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Synthesis and Antimycobacterial Activity of New Quinoxaline-2-carboxamide 1,4-di-*N*-Oxide Derivatives

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Abstract—As a continuation of our research and with the aim of obtaining new antituberculosis agents which can improve the current chemotherapeutic antituberculosis treatments, new series of quinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives were synthesized and evaluated for in vitro antituberculosis activity against *Mycobacterium tuberculosis* strain H₃₇Rv, using the radiometric BACTEC 460-TB methodology. Active compounds were also screened by serial dilution to assess toxicity to a VERO cell line. The results indicate that some compounds exhibited a good antituberculosis activity and the arylcarboxamide analogues **3**, **8**, and **9** were the most active compounds (EC₉₀/MIC1). Also, the cytotoxic effects indicate that these compounds have a good Selectivity Index (SI).

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Introduction

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis*, still remains the leading cause of worldwide death among infectious diseases. One-third of the population is infected with *M. tuberculosis* and the World Health Organization (WHO) estimates that within the next 20 years about 30 million people will be infected with the bacillus.¹

The mycobacterial cell wall is the site of action of many of the first line antimycobacterial agents and contains numerous components that are presumed to be required for cell viability or survival in the host and therefore, are attractive drug targets.²

Current frontline therapy consists of administering one of three drugs (isoniazid, rifampin and pyrazinamide) for 2 months followed by 4 months of follow up therapy with isoniazid and rifampin.³ But the ineffectiveness of the present therapy is found to be responsible for a very long duration of the therapy as well as the emergence of resistance to these drugs. Thus, the problem arising due to MDR-TB requires the development of new therapeutic agents that have unique mechanism of action from presently used antitubercular drugs in order to treat drug resistant forms of the disease.⁴

As a result of the antituberculosis research project, our group has had several papers published in which the synthesis and biological assessment of a large amount of quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives have been described.^{5,6} Therefore, for example, different 7-chloro-3-(*p*-substituted)phenylaminoquinoxaline-2-carbonitrile 1,4-di-*N*-oxides (**I**, Fig. 1) have been shown to possess *M. tuberculosis* growth inhibition values of 99%⁷ and 6,7-dichloro-2-ethoxycarbonyl-3-methylquinoxaline 1,4-di-*N*-oxide (**II**, Fig. 1) and 3-acetamide-6,7-dichloroquinoxaline-2-carbonitrile 1,4-di-*N*-oxide derivatives produced growth inhibition values of 100%.^{8,9} On the other hand, we observed that the lack of the two *N*-oxide groups generally led to the loss of the antimycobacterial activity.^{9,10}

As a consequence of this research and with the aim of obtaining new and more potent antituberculosis compounds which can improve the current chemotherapeutic antituberculosis treatments, we have synthesized and evaluated 31 new carbonylquinoxaline 1,4-di-*N*-oxide derivatives possessing different amine groups in the 2-position and methyl group in the 3-position (**III**, Fig. 1).

Chemistry

The aforementioned compounds were prepared according to the synthetic sequences illustrated in Scheme 1. The starting compounds, 5-substituted benzofuroxane

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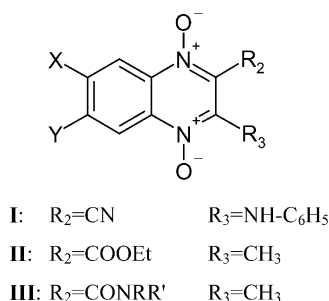


Figure 1.

or 5,6-disubstituted benzofuroxane were obtained by previously described methods.⁶

The synthesis of compounds **1–14** was carried out by reaction of the appropriate benzofuroxane with the corresponding 3-oxobutylamide, using triethylamine. The 2-carboxamide derivatives with general structure 2-carboxypiperazine, namely **15–31**, were obtained by the reaction of benzofuroxane with the corresponding *N*-(3-oxobutyl)-piperazine, using morpholine as catalyst.

The intermediates *N*-(3-oxobutyl)-piperazine were prepared according to the synthetic procedure for similar compounds.¹¹ The methyl acetoacetate was heated with the corresponding piperazine in the presence of 2-hydroxypyridine as catalyst in a bath at 169 °C for 5 h, under nitrogen atmosphere as illustrated in Scheme 2. The partly cooled mixture was then stirred into hot water. The resulting suspension was dissolved in di-

chloromethane. The organic solvent was eliminated under pressure and a solid or oil was obtained.

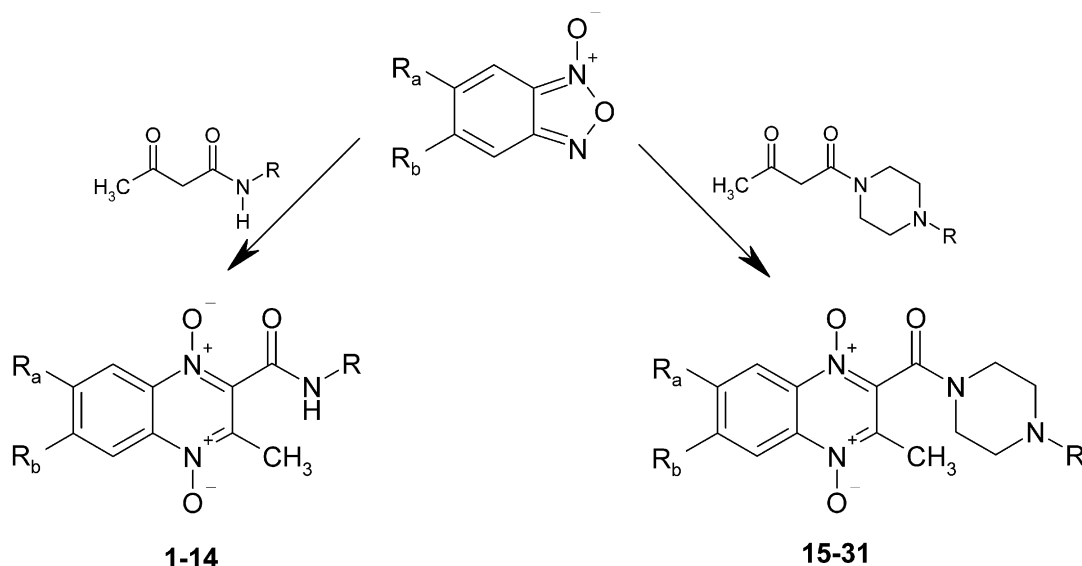
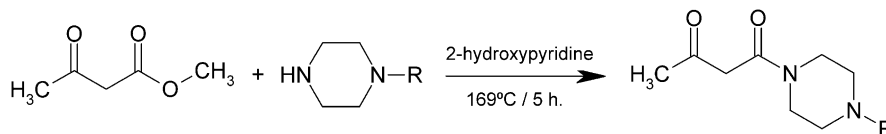
Formation of isomeric quinoxaline 1,4-di-*N*-oxides was observed in the case of monosubstituted benzofuroxanes. According to previous reports,¹² we have observed that 7-substituted quinoxaline 1,4-di-*N*-oxides were prevailing over the 6-isomer, or the only 7-isomer formed in the case of methoxy substituent. In practice, the workup and purification allows isolation of the 7 isomer.¹³

All of the compounds were chemically characterized by thin-layer chromatography (TLC), melting point, infrared (IR) and nuclear magnetic resonance (¹H NMR) spectra as well as elemental microanalysis.

Pharmacology

In vitro evaluation of the antituberculosis activity was carried out at the GWL Hansen's Disease Center within the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program for the discovery of novel drugs for the treatment of tuberculosis. Under the direction of the US National Institute of Allergy and Infectious Disease (NIAID), Southern Research Institute coordinates the overall program.

The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*. Biological tests have been performed according to the previously described method.^{14,15}

Scheme 1. Synthesis of compounds **1–31**.Scheme 2. Synthesis of the intermediates *N*-(3-oxobutyl)-piperazine derivatives.

Results and Discussion

The results of the in vitro evaluation of antituberculosis activity are reported in Tables 1–3. As described in previous papers, the presence of 1,4-di-*N*-oxides is essential for the activity of the compounds.⁶ In general, most compounds with a *N*-arylcarboxamide group in position 2, **1**–**10**, have been shown to possess good antimycobacterial activity in the run of the first level screening, with growth inhibition values ranging from 98 to 100%. Only compounds **1**, **2**, **6** and **7** showed low or no activity (0%, 62%, 22% and 70% growth inhibition, respectively). On the contrary, 2-piperazinylcarbonyl quinoxaline derivatives **15**–**31**, showed no activity (Table 1).

Compounds with a chloro in the 7- or 6,7-positions (**4**, **5**, **9** and **14**) and corresponding unsubstituted derivatives (**3** and **8**) showed the best activity. An electron-releasing group (CH₃) on the benzene moiety reduces the activity.

Second level assays and cytotoxicity results are summarized in Table 2. All of the compounds that were

active in the first level screening were then tested to determine the actual MIC. MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Therefore, compounds **3**, **4**, **5**, and **14** have proven to be the most efficient ones, with MIC values ranging from 0.78 to 3.13 μM. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC₅₀) in VERO cells, and the Selectivity Index (SI = IC₅₀/MIC) was calculated.

Three compounds, **3**, **8** and **9**, with a high SI value (> 40.06, > 10 and > 10, respectively), were then tested for efficacy in vitro in a TB-infected macrophage model, showing good values of EC₉₀ and EC₉₉. Macrophage assay results are reported in Table 3.

On the basis of the obtained data we can remark the interesting antitubercular properties showed by some *N*-phenylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives, namely **3**, **8** and **9**. These compounds have shown very good activity in macrophage assay (EC₉₀/MIC = 0.89, 0.42 and 0.14, respectively). SDR-MIC and MBC studies are scheduled. The activity is significantly

Table 1. Results of the first antituberculosis screening

Compd	R	R _a	R _b	MIC (μg/mL) ^a	GI (%) ^b
1	NH–C ₆ H ₅	CH ₃	CH ₃	> 6.25	0
2	NH–C ₆ H ₅	CH ₃	H	> 6.25	62
3	NH–C ₆ H ₅	H	H	< 6.25	100
4	NH–C ₆ H ₅	Cl	H	< 6.25	99
5	NH–C ₆ H ₅	Cl	Cl	< 6.25	100
6	2'–CH ₃ –C ₆ H ₄ –NH	CH ₃	CH ₃	> 6.25	22
7	2'–CH ₃ –C ₆ H ₄ –NH	CH ₃	H	> 6.25	70
8	2'–CH ₃ –C ₆ H ₄ –NH	H	H	< 6.25	98
9	2'–CH ₃ –C ₆ H ₄ –NH	Cl	H	< 6.25	98
10	2'–CH ₃ –C ₆ H ₄ –NH	Cl	Cl	> 6.25	100
11	NH–C(CH ₃) ₃	CH ₃	CH ₃	> 6.25	0
12	NH–C(CH ₃) ₃	H	H	> 6.25	10
13	NH–C(CH ₃) ₃	Cl	H	> 6.25	51
14	NH–C(CH ₃) ₃	Cl	Cl	< 6.25	100
15	4–C ₆ H ₅ –Piperazin-1-yl	CH ₃	CH ₃	> 6.25	0
16	4–C ₆ H ₅ –Piperazin-1-yl	CH ₃	H	> 6.25	0
17	4–C ₆ H ₅ –Piperazin-1-yl	Cl	Cl	> 6.25	0
18	4-(4'–Chlorophenyl)piperazin-1-yl	CH ₃	CH ₃	> 6.25	0
19	4-(4'–Chlorophenyl)piperazin-1-yl	H	H	> 6.25	0
20	4-(4'–Nitrophenyl)piperazin-1-yl	CH ₃	CH ₃	> 6.25	0
21	4-(4'–Nitrophenyl)piperazin-1-yl	CH ₃	H	> 6.25	0
22	4-(4'–Nitrophenyl)piperazin-1-yl	H	H	> 6.25	0
23	4-(4'–Nitrophenyl)piperazin-1-yl	Cl	Cl	> 6.25	0
24	4-(4'–Nitrophenyl)piperazin-1-yl	OCH ₃	H	> 6.25	0
25	4-(2'–Methoxyphenyl)piperazin-1-yl	CH ₃	CH ₃	> 6.25	0
26	4-(2'–Methoxyphenyl)piperazin-1-yl	Cl	Cl	> 6.25	0
27	4-(4'–Methoxyphenyl)piperazin-1-yl	H	H	> 6.25	0
28	4-(4'–Methoxyphenyl)piperazin-1-yl	Cl	Cl	> 6.25	0
29	4-Benzylpiperazin-1-yl	CH ₃	CH ₃	> 6.25	0
30	4-Benzylpiperazin-1-yl	H	H	> 6.25	0
31	4-Benzylpiperazin-1-yl	Cl	Cl	> 6.25	0

^aMIC of Rifampin: 0.125 μg/mL versus *M. tuberculosis* H₃₇Rv (97% inhibition).

^bGrowth Inhibition of virulent H₃₇Rv strain of *M. tuberculosis*. According to the TAACF program, compounds effecting less than 90% inhibition are considered to be inactive.

Table 2. Results of second level and cytotoxicity antituberculosis assays

Compd	MIC (μM) ^a	IC ₅₀ (μM) ^b	SI (IC ₅₀ /MIC) ^c
3	3.13	1	> 40.06
4	1.56	> 10	> 6.41
5	0.78	Insoluble	Insoluble
8	6.25	> 62.5	> 10
9	6.25	> 62.5	> 10
14	1.56	> 10	> 6.41

^aActual minimum inhibitory concentration (MABA assay).^bMeasurement of cytotoxicity in VERO cells.^cSelectivity Index.**Table 3.** Results of macrophage assay

Compd	EC ₉₀ ^a	EC ₉₉ ^a	EC ₉₀ /MIC ^b
3	2.79	10.07	0.89
8	2.60	11.08	0.42
9	0.86	6.75	0.14

^aThe EC₉₀ and EC₉₉ are defined as the concentrations effecting 90 and 99% reduction in residual mycobacterial growth after 7 days, as compared to untreated controls.^bCompounds with EC₉₀ > 16×MIC are considered inactive.

affected by substituents, both on position 7 of quinoxaline nucleus and position 2' of the phenyl ring. Compounds with a chloro in 7 position and corresponding unsubstituted derivatives showed the best activity. With regard to the benzene moiety, greater effectiveness resulted for *ortho*-methyl substituents.

Conclusion

In conclusion, we can assert that the promising results obtained for the new *N*-phenylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives presented in this paper make them possible candidates for the treatment of tuberculosis and encourage us to advance in the synthesis and evaluation of new derivatives.

Experimental

Chemistry

Melting points were determined with a Mettler FP82+FP80 apparatus (Greifensee, Switzerland) and have not been corrected. The ¹H NMR spectra were recorded on a Bruker AC-200E (Rheinstetten, Germany) using TMS as the internal standard. The IR spectra were performed on a Perkin Elmer 1600 FTIR (Norwalk, CT, USA) in KBr pellets. Elemental microanalyses were obtained on a Elemental Analyzer (Carlo Erba 1106, Milan, Italy) from vacuum-dried samples; for each compound, the calculated and found values are being reported.

Alugram® SIL G/UV₂₅₄ (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG, Postfach 101352, D-52313 Düren, Germany) was used for Thin Layer Chromatography. HPLC conditions: Column Nova Pack C18 60

A 4 μm (3.9×150 mm); mobile phase: acetonitrile/iso-propanol 50/50; flux: 1 mL/min.

Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A., (Alcobendas, Madrid), Acros Organics (Janssen Pharmaceuticaaan 3a, 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

General procedure for compounds 1–14

The corresponding 3-oxobutylamide (4.8 mmol) was added to a solution of the appropriate benzofuroxane (2.4 mmol) in dry chloroform (35 mL). The mixture was allowed to stand at 0 °C. Next, triethylamine was added drop by drop (0.1 mL). The solution was stirred at room temperature for 24 h. The solvent was eliminated under pressure. The resulting solid was washed with ethyl ether (or *n*-hexane). The obtained yellow precipitate was purified by recrystallization using methanol. Yields: 5–64%.

3,6,7-Trimethyl-*N*-phenylquinoxaline-2-carboxamide 1,4-dioxide (1). Mp 229–230 °C. IR (KBr) ν 3250, 1682, 1334 cm^{-1} ; ¹H NMR (DMSO-*d*₆) δ 2.50 (s, 3H, C₃–CH₃), 2.54 (s, 6H, 2CH₃–Ar), 7.21 (t, 1H, H_{4'}), 7.45 (t, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 7.7$ Hz), 7.71 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 7.9$ Hz), 8.29 (s, 1H, H₅), 8.33 (s, 1H, H₈), 11.03 (s, 1H, NH) ppm. Anal. (C₁₈H₁₇N₃O₃) C, H, N; C (%): calcd: 66.87; found: 66.51. H (%): calcd: 5.26; found: 5.15. N (%): calcd: 13.00; found: 13.05 (33% yield).

3,7-Dimethyl-*N*-phenylquinoxaline-2-carboxamide 1,4-dioxide (2). Mp 206–207 °C. IR (KBr) ν 3249, 1687, 1554, 1333 cm^{-1} ; ¹H NMR (DMSO-*d*₆) δ 2.47 (s, 3H, C₃–CH₃), 2.59 (s, 3H, CH₃–Ar), 7.20 (t, 1H, H_{4'}), 7.41 (t, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 7.7$ Hz); 7.67 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 8.0$ Hz), 7.85 (d, 1H, H₆, $J_{6-5} = 8.0$ Hz), 8.29 (s, 1H, H₈), 8.41 (d, 1H, H₅), 10.98 (s, 1H, NH) ppm. Anal. (C₁₇H₁₅N₃O₃) C, H, N; C (%): calcd: 66.02; found: 66.05. H (%): calcd: 4.85; found: 4.75. N (%): calcd: 13.59; found: 13.35 (35% yield).

3-Methyl-*N*-phenylquinoxaline-2-carboxamide 1,4-dioxide (3). Mp 225–226 °C. IR (KBr) ν 3251, 1686, 1309, 757 cm^{-1} . ¹H NMR (DMSO-*d*₆) δ 2.51 (s, 3H, C₃–CH₃), 7.20 (t, 1H, H_{4'}, $J_{4'-3'} = 6.9$ Hz), 7.43 (t, 2H, H_{3'} + H_{5'}), 7.70 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 8.2$ Hz), 8.04–8.08 (m, 2H, H₆ + H₇), 8.49–8.57 (m, 2H, H₅ + H₈), 11.00 (s, 1H, NH) ppm. Anal. (C₁₆H₁₃N₃O₃) C, H, N; C (%): calcd: 65.08; found: 64.60. H (%): calcd: 4.36; found: 4.38. N (%): calcd: 14.24; found: 14.20 (33% yield).

7-Chloro-3-methyl-*N*-phenylquinoxaline-2-carboxamide 1,4-dioxide (4). Mp 189–190 °C. IR (KBr) ν 3248, 1677, 1333, 743 cm^{-1} . ¹H NMR (DMSO-*d*₆) δ 2.49 (s, 3H, C₃–CH₃), 7.20 (t, 1H, H_{4'}, $J_{4'-3'} = 7.2$ Hz), 7.42 (t, 2H, H_{3'} + H_{5'}), 7.66 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 8.0$ Hz), 8.05 (d, 1H, H₆, $J_{6-5} = 7.3$ Hz); 8.49 (s, 1H, H₈), 8.54 (d, 1H, H₅), 11.01 (s, 1H, NH) ppm. Anal. (C₁₆H₁₂ClN₃O₃) C, H, N; C (%): calcd: 58.27; found: 58.21. H (%):

calcd: 3.64; found: 3.90. N (%): calcd: 12.75; found: 12.61. HPLC: R_t = 3.06 min (27% yield).

6,7-Dichloro-3-methyl-*N*-phenylquinoxaline-2-carboxamide 1,4-dioxide (5). Mp 213–214 °C. IR (KBr) ν 3255, 1677, 1312, 743 cm^{-1} . ^1H NMR (DMSO- d_6) δ 2.49 (s, 3H, $\text{C}_3\text{-CH}_3$), 7.19 (t, 1H, $\text{H}_{4'}$, $J_{4'-3'} = 7.3$ Hz), 7.41 (t, 2H, $\text{H}_{3'} + \text{H}_{5'}$), 7.66 (d, 2H, $\text{H}_{2'} + \text{H}_{6'}$, $J_{2'-3'} = 7.9$ Hz), 8.68 (s, 1H, H_8), 8.70 (s, 1H, H_5), 10.97 (s, 1H, NH) ppm. Anal. ($\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_3$) C, H, N; C (%): calcd: 52.75; found: 53.26. H (%): calcd: 3.02; found: 3.13. N (%): calcd: 11.54; found: 11.24 (15% yield).

3,6,7-Trimethyl-*N*-(2-methylphenyl)quinoxaline-2-carboxamide 1,4-dioxide (6). Mp 228–229 °C. IR (KBr) ν 3262, 1664, 1590, 1333 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 2.31 (s, 3H, $\text{C}_{2'}\text{-CH}_3$), 2.52 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.54 (s, 6H, $2\text{CH}_3\text{-Ar}$), 7.16–7.32 (m, 3H, $\text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'}$), 7.64 (d, 1H, $\text{H}_{6'}$, $J_{6'-5'} = 6.4$ Hz); 8.30 (s, 1H, H_5), 8.31 (s, 1H, H_8), 10.35 (s, 1H, NH) ppm. Anal. ($\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_3$) C, H, N; C (%): calcd: 67.65; found: 67.58. H (%): calcd: 5.64; found: 5.43. N (%): calcd: 12.46; found: 12.15. HPLC: R_t = 3.09 min (38% yield).

3,7-Dimethyl-*N*-(2-methylphenyl)quinoxaline-2-carboxamide 1,4-dioxide (7). Mp 196–197 °C. IR (KBr) ν 3260, 1659, 1544, 1331 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 2.33 (s, 3H, $\text{C}_{2'}\text{-CH}_3$), 2.57 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.64 (s, 3H, Ar-CH_3), 7.22–7.35 (m, 3H, $\text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'}$), 7.69 (d, 1H, $\text{H}_{6'}$, $J_{6'-5'} = 7.4$ Hz); 7.89 (d, 1H, H_6 , $J_{6-5} = 8.0$ Hz), 8.36 (s, 1H, H_8), 8.46 (d, 1H, H_5 , $J_{5-6} = 8.0$ Hz), 10.35 (s, 1H, NH) ppm. Anal. ($\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_3$) C, H, N; C (%): calcd: 66.87; found: 66.77. H (%): calcd: 5.26; found: 5.41. N (%): calcd: 13.00; found: 12.61. HPLC: R_t = 3.14 min (46% yield).

3-Methyl-*N*-(2-methylphenyl)quinoxaline-2-carboxamide 1,4-dioxide (8). Mp 195–196 °C. IR (KBr) ν 3254, 1663, 1339, 751 cm^{-1} . ^1H NMR (DMSO- d_6) δ 2.28 (s, 3H, $\text{C}_{2'}\text{-CH}_3$), 2.48 (s, 3H, $\text{C}_3\text{-CH}_3$), 7.12–7.29 (m, 3H, $\text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'}$), 7.63 (d, 1H, $\text{H}_{6'}$, $J_{6'-5'} = 8.0$ Hz), 7.96–8.04 (m, 2H, $\text{H}_6 + \text{H}_7$), 8.48–8.54 (m, 2H, $\text{H}_5 + \text{H}_8$) ppm. Anal. ($\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_3$) C, H, N; C (%): calcd: 66.88; found: 66.55. H (%): calcd: 4.92; found: 4.77. N (%): calcd: 13.77; found: 13.47. HPLC: R_t = 1.38 min (64% yield).

7-Chloro-3-methyl-*N*-(2-methylphenyl)quinoxaline-2-carboxamide 1,4-dioxide (9). Mp 202–203 °C. IR (KBr) ν 3249, 1658, 1326, 757 cm^{-1} . ^1H NMR (DMSO- d_6) δ 2.29 (s, 3H, $\text{C}_{2'}\text{-CH}_3$), 2.54 (s, 3H, $\text{C}_3\text{-CH}_3$), 7.07–7.39 (m, 4H, $\text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'} + \text{H}_{6'}$), 7.64 (d, 1H, H_6 , $J_{6-5} = 7.1$ Hz), 8.04 (s, 1H, H_8), 8.53 (d, 1H, H_5), 10.32 (s, 1H, NH) ppm. Anal. ($\text{C}_{17}\text{H}_{14}\text{ClN}_3\text{O}_3 \cdot 1/4\text{H}_2\text{O}$) C, H, N; C (%): calcd: 58.62; found: 58.93. H (%): calcd: 4.17; found: 4.11. N (%): calcd: 12.07; found: 11.67. HPLC: R_t = 3.11 min (7% yield).

6,7-Dichloro-3-methyl-*N*-(2-methylphenyl)quinoxaline-2-carboxamide 1,4-dioxide (10). Mp 224–225 °C. IR (KBr) ν 3261, 1672, 1327, 758 cm^{-1} . ^1H NMR (DMSO- d_6) δ 2.30 (s, 3H, $\text{C}_{2'}\text{-CH}_3$), 2.52 (s, 3H, $\text{C}_3\text{-CH}_3$), 7.20–7.33 (m, 3H, $\text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'}$), 7.64 (d, 1H, $\text{H}_{6'}$,

$J_{6'-5'} = 6.4$ Hz), 8.73 (bs, 2H, $\text{H}_5 + \text{H}_8$), 10.37 (s, 1H, NH) ppm. Anal. ($\text{C}_{17}\text{H}_{13}\text{Cl}_2\text{N}_3\text{O}_3$) C, H, N; C (%): calcd: 53.97; found: 53.66. H (%): calcd: 3.44; found: 3.34. N (%): calcd: 11.11; found: 10.91 (14% yield).

***N*-(*tert*-Butyl)-3,6,7-trimethylquinoxaline-2-carboxamide 1,4-dioxide (11).** Mp 261–262 °C. IR (KBr) ν 3256, 1686, 1544, 1331 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 1.36 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.39 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.47 (s, 6H, $2\text{CH}_3\text{-Ar}$), 8.18 (s, 1H, H_5), 8.24 (s, 1H, H_8), 8.51 (s, 1H, NH) ppm. Anal. ($\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_3 \cdot 1/4\text{H}_2\text{O}$) C, H, N; C (%): calcd: 62.43; found: 62.39. H (%): calcd: 6.99; found: 7.18. N (%): calcd: 13.66; found: 13.80. HPLC: R_t = 2.61 min (34% yield).

***N*-(*tert*-Butyl)-3-methylquinoxaline-2-carboxamide 1,4-dioxide (12).** Mp 213–214 °C. IR (KBr) ν 3260, 1685, 1333 cm^{-1} . ^1H NMR (DMSO- d_6) δ 1.39 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.44 (s, 3H, $\text{C}_3\text{-CH}_3$), 7.96–8.00 (m, 2H, $\text{H}_6 + \text{H}_7$), 8.48–8.53 (m, 2H, $\text{H}_5 + \text{H}_8$), 8.58 (s, 1H, NH) ppm. Anal. ($\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_3 \cdot 1/4\text{H}_2\text{O}$) C, H, N; C (%): calcd: 60.11; found: 59.70. H (%): calcd: 6.26; found: 6.51. N (%): calcd: 15.02; found: 14.62. HPLC: R_t = 3.07 min (5% yield).

7-Chloro-*N*-(*tert*-butyl)-3-methylquinoxaline-2-carboxamide 1,4-dioxide (13). Mp 209–210 °C. IR (KBr) ν 3248, 1673, 1327, 802 cm^{-1} . ^1H NMR (DMSO- d_6) δ 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.42 (s, 3H, $\text{C}_3\text{-CH}_3$), 8.01 (d, 1H, H_6 , $J_{6-5} = 7.0$ Hz), 8.44 (s, 1H, H_8), 8.52 (d, 1H, H_5), 8.46 (s, 1H, NH). Anal. ($\text{C}_{14}\text{H}_{16}\text{ClN}_3\text{O}_3 \cdot 1/4\text{H}_2\text{O}$) C, H, N; C (%): calcd: 53.51; found: 53.73. H (%): calcd: 5.25; found: 5.10. N (%): calcd: 13.37; found: 13.22. HPLC: R_t = 3.14 min (6% yield).

6,7-Dichloro-*N*-(*tert*-butyl)-3-methylquinoxaline-2-carboxamide 1,4-dioxide (14). Mp 220–221 °C. IR (KBr) ν 3254, 2968, 1681, 1329, 757 cm^{-1} . ^1H NMR (DMSO- d_6) δ 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.42 (s, 3H, $\text{C}_3\text{-CH}_3$), 8.54 (s, 1H, NH), 8.63 (s, 1H, H_5), 8.66 (s, 1H, H_8) ppm. Anal. ($\text{C}_{14}\text{H}_{15}\text{Cl}_2\text{N}_3\text{O}_3$) C, H, N; C (%): calcd: 48.84; found: 49.03. H (%): calcd: 4.36; found: 4.39. N (%): calcd: 12.21; found: 11.88 (34% yield).

General procedure for compounds 15–31

The corresponding *N*-(3-oxobutyl)piperazine (4.8 mmol) was added to a solution of the appropriate benzofuroxane (2.4 mmol) in dry chloroform (35 mL). The mixture was allowed to stand at 0 °C. Next, morpholine was added drop by drop (0.1 mL). The solution was stirred at room temperature for 48 h. The solvent was eliminated under pressure. The resulting solid was washed with ethyl ether (or methanol). The compounds were purified by recrystallization using methanol. Yields: 4–44%.

3,6,7-Trimethyl-2-(4-phenylpiperazin-1-yl)carbonylquinoxaline 1,4-dioxide (15). Mp 221–222 °C. IR (KBr) ν 1652, 1598, 1474, 1327, 1012 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 2.46 (s, 3H, $\text{C}_3\text{-CH}_3$), 2.54 (s, 6H, $2\text{CH}_3\text{-Ar}$), 3.03–4.04 (m, 8H, 4CH_2 piperazine), 6.86 (t, 1H, $\text{H}_{4'}$), 7.00 (d, 2H, $\text{H}_{2'} + \text{H}_{6'}$, $J_{2'-3'} = 8.2$ Hz), 7.27 (t, 2H, $\text{H}_{3'} + \text{H}_{5'}$,

$J_{3'-4'} = 7.6$ Hz), 8.25 (s, 1H, H₅), 8.31 (s, 1H, H₈) ppm. Anal. (C₂₂H₂₄N₄O₃) C, H, N; C (%): calcd: 67.34; found: 67.41. H (%): calcd: 6.12; found: 6.06. N (%): calcd: 14.28; found: 14.01. HPLC: $R_t = 3.01$ min (44% yield).

3,7-Dimethyl-2-(4-phenylpiperazin-1-yl)carbonylquinoxaline 1,4-dioxide (16). Mp 232–233 °C. IR (KBr) ν 1646, 1523, 1327, 1011 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.42 (s, 3H, C₃–CH₃), 2.58 (s, 3H, CH₃–Ar), 2.97–3.94 (m, 8H, 4CH₂ piperazine), 6.84 (t, 1H, H_{4'}), 6.96 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 8.0$ Hz), 7.23 (t, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 7.2$ Hz), 7.81 (d, 1H, H₆, $J_{6-5} = 8.4$ Hz), 8.24 (s, 1H, H₈), 8.38 (d, 1H, H₅) ppm. Anal. (C₂₁H₂₂N₄O₃) C, H, N; C (%): calcd: 66.66; found: 66.31. H (%): calcd: 5.82; found: 5.72. N (%): calcd: 14.81; found: 14.49. HPLC: $R_t = 3.02$ min (30% yield).

6,7-Dichloro-3-methyl-2-(4-phenylpiperazin-1-yl)carbonylquinoxaline 1,4-dioxide (17). Mp 221–222 °C. IR (KBr) ν 3067, 1655, 1324, 882. ¹H NMR (DMSO-*d*₆) δ 2.43 (s, 3H, C₃–CH₃), 2.95–3.92 (m, 8H, 4CH₂ piperazine), 6.83 (t, 1H, H_{4'}, $J_{4'-3'} = 7.1$ Hz), 6.98 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 8.0$ Hz), 7.24 (t, 2H, H_{3'} + H_{5'}), 8.63 (s, 1H, H₅), 8.67 (s, 1H, H₈) ppm. Anal. (C₂₀H₁₈Cl₂N₄O₃) C, H, N; C (%): calcd: 55.43; found: 55.71. H (%): calcd: 4.16; found: 4.23. N (%): calcd: 12.93; found: 12.75. HPLC: $R_t = 3.14$ min (12% yield).

3,6,7-Trimethyl-2-4-(4-chlorophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (18). Mp 214–215 °C. IR (KBr) ν 1646, 1496, 1329, 1000 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.41 (s, 3H, C₃–CH₃), 2.50 (s, 6H, 2CH₃–Ar), 3.01–4.00 (m, 8H, 4CH₂ piperazine), 6.98 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 7.4$ Hz), 7.26 (d, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 7.0$ Hz), 8.21 (s, 1H, H₅), 8.27 (s, 1H, H₈) ppm. Anal. (C₂₂H₂₃ClN₄O₃·1/4H₂O) C, H, N; C (%): calcd: 61.26; found: 61.39. H (%): calcd: 5.45; found: 5.47. N (%): calcd: 12.99; found: 12.46. HPLC: $R_t = 3.05$ min (5% yield).

3-Methyl-2-4-(4-chlorophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (19). Mp 216–217 °C. IR (KBr) ν 1648, 1496, 1330, 1232 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.44 (s, 3H, CH₃), 3.03–4.00 (m, 8H, 4CH₂ piperazine), 6.99 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 7.3$ Hz), 7.26 (d, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 7.4$ Hz), 7.95–8.02 (m, 2H, H₆ + H₇), 8.43–8.52 (m, 2H, H₅ + H₈) ppm. Anal. (C₂₀H₁₉ClN₄O₃·1/2H₂O) C, H, N; C (%): calcd: 58.90; found: 59.01. H (%): calcd: 4.90; found: 4.82. N (%): calcd: 13.74; found: 13.25. HPLC: $R_t = 3.02$ min (4% yield).

3,6,7-Trimethyl-2-4-(4-nitrophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (20). Mp 235–236 °C. IR (KBr) ν 1645, 1595, 1493, 1330 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.42 (s, 3H, C₃–CH₃), 2.49 (s, 3H, C₆ or C₇–CH₃), 2.50 (s, 3H, C₇ or C₆–CH₃), 3.38–3.89 (m, 8H, 4CH₂ piperazine), 7.04 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 9.4$ Hz), 8.07 (d, 2H, H_{3'} + H_{5'}), 8.21 (s, 1H, H₅), 8.27 (s, 1H, H₈) ppm. Anal. (C₂₂H₂₃N₅O₅·1/2H₂O) C, H, N; C (%): calcd: 59.19; found: 59.10. H (%): calcd: 5.38; found: 5.40. N (%): calcd: 15.69; found: 15.59 (15% yield).

3,7-Dimethyl-2-4-(4-nitrophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (21). Mp 274–275 °C. IR (KBr) ν 1642, 1598, 1492, 1332, 1243 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.43 (s, 3H, C₃–CH₃), 2.60 (s, 3H, CH₃–Ar), 3.46–3.92 (m, 8H, 4CH₂ piperazine), 7.07 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 8.9$ Hz), 7.83 (d, 1H, H₆, $J_{6-5} = 8.0$ Hz), 8.09 (d, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 8.8$ Hz), 8.26 (s, 1H, H₈) ppm. Anal. (C₂₁H₂₁N₅O₅·1/2H₂O) C, H, N; C (%): calcd: 58.33; found: 58.31. H (%): calcd: 5.09; found: 4.95. N (%): calcd: 16.20; found: 15.94 (29% yield).

3-Methyl-2-4-(4-nitrophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (22). Mp 220–221 °C. IR (KBr) ν 1643, 1597, 1334, 1241 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.42 (s, 3H, CH₃), 3.49–3.89 (m, 8H, 4CH₂ piperazine), 7.04 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 9.2$ Hz), 7.94–7.99 (m, 2H, H₆ + H₇), 8.07 (d, 2H, H_{3'} + H_{5'}), 8.41–8.51 (m, 2H, H₅ + H₈) ppm. Anal. (C₂₀H₁₉N₅O₅·1/2H₂O) C, H, N; C (%): calcd: 57.41; found: 57.26. H (%): calcd: 4.78; found: 4.62. N (%): calcd: 16.74; found: 16.64 (5% yield).

6,7-Dichloro-3-methyl-2-4-(4-nitrophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (23). Mp 223–224 °C. IR (KBr) ν 1656, 1325 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.43 (s, 3H, C₃–CH₃), 3.45–3.89 (m, 8H, 4CH₂ piperazine), 7.07 (d, 2H, H_{3'} + H_{5'}, $J_{3'-2'} = 9.4$ Hz), 8.08 (d, 2H, H_{2'} + H_{6'}), 8.62 (s, 1H, H₅), 8.67 (s, 1H, H₈) ppm. Anal. (C₂₀H₁₇Cl₂N₅O₅) C, H, N; C (%): calcd: 50.21; found: 50.87. H (%): calcd: 3.55; found: 3.62. N (%): calcd: 14.64; found: 14.48. HPLC: $R_t = 3.01$ min (16% yield).

3-Methyl-7-methoxy-2-4-(4-nitrophenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (24). Mp 264–265 °C. IR (KBr) ν 1643, 1598, 1492, 1330, 1241 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.42 (s, 3H, CH₃), 3.57–3.78 (m, 8H, 4CH₂ piperazine), 4.00 (s, 3H, OCH₃), 7.07 (d, 2H, H_{2'} + H_{6'}, $J_{2'-3'} = 7.9$ Hz), 7.60 (d, 1H, H₆, $J_{6-5} = 8.9$ Hz), 7.77 (s, 1H, H₈), 8.09 (d, 2H, H_{3'} + H_{5'}, $J_{3'-4'} = 8.3$ Hz), 8.42 (d, 1H, H₅) ppm. Anal. (C₂₁H₂₁N₅O₆·H₂O) C, H, N; C (%): calcd: 55.14; found: 55.34. H (%): calcd: 5.03; found: 4.81. N (%): calcd: 15.31; found: 14.98 (33% yield).

3,6,7-Trimethyl-2-4-(2-methoxyphenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (25). Mp 229–230 °C. IR (KBr) ν 1650, 1443, 1328, 1241 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 2.42 (s, 3H, C₃–CH₃), 2.49 (s, 6H, 2CH₃–Ar), 2.67–4.05 (m, 8H, 4CH₂ piperazine), 3.77 (s, 3H, OCH₃), 6.88–6.96 (m, 4H, H_{3'} + H_{4'} + H_{5'} + H_{6'}), 8.21 (s, 1H, H₅), 8.27 (s, 1H, H₈) ppm. Anal. (C₂₃H₂₆N₄O₄·1/2H₂O) C, H, N; C (%): calcd: 64.03; found: 64.02. H (%): calcd: 6.26; found: 6.02. N (%): calcd: 12.99; found: 12.80 (4% yield).

6,7-Dichloro-3-methyl-2-4-(2-methoxyphenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (26). Mp 212–213 °C. IR (KBr) ν 3065, 1649, 1462, 1323. ¹H NMR (DMSO-*d*₆) δ 2.44 (s, 3H, C₃–CH₃), 3.78 (s, 3H, OCH₃), 2.84–3.92 (m, 8H, 4CH₂ piperazine), 6.90–6.96 (m, 4H, H_{3'} + H_{4'} + H_{5'} + H_{6'}), 8.63 (s, 1H, H₅), 8.65 (s, 1H, H₈) ppm. Anal. (C₂₁H₂₀Cl₂N₄O₄) C, H, N; C (%): calcd:

54.42; found: 53.91. H (%): calcd: 4.32; found: 4.43. N (%): calcd: 12.10; found: 11.80. HPLC: R_t = 1.58 min (19% yield).

3-Methyl-2-4-(4-methoxyphenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (27). Mp 216–217 °C. IR (KBr) ν 3065, 1649, 1322, 1240, 831 cm^{-1} . ^1H NMR (DMSO- d_6) δ 2.48 (s, 3H, $\text{C}_3\text{--CH}_3$), 2.88–3.99 (m, 8H, 4CH₂ piperazine), 3.72 (s, 3H, OCH₃), 6.87 (d, 2H, $\text{H}_{2'} + \text{H}_{6'}$, $J_{2'-3'} = 9.0$ Hz), 6.97 (d, 2H, $\text{H}_{3'} + \text{H}_{5'}$), 7.98–8.04 (m, 2H, $\text{H}_6 + \text{H}_7$), 8.46–8.56 (m, 2H, $\text{H}_5 + \text{H}_8$) ppm. Anal. ($\text{C}_{21}\text{H}_{22}\text{N}_4\text{O}_4$) C, H, N; C (%): calcd: 63.96; found: 63.80. H (%): calcd: 5.58; found: 5.30. N (%): calcd: 14.21; found: 13.81. HPLC: R_t = 2.36 min (14% yield).

6,7-Dichloro-3-methyl-2-4-(4-methoxyphenyl)piperazin-1-ylcarbonylquinoxaline 1,4-dioxide (28). Mp 219–220 °C. IR (KBr) ν 3065, 1657, 1326, 1240, 831 cm^{-1} . ^1H NMR (DMSO- d_6) δ 2.47 (s, 3H, $\text{C}_3\text{--CH}_3$), 2.88–3.90 (m, 8H, 4CH₂ piperazine), 3.72 (s, 3H, OCH₃), 6.87 (d, 2H, $\text{H}_{2'} + \text{H}_{6'}$, $J_{2'-3'} = 9.1$ Hz), 6.98 (d, 2H, $\text{H}_{3'} + \text{H}_{5'}$), 8.67 (s, 1H, H_5), 8.71 (s, 1H, H_8) ppm. Anal. ($\text{C}_{21}\text{H}_{20}\text{Cl}_2\text{N}_4\text{O}_4$) C, H, N; C (%): calcd: 54.42; found: 54.15. H (%): calcd: 4.32; found: 4.35. N (%): calcd: 12.10; found: 12.01. HPLC: R_t = 2.40 min (16% yield).

3,6,7-Trimethyl-2-(4-benzylpiperazin-1-yl)carbonylquinoxaline 1,4-dioxide (29). Mp 210–211 °C. IR (KBr) ν 1649, 1474, 1330, 1019 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 2.41 (s, 3H, $\text{C}_3\text{--CH}_3$), 2.51 (s, 6H, 2CH₃–Ar), 2.40–3.80 (m, 8H, 4CH₂ piperazine), 3.55 (s, 2H, NCH₂Ph), 7.34 (bs, 5H, $\text{H}_{2'} + \text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'} + \text{H}_{6'}$), 8.22 (s, 1H, H_5), 8.27 (s, 1H, H_8) ppm. Anal. ($\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_3 \cdot 1/2\text{H}_2\text{O}$) C, H, N; C (%): calcd: 66.50; found: 66.31. H (%): calcd: 6.50; found: 6.77. N (%): calcd: 13.49; found: 13.52. HPLC: R_t = 3.26 min (11% yield).

3-Methyl-2-(4-benzylpiperazin-1-yl)carbonylquinoxaline 1,4-dioxide (30). Mp 194–195 °C. IR (KBr) ν 1655, 1473, 1331, 995 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 2.41 (s, 3H, CH₃), 2.25–3.85 (m, 8H, 4CH₂ piperazine), 3.52 (s, 2H, NCH₂Ph), 7.32 (bs, 5H, $\text{H}_{2'} + \text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'} + \text{H}_{6'}$), 7.93–7.98 (m, 2H, $\text{H}_6 + \text{H}_7$), 8.40–8.50 (m, 2H, $\text{H}_5 + \text{H}_8$) ppm. Anal. ($\text{C}_{21}\text{H}_{22}\text{O}_3\text{N}_4 \cdot 1/2\text{H}_2\text{O}$) C, H, N; C (%): calcd: 65.88; found: 65.52. H (%): calcd: 5.88; found: 6.22. N (%): calcd: 14.64; found: 14.97. HPLC: R_t = 3.33 min (35% yield).

6,7-Dichloro-3-methyl-2-(4-benzylpiperazin-1-yl)carbonylquinoxaline 1,4-dioxide (31). Mp 182–183 °C. IR (KBr) ν 1649, 1442, 1321. ^1H NMR (DMSO- d_6) δ 2.39 (s, 3H, $\text{C}_3\text{--CH}_3$), 2.42–3.79 (m, 8H, 4CH₂ piperazine), 3.53 (s, 2H, NCH₂Ph), 7.31 (bs, 5H, $\text{H}_{2'} + \text{H}_{3'} + \text{H}_{4'} + \text{H}_{5'} + \text{H}_{6'}$), 8.61 (d, 1H, H_5), 8.65 (d, 1H, H_8) ppm. Anal. ($\text{C}_{21}\text{H}_{20}\text{Cl}_2\text{N}_4\text{O}_3$) C, H, N; C (%): calcd: 56.38; found: 56.51. H (%): calcd: 4.47; found: 4.47. N (%): calcd: 12.53; found: 12.43. HPLC: R_t = 1.8 min (29% yield).

Biological evaluation

In vitro evaluation of anti-tuberculosis activity. Primary screening was conducted at 6.25 $\mu\text{g/mL}$ against *M. tuberculosis* H₃₇Rv (ATCC 27294) in BACTEC 12B

medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).¹⁴ Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.¹⁶ Compounds showing $\geq 90\%$ inhibition in the primary screening were considered active and then re-tested at lower concentration against *M. tuberculosis* H₃₇Rv in order to determine the actual minimum inhibitory concentration (MIC), using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% with respect to the controls.

Compounds were also tested for cytotoxicity (IC₅₀) in VERO cell line at a concentration equal to and greater than the MIC for *M. tuberculosis* H₃₇Rv. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radio-active Cell Proliferation assay. The Selectivity Index was also determined; it is considered significant when > 10 . Rifampin (RMP) was used as reference compound.

Macrophage assay. Compounds with a MIC ≤ 6.25 $\mu\text{g/mL}$ and a SI > 10 were then tested to evaluate efficacy in vitro in a TB-infected macrophage model.¹⁵ The EC₉₀ and EC₉₉ are defined as the concentrations effecting 90 and 99% reduction in residual mycobacterial growth after 7 days, compared to untreated controls. Compounds with EC₉₀ $> 16 \times \text{MIC}$ are considered inactive.

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