayed, F. A. Kamal, A.-E. Salah, and B. Razika, *J. Carbohydr. Chem.*, **26**, 1 (2007).
27. Q. Jairo, C. Debora, I. Braulio, A. Rodrigo, C. Silvia, N. Manuel, and C. Justo, *J. Heterocycl. Chem.*, **45**, 155 (2008).
28. X. Deng and N. S. Mani, *Org. Lett.*, **8**, 3505 (2006).
29. L. Pezdirc, V. Groselj, A. Meden, B. Stanovnik, and J. Svete, *J. Heterocycl. Chem.*, **45**, 181 (2008).
30. P. Wang, Z. Xie, Z. Hong, J. Tang, O. Wong, C.-S. Lee, N. Wong, and S. Lee, *J. Mat. Chem.*, **13**, 1894 (2003).
31. S. M. Allin, W. R. Bowman, M. R. J. Elsegood, V. Mckee, R. Karim, and S. S. Rahman, *Tetrahedron*, **61**, 2689 (2005).
32. C. A. Obafemi and W. Pfeleiderer, *Helv. Chim. Acta.*, **77**, 1549 (1994).
33. L. P. Carrod and F. D. Grady, *Antibiotics and Chemotherapy*, 3rd ed., Churchill Livingstone, Edinburgh, 1972, p. 477.
34. A. D. Russell and J. R. Furr, *J. Appl. Bacteriol.*, **43**, 253 (1977).
35. N. Rashed, A. M. El Massry, E.-S. H. El Ashry, A. Amer, and H. Zimmer, *J. Heterocycl. Chem.*, **27**, 691 (1990).
36. G. W. H. Cheeseman and M. Rafiq, *J. Chem. Soc., C*, 452 (1971).