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A FACILE SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL QUINOXALINE-BENZOFURAN HYBRIDS

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Abstract – In the present work, a simple and facile synthesis of a series of new type of quinoxaline-benzofuran hybrids, i.e., 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acids has been achieved using the newly-synthesized ethyl 3-bromomethylquinoxaline-2-carboxylate as substrate through ultrasound-assisted one-pot sequential Rap-Stoermer type reaction with various salicylaldehydes followed by ester hydrolysis. A preliminary screening for their antibacterial activities against five bacterial strains revealed that compounds with *tert*-butyl and halo (Cl and Br) substituents exhibited promising inhibitory activity against *B. subtilis* with the MIC values of 15.625 and 7.8125 µg/mL, respectively, being equipotent or even better than the reference Ciprofloxacin.

Among nitrogen-containing heterocycles, quinoxaline ring is an important structural motif present as pivotal skeleton in numerous significant natural products and pharmacologically relevant compounds with significant biological properties such as anticancer,¹ antimycobacterial² and antibacterial activities.³ In this regard, a recent advance on pharmacological activities of quinoxaline derivatives has been reviewed by Tariq *et al.*⁴ Due to their striking biological activities and in order to have structurally diversified molecules for bio-screening, considerable synthetic efforts have been devoted surrounding the quinoxaline ring for further modification and functionalization by both organic and medicinal chemists with the aim of enhancing the potency of this class of compounds.⁵⁻⁷ Especially, in recent years the synthesis and antibacterial activity evaluation of various quinoxaline-heterocycle hybrids have frequently been reported for current antibacterial drug studies. For example, Kamila *et al.*⁸ reported an easy access to novel series of quinoxaline-indole hybrids, which exhibited important antibacterial activity. Recently,

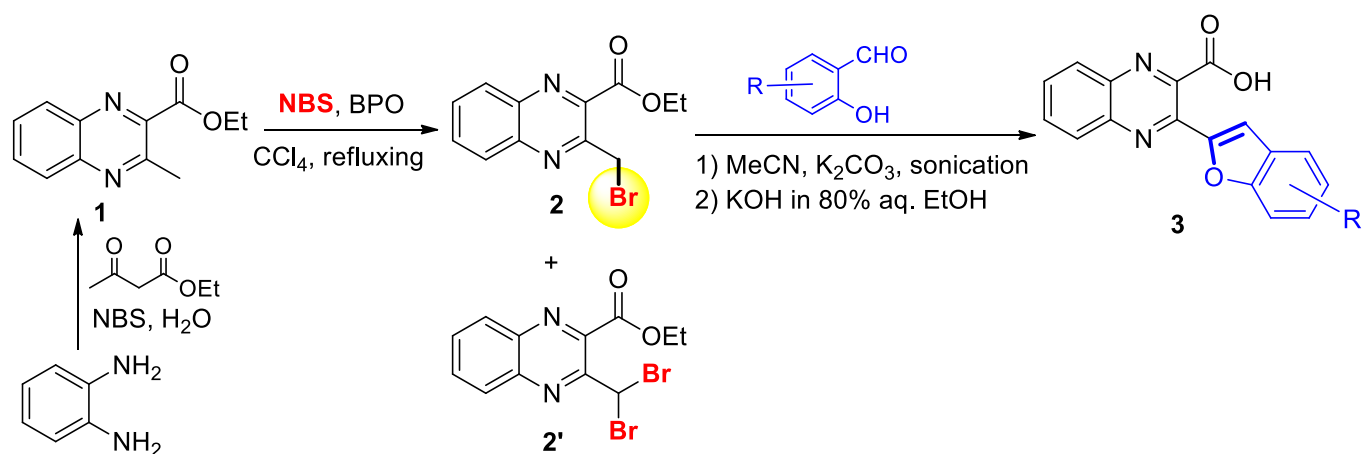
El-Attar *et al.*⁹ described the synthesis and antibacterial activity of structurally intriguing quinoxaline-pyrazole and quinoxaline-thiazole hybrids. In this context, Patel *et al.*¹⁰ synthesized newer quinoxaline-oxadiazole hybrids with potent antibacterial and antifungal properties.

On the other hand, benzofuran has been an attractive synthetic target because this moiety is one of the privileged medicinal scaffold and has a wide application in the design and synthesis of diverse bioactive molecules.¹¹ Especially, a review by Hiremathad *et al.*¹² on recent progress of the antibacterial and antimycobacterial activities of benzofuran derivatives highlighted their importance in the development of new pharmaceutical products to overcome the increasing danger of drug-resistant problems. As a consequence, the efficient incorporation of benzofuran unit into organic molecules for the design and synthesis of more novel and interesting types of benzofuran-containing compounds has been a topic of great synthetic interest.¹³⁻¹⁵ In addition, Maresca *et al.*¹⁶ have reported that many carboxylic acids incorporating various aromatic/heterocyclic scaffold showed significant potential for developing antibacterial agents with a diverse mechanism of action compared to the clinically used drugs.

Keeping these findings in mind and in view of molecular hybridization playing a prominent role in medicinal and combinatorial chemistry,¹⁷ we felt it would be an attractive idea to incorporate benzofuran ring, quinoxaline moiety and carboxyl functional group in one molecular platform, because it might lead to a new dimension of structural diversity as potential candidates for biological evaluation. A literature survey revealed that there is no related studies reported concerning such synthesis, probably due to the lack of a suitable synthetic method to construct them. Thus, we would like to report herein the synthesis and antibacterial evaluation of a series of structurally intriguing benzofuran-quinoxaline-2-carboxylic acid hybrids, namely 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acid derivatives.

In 2011, Kumar *et al.*¹⁸ reported a facile NBS-mediated one-pot reaction of phenylenediamine with ethyl acetoacetate for the synthesis of ethyl 3-methylquinoxaline-2-carboxylate (**1**), but to our knowledge, the further transformation by NBS radical bromination reaction at its 3-methyl position has never reported. As well known, halomethyl-functionalized aromatic heterocycles have been widely served as versatile building blocks for the construction of various intriguing and complex small molecules.^{19,20} For example, Wang's group have reported the use of 2-chloromethylpyrido[1,2-*a*]pyrimidines as important building blocks for the construction various bioactive polycyclic-fused heterocycle systems.²¹⁻²³ In this context, our research group has gained ability in the application of ethyl 2-(halomethyl)quinoline-3-carboxylates as the key intermediates for the flexible synthesis of diversely substituted quinoline-based hybrids.²⁴⁻²⁷ Building on the evolving expertise, we envisioned that if the 3-bromomethyl functionalization of quinoxaline **1** could be achieved, the resulting ethyl 3-(bromomethyl)quinoxaline-2-carboxylate might open opportunity for the construction of the desired 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acid hybrids. Accordingly, based on the synthetic plan we devised a synthetic route as outlined in Scheme 1,

which did not involve the use of expensive reagents or catalysts and would contribute to the usefulness of this method.



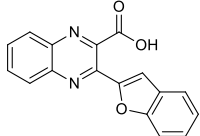
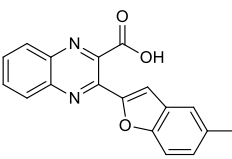
Scheme 1. Synthetic route for 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acids (**3**)

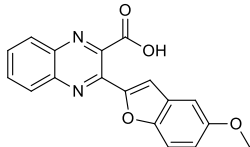
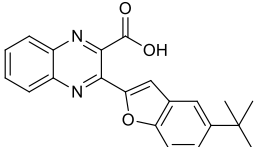
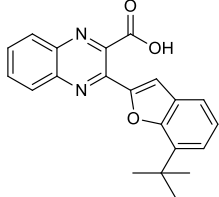
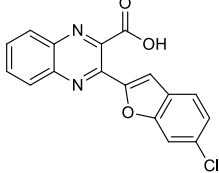
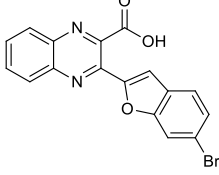
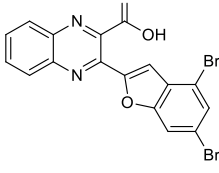
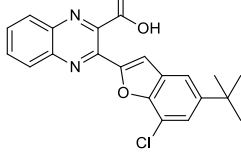
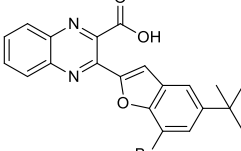
Thus, the substrate **1**, readily available following the protocol of the literature,¹⁸ was first subjected to radical bromination reaction with 1.2 equiv. of NBS in refluxing CCl₄ with the presence of catalytic amount of benzoyl peroxide (BPO) as initiator. We observed that the order of addition of NBS had an obvious influence on the product yield. When the amount of 1.2 equiv. of NBS was added simultaneously together with the substrate **1** to CCl₄ solution, the radical bromination reaction was suffered from low yield because the desired monobromo product **2** was always accompanied by the excess *gem*-dibromo byproduct **2'** and small amount of unreacted substrate **1**. Moreover, due to the close polarities of the three compounds, their isolation became very tedious and cumbersome. In order to characterize the structures of **2** and **2'**, we attempted to isolate them by careful column chromatography over silica gel using petroleum ether/ethyl acetate mixture as eluent (15:1, v/v). After repeating the column chromatography separation three times the desired monobrominated product **2** was obtained in 49% yield, along with 30% of the dibromide byproduct **2'**, 12% unreacted **1** as well as small quantities of tarry products. This is a problem that is common to numerous radical bromination protocols.²⁸ We found that if the NBS was added in batches in the amount of 1/3 portions of 1.2 equiv. every 1.5~2.0 h to the gently refluxing CCl₄ solution,²⁷ a 22% increase in the product yield to 71% was achieved with a small amount of byproduct after the reaction was complete. Presumably, the resulting Br₂ derived from NBS remain in a low concentration throughout the course of the bromination reaction, thereby restraining the side reaction and leading to the formation of the desired product in a higher yield. The newly resulting **2** represents a valuable scaffold and would be highly useful in the field of synthetic organic chemistry. Its structure was easily confirmed by the ¹H and ¹³C NMR spectral data. In its ¹H NMR spectrum no signal attributable to the methyl protons of its precursor was observed, but instead a two-proton singlet at 5.16 ppm was found,

readily recognizable as arising from bromomethyl protons, supporting the signal of its ^{13}C NMR spectrum at 62.98 ppm for the methylene carbon. Similarly, the structure of the dibromide byproduct **2'** was also readily established according to its spectral data (see Experimental section).

Having the desired monobrominated **2** in hand, our attention was focused on its reaction with various salicylaldehydes for building the targeted products 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acids (**3**). Initially, we investigated the reaction with salicylaldehyde according to our previously reported method.²⁴ However, this purported approach was unsatisfactory in this case and the expected 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acid (**3a**) was obtained only in a modest yield of 51% even after refluxing 24 h. Further attempts by switching the solvents or bases were also to no avail. After these unfruitful attempts, we were delight to find that upon the reaction was adopted in conjunction with ultrasonic irradiation with the conditions otherwise remaining unchanged, an obvious improvement in terms of product yield and reaction time could be achieved with **3a** being obtained in a good yield of 77% within 6 h. The possible reason for this might be due to the phenomenon of cavitation produced by ultrasound, which generates the inducing high local temperature and pressure when it collapses,²⁹ and thus activate the reaction mixture and push forward, thereby resulting in an increase in the reaction rate. Subsequently, different substituted salicylaldehydes available in our laboratory containing mono- or di-substituents with differing electronic properties were subjected to the reaction sequence in a similar fashion. As expected, these salicylaldehydes were equally amenable to the reaction process without any experimental difficulties, successfully furnishing the corresponding **3b-j** in satisfactory yields of 62-79% as listed in Table 1. However, upon salialdehydes bearing strong electron-withdrawing groups such as F, CN, and NO_2 , the reaction produced intractable mixtures as observed on TLC, from which we could not obtain the desired products in appreciable yields. Work is currently ongoing and more studies toward extending the reaction scope will be part of our future efforts.

Table 1. Yields and physical properties of the compounds **3a-j**

Entry	Product	3a-j	Time /h	Yield /% ^a	Mp/°C
1		3a	6	77	153-154
2		3b	6	74	182-183

3		3c	6	71	170-171
4		3d	6	73	154-155
5		3e	8	65	153-154
6		3f	6	76	165-166
7		3g	6	78	204-205
8		3h	6	79	209-210
9		3i	10	62	159-161
10		3j	10	67	150-151

^aIsolated yield.

To the best of our knowledge, all these newly synthesized compounds **3a-j** have never been reported, and their structures were explicitly characterized based on the spectral and analytical data. As an example, the ¹H NMR spectrum of **3a** exhibited the absence of the singlet signal of bromomethyl protons and the

triplet and quartet signal set due to the ethyl ester protons, but instead contained a readily recognizable furan proton singlet at 7.74 ppm and a broad carboxyl proton singlet at 14.44 ppm, along with the signals for 8 aromatic protons between 7.34 and 8.18 ppm, which was consistent with the introduction of the nascent benzofuran ring moiety to the quinoxaline ring. Further, its ^{13}C NMR spectrum was also in good agreement with the assigned structure **3a**, which revealed the presence of 16 signals at 122.55-153.81 ppm due to the aromatic carbons together with carboxyl carbon at 166.64 ppm.

After successfully synthesizing a series of the desired benzofuran-quinoxaline hybrids, a preliminary evaluation for their *in vitro* antibacterial activities against *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) as Gram (+), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) as Gram (–) and *Mycobacterium smegmatis* (*M. smegmatis*) was assayed by measuring their minimum inhibitory concentrations (MICs). The results were recorded in Table 2.

Table 2. *In vitro* antibacterial activity of **3a-j** [MIC/($\mu\text{g mL}^{-1}$)]

Compd.	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>M. smegmatis</i>
3a	125	125	250	125	250
3b	125	125	250	250	250
3c	250	250	250	250	250
3d	31.25	125	125	125	250
3e	125	250	125	250	250
3f	125	125	250	250	250
3g	62.5	125	250	250	250
3h	62.5	62.5	250	125	250
3i	15.625	125	125	250	250
3j	7.8125	62.5	250	250	250
Ciprofloxacin	15.625	15.625	15.625	15.625	– ^a
Rifampicin	–	–	–	–	15.625

^a – Means no test.

As shown in Table 2, all these newly-synthesized compounds **3a-j** exhibited moderate to good *in vitro* antibacterial activity against *B. subtilis*, weak activity against *S. aureus* and poor activity against the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and *M. smegmatis*. For the *B. subtilis*, it was observed that the introduction of methyl group as in compound **3b** resulted in no obvious improvement in its activity compared with the unsubstituted compound **3a**, while the presence of methoxy group as in

compound **3c** disfavored its inhibitory activity. Interestingly, the 5-*tert*-butyl substituted **3d** was found to be beneficial for the inhibitory activity, exhibiting higher MIC value (31.25 ug/mL) than **3a-c**. The isomer **3e**, although also contains a *tert*-butyl moiety, showed a relatively low activity profile (125 ug/mL). This observation might suggest that the introduction of *tert*-butyl substituent at the 5-position of benzofuran ring was more favorable for enhancing the inhibitory activity. Especially, we noticed that compounds **3i** and **3j** with 5-*tert*-butyl and 7-halo (Cl and Br) substitution showed more significant inhibitory activity against *B. subtilis* with the MIC values of 15.625 ug/mL and 7.8125 ug/mL, respectively, being equipotent or even better than the reference drug. This insight might provide valuable information for further optimization of the series of derivatives. Our next efforts will mainly focus on the structural optimization and structural activity relationship study by exploring more structural diversity towards the ultimate goal of providing intriguing lead compounds for the development of new and effective antibacterial agents.

In summary, we have demonstrated the first synthesis of ethyl 3-bromomethylquinoxaline-2-carboxylate as a new type of building block for the flexible construction of a series of hitherto unreported 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acid hybrids. The synthetic protocol described here might be attractive as it is simple and does not involve the use of expensive reagents or catalysts. Antibacterial activity of these newly synthesized products has been studied by employing five bacterial strains, among which **3i** and **3j** bearing both 5-*tert*-butyl and 7-halo substituents were found to be promising with respect to the inhibitory activity against *B. subtilis* with MIC values of 15.625 μ g/mL and 7.8125 μ g/mL, respectively, being equipotent or even better than the reference Ciprofloxacin. In addition, due to the presence of a derivatizable carboxyl functional group, further studies on the structural modification and optimization for the possible improvements in their biological activities represent an intriguing goal that are ongoing in our laboratory and the results will be communicated in due course.

EXPERIMENTAL

All chemicals (analytical grade) were commercially available and used without further purification. The melting points were determined by using WRS-1B melting points apparatus and were uncorrected. Ultrasonication was performed in a KQ-250B medical ultrasound cleaner with a frequency of 40 kHz and an output power of 250 W (built-in heating 30-80 °C thermostatically adjustable). ^1H and ^{13}C NMR spectra were recorded on an Agilent 400-MR spectrometer (400 and 100 MHz, respectively) using CDCl_3 (compounds **2** and **2'**) or $\text{DMSO}-d_6$ (compounds **3a-j**) as the solvent. The reported chemical shifts (δ values) are given in parts per million downfield from tetramethylsilane (TMS) as the internal standard (NMR abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, J = coupling constant). HRMS (ESI) data were acquired on a Bruker Customer micrOTOF-Q

125 high resolution mass spectrometer using ESI ionization. Elemental analyses were performed for C, H, and N using an Elementar vario EL III element analyzer. The progress of reactions was monitored by thin-layer chromatography (TLC) on silica gel GF254 (200-400 mesh) using EtOAc – petroleum ether as the eluent.

Procedure for the NBS-mediated radical bromination reaction of ethyl 3-methylquinoxaline-2-carboxylate (1). Ethyl 3-methylquinoxaline-2-carboxylate (**1**) (10.812 g, 50 mmol) was added to CCl₄ (500 mL) and heated with stirring until refluxed gently. A catalytic amount of benzoyl peroxide (BPO) (0.302 g, 1.25 mmol) as initiator was then added to the reaction mixture. After that, a slightly excessive amount of NBS (10.678 g, 60 mmol) was added carefully in three batches to the gently refluxing CCl₄ solution, i. e., 3.56 g (20 mmol) portions of 10.678 g (60 mmol) NBS were added every 1.5~2.0 h. After the addition was complete, the mixture continued to reflux gently till the disappearance of **1** (as monitored by TLC). The reaction mixture was cooled to room temperature and the precipitated succinimide was filtered off. The obtained filtrate was washed with water and dried over Na₂SO₄. Evaporation of the solvent under reduced pressure afforded a crude solid product, which was subjected to column chromatography over silica gel (200-400 mesh) using petroleum ether/EtOAc mixture (10:1, v/v) as eluent to give 10.46 g of product **2** and 3.37 g of **2'**.

Ethyl 3-(bromomethyl)quinoxaline-2-carboxylate (2): a white solid; mp 96-98 °C; yield 71%; ¹H NMR (400 MHz, CDCl₃) δ: 1.54 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃), 4.63 (q, *J* = 7.2 Hz, 2H, OCH₂CH₃), 5.16 (s, 2H, CH₂Br), 7.86-7.91 (m, 2H, Quin-H), 8.12 (d, *J* = 7.2 Hz, 1H, Quin-H), 8.25 (d, *J* = 7.2 Hz, 1H, Quin-H); ¹³C NMR (100 MHz, CDCl₃) δ: 14.20, 31.54, 62.98, 128.99, 129.88, 131.27, 132.41, 140.59, 141.87, 143.62, 151.23, 164.85. Anal. Calcd for C₁₂H₁₁BrN₂O₂: C, 48.84; H, 3.76; N, 9.49. Found: C, 48.56; H, 3.87; N, 9.78.

Ethyl 3-(dibromomethyl)quinoxaline-2-carboxylate (2'): a white solid; mp 91-92 °C; yield 18%; ¹H NMR (400 MHz, CDCl₃) δ: 1.46 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃), 4.54 (q, *J* = 7.2 Hz, 2H, OCH₂CH₃), 7.81-7.91 (m, 2H, Quin-H), 7.87 (s, 1H, CHBr₂), 8.20 (d, *J* = 8.4 Hz, 2H, Quinoxal-H); ¹³C NMR (100 MHz, CDCl₃) δ: 14.21, 39.01, 63.31, 129.35, 129.82, 132.10, 132.95, 138.89, 141.06, 142.34, 151.14, 164.53. Anal. Calcd for C₁₂H₁₀Br₂N₂O₂: C, 38.53; H, 2.69; N, 7.49. Found: C, 38.24; H, 2.93; N, 7.28.

General procedure for the preparation of 3-(benzofuran-2-yl)quinoxaline-2-carboxylic acids (3a-j): To a stirred solution of ethyl 3-(bromomethyl)quinoxaline-2-carboxylate (0.295 g, 1.0 mmol) in MeCN (20 mL) were added respective salicylaldehyde and anhydrous K₂CO₃ (0.552 g, 4.0 mmol). The resulting mixture was heated in an ultrasonic bath at 80 °C for 4~6 h. After the reaction was complete as monitored by TLC, MeCN was evaporated to dryness, and a solution of KOH (2.24 g, 40.0 mmol) in 80% aq. EtOH (20 mL) was added directly to the residue. The resulting mixture was continued to reflux for 2~4 h and then cooled to room temperature followed by acidification with 1 M HCl solution. The precipitated crude

product was purified by recrystallization from EtOH to give the pure compounds **3a-j**. The yields, physical properties, and spectral and analytical data are given below.

3-(Benzofuran-2-yl)quinoxaline-2-carboxylic acid (3a): a yellow solid; mp 153-154 °C; yield 77%; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.34 (t, *J* = 7.6 Hz, 1H, Ben-H), 7.45 (t, *J* = 8.0 Hz, 1H, Ben-H), 7.66 (d, *J* = 8.4 Hz, 1H, Ben-H), 7.74 (s, 1H, Furan-H), 7.82 (d, *J* = 7.6 Hz, 1H, Ben-H), 7.93 (t, *J* = 7.6 Hz, 1H, Quin-H), 7.97 (t, *J* = 7.6 Hz, 1H, Quin-H), 8.15 (d, *J* = 8.0 Hz, 1H, Quin-H), 8.18 (d, *J* = 8.0 Hz, 1H, Quin-H), 14.44 (s br, 1H, COOH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 122.55, 127.29, 128.38, 128.75, 128.83, 129.10, 130.28, 131.92, 135.46, 136.38, 139.07, 141.56, 144.96, 147.38, 149.72, 153.81, 166.64. HRMS: Calcd for C₁₇H₁₀N₂NaO₃ [M+Na]⁺: 313.0584. Found: 313.0560.

3-(5-Methylbenzofuran-2-yl)quinoxaline-2-carboxylic acid (3b): a yellow solid; mp 182-183 °C; yield 74%; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 2.44 (s, 3H, Me), 7.15 (d, *J* = 7.6 Hz, 1H, Ben-H), 7.45 (s, 1H, Ben-H), 7.67 (s, 1H, Furan-H), 7.65 (d, *J* = 7.6 Hz, 1H, Ben-H), 7.90 (t, *J* = 8.4 Hz, 1H, Quin-H), 7.95 (t, *J* = 8.0 Hz, 1H, Quin-H), 8.13 (d, *J* = 8.4 Hz, 1H, Quin-H), 8.16 (d, *J* = 8.4 Hz, 1H, Quin-H), 14.40 (s br, 1H, COOH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 21.86, 109.54, 111.95, 122.42, 125.76, 125.78, 129.31, 129.32, 131.85, 132.52, 137.30, 139.56, 140.53, 141.52, 146.16, 151.24, 155.92, 167.91. Anal. Calcd for C₁₈H₁₂N₂O₃: C, 71.05; H, 3.97; N, 9.21. Found: C, 71.24; H, 3.88; N, 9.05.

3-(5-Methoxybenzofuran-2-yl)quinoxaline-2-carboxylic acid (3c): a yellow solid; mp 170-171 °C; yield 71%; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.78 (s, 3H, OMe), 7.02 (dd, *J* = 8.4, 2.4 Hz, 1H, Ben-H), 7.29 (d, *J* = 2.4 Hz, 1H, Ben-H), 7.55 (d, *J* = 8.4 Hz, 1H, Ben-H), 7.66 (s, 1H, Furan-H), 7.91 (t, *J* = 8.0 Hz, 1H, Quin-H), 7.95 (t, *J* = 7.6 Hz, 1H, Quin-H), 8.13 (d, *J* = 8.4 Hz, 1H, Quin-H), 8.16 (d, *J* = 8.4 Hz, 1H, Quin-H), 14.39 (s br, 1H, COOH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 56.04, 104.38, 109.65, 112.65, 116.30, 128.88, 129.33, 129.34, 131.94, 132.54, 139.64, 140.42, 141.51, 146.15, 150.50, 152.47, 156.57, 167.89. Anal. Calcd for C₁₈H₁₂N₂O₄: C, 67.50; H, 3.78; N, 8.75. Found: C, 67.38; H, 3.69; N, 8.66.

3-(5-(*tert*-Butyl)benzofuran-2-yl)quinoxaline-2-carboxylic acid (3d): a brown solid; mp 154-155 °C; yield 73%; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 1.29 (s, 9H, *t*-Bu), 7.48 (d, *J* = 8.4 Hz, 1H, Ben-H), 7.55 (d, *J* = 8.4 Hz, 1H, Ben-H), 7.68 (s, 1H, Ben-H), 7.74 (s, 1H, Furan-H), 7.89 (t, *J* = 8.0 Hz, 1H, Quin-H), 7.93 (t, *J* = 8.0 Hz, 1H, Quin-H), 8.13 (d, *J* = 8.4 Hz, 1H, Quin-H), 8.16 (d, *J* = 8.4 Hz, 1H, Quin-H), 14.44 (s br, 1H, COOH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 31.91, 34.96, 109.75, 111.36, 118.74, 125.06, 127.96, 129.32, 131.88, 132.50, 137.22, 139.62, 140.57, 141.51, 146.21, 146.84, 151.96, 153.80, 167.94. HRMS: Calcd for C₂₁H₁₉N₂O₃ [M+H]⁺: 347.1390. Found: 347.1404.

3-(7-(*tert*-Butyl)benzofuran-2-yl)quinoxaline-2-carboxylic acid (3e): a brown solid; mp 153-154 °C; yield 65%; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 1.52 (s, 9H, *t*-Bu), 7.28 (t, *J* = 8.4 Hz, 1H, Ben-H), 7.31 (t, *J* = 8.0 Hz, 1H, Ben-H), 7.67 (t, *J* = 8.0 Hz, 1H, Ben-H), 7.87 (s, 1H, Furan-H), 7.96 (d, *J* = 7.6 Hz, 1H, Quin-H), 8.00 (d, *J* = 7.6 Hz, 1H, Quin-H), 8.19 (d, *J* = 8.0 Hz, 2H, Quin-H), 14.29 (s br, 1H, COOH);

^{13}C NMR (100 MHz, DMSO- d_6) δ : 30.14, 34.29, 109.44, 120.78, 123.25, 124.28, 128.76, 129.30, 129.33, 131.92, 132.57, 135.40, 139.76, 140.53, 141.60, 145.94, 151.71, 153.96, 167.46. Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_3$: C, 72.82; H, 5.24; N, 8.09. Found: C, 72.54; H, 5.35; N, 8.38.

3-(6-Chlorobenzofuran-2-yl)quinoxaline-2-carboxylic acid (3f): a yellow solid; mp 165-166 °C; yield 76%; ^1H NMR (400 MHz, DMSO- d_6) δ : 7.04 (dd, $J = 8.8, 2.4$ Hz, 1H, Ben-H), 7.30 (d, $J = 2.4$ Hz, 1H, Ben-H), 7.58 (d, $J = 8.8$ Hz, 1H, Ben-H), 7.68 (s, 1H, Furan-H), 7.92 (t, $J = 7.6$ Hz, 1H, Quin-H), 7.97 (t, $J = 7.6$ Hz, 1H, Quin-H), 8.16 (d, $J = 8.0$ Hz, 1H, Quin-H), 8.18 (d, $J = 8.4$ Hz, 1H, Quin-H), 14.50 (s br, 1H, COOH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 109.01, 113.79, 122.26, 126.89, 128.60, 129.42, 129.77, 132.26, 132.65, 139.80, 140.08, 141.44, 143.56, 146.17, 153.28, 153.86, 167.77. HRMS: Calcd for $\text{C}_{17}\text{H}_9\text{ClN}_2\text{NaO}_3$ [$\text{M}+\text{Na}$] $^+$: 347.0194. Found: 347.0189.

3-(6-Bromobenzofuran-2-yl)quinoxaline-2-carboxylic acid (3g): a brown solid; mp 204-205 °C; yield 78%; ^1H NMR (400 MHz, DMSO- d_6) δ : 7.63 (d, $J = 8.4$ Hz, 1H, Ben-H), 7.71 (d, $J = 8.4$ Hz, 1H, Ben-H), 7.75 (s, 1H, Furan-H), 8.00 (t, $J = 8.0$ Hz, 1H, Quin-H), 8.04 (t, $J = 8.0$ Hz, 1H, Quin-H), 8.10 (s, 1H, Ben-H), 8.22 (d, $J = 8.4$ Hz, 1H, Quin-H), 8.24 (d, $J = 8.4$ Hz, 1H, Quin-H), 14.50 (s br, 1H, COOH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 108.89, 114.11, 116.55, 125.35, 129.40, 129.46, 129.60, 130.43, 132.36, 132.76, 139.83, 140.10, 141.48, 146.13, 153.09, 154.22, 167.76. Anal. Calcd for $\text{C}_{17}\text{H}_9\text{BrN}_2\text{O}_3$: C, 55.31; H, 2.46; N, 7.59. Found: C, 55.10; H, 2.34; N, 7.48.

3-(4,6-Dibromobenzofuran-2-yl)quinoxaline-2-carboxylic acid (3h): a brown solid; mp 209-210 °C; yield 79%; ^1H NMR (400 MHz, DMSO- d_6) δ : 7.81 (s, 1H, Furan-H), 7.89 (s, 1H, Ben-H), 7.99-8.03 (m, 2H, Quin-H), 8.06 (s, 1H, Ben-H), 8.19-8.22 (m, 2H, Quin-H), 14.50 (s br, 1H, COOH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 105.07, 109.39, 114.79, 124.88, 129.42, 129.49, 131.07, 131.18, 132.53, 132.79, 139.80, 140.00, 141.39, 146.24, 151.70, 153.74, 167.51. Anal. Calcd for $\text{C}_{17}\text{H}_8\text{Br}_2\text{N}_2\text{O}_3$: C, 45.57; H, 1.80; N, 6.25. Found: C, 45.34; H, 1.68; N, 5.98.

3-(5-(*tert*-Butyl)-7-chlorobenzofuran-2-yl)quinoxaline-2-carboxylic acid (3i): a yellow solid; mp 159-161 °C; yield 62%; ^1H NMR (400 MHz, DMSO- d_6) δ : 1.36 (s, 9H, *t*-Bu), 7.61 (s, 1H, Ben-H), 7.79 (s, 1H, Ben-H), 7.80 (s, 1H, Furan-H), 7.98 (t, $J = 7.6$ Hz, 1H, Quin-H), 8.02 (t, $J = 7.6$ Hz, 1H, Quin-H), 8.20 (d, $J = 8.4$ Hz, 1H, Quin-H), 8.24 (d, $J = 8.4$ Hz, 1H, Quin-H), 14.32 (s br, 1H, COOH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 31.73, 35.24, 110.09, 115.56, 117.95, 124.66, 129.38, 129.41, 129.70, 132.21, 132.64, 139.83, 140.22, 141.43, 146.29, 148.73, 149.31, 152.83, 167.69. Anal. Calcd for $\text{C}_{21}\text{H}_{17}\text{ClN}_2\text{O}_3$: C, 66.23; H, 4.50; N, 7.36. Found: C, 66.13, H, 4.45; N, 7.15.

3-(7-Bromo-5-(*tert*-butyl)benzofuran-2-yl)quinoxaline-2-carboxylic acid (3j): a brown solid; mp 150-151 °C; yield 67%; ^1H NMR (400 MHz, DMSO- d_6) δ : 1.31 (s, 9H, *t*-Bu), 7.67 (d, $J = 1.6$ Hz, 1H, Ben-H), 7.76 (d, $J = 1.6$ Hz, 1H, Ben-H), 7.77 (s, 1H, Furan-H), 7.92 (td, $J = 7.6, 2.0$ Hz, 1H, Quin-H), 7.96 (td, $J = 7.6, 2.0$ Hz, 1H, Quin-H), 8.15 (dd, $J = 8.4, 2.0$ Hz, 1H, Quin-H), 8.18 (dd, $J = 8.4, 2.0$ Hz,

1H, Quin-H), 14.26 (s br, 1H, COOH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 31.70, 35.29, 109.96, 111.03, 111.19, 114.72, 129.38, 131.33, 132.22, 132.67, 139.81, 140.18, 141.45, 145.77, 146.10, 148.23, 148.85, 153.02, 167.73. HRMS: Calcd for C₂₁H₁₈BrN₂O₃ [M+H]⁺: 425.0501. Found: 425.0495.

Antibacterial activity test. All the newly-synthesized compounds herein were screened for their potential *in vitro* antibacterial activities against *B. subtilis* [CMCC (B) 63501], *S. aureus* [CMCC (B) 26003], *E. coli* [CMCC (B) 44102] and *P. aeruginosac* [CMCC (B) 10104] and anti-tubercular activity against *M. smegmatis* [CGMCC 1.2621] by the broth microdilution assay. Each of the test compounds was dissolved in DMSO and then was serially diluted in five concentrations at 2-fold dilutions (250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625 μg/mL) to determine the MICs. Rifampicin and Ciprofloxacin were used as the reference standards.

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