Synthesis of New Quinoxaline-2-carboxylate 1,4-Dioxide Derivatives as Anti-*Mycobacterium tuberculosis* Agents

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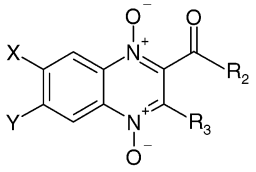
Twenty-nine new 6(7)-substituted quinoxaline-2-carboxylate 1,4-dioxide derivatives were synthesized and evaluated for in vitro antituberculosis activity. In general, the in vitro activity is significantly affected by substituents on the quinoxaline nucleus. It has been observed that the presence of a chloro, methyl, or methoxy group in position 7 of the benzene moiety reduces the MIC and IC_{50} values. However, antituberculosis activity principally depends on the substituents in the carboxylate group, improving in the following order: benzyl > ethyl > 2-methoxyethyl > allyl > *tert*-butyl. Fourteen compounds have been selected for macrophage assay, and the results show that ethyl and benzyl 3-methylquinoxaline-2-carboxylate 1,4-dioxide derivatives with the chlorine group in position 7 of the benzene moiety (compounds **10** and **26**) and the unsubstituted derivatives (compounds **11** and **27**) have good antitubercular activity, including activity in macrophages. In addition, compounds **7** and **28** (the only ones tested up to now) are active against drug-resistant strains of *M. tuberculosis* $H_{37}Rv$. In conclusion, the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.

Introduction

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis,* still remains the leading cause of worldwide death among infectious diseases. The statistics indicate that 3 million people throughout the world die annually from tuberculosis,[1,2] and there are an estimated 8 million new cases each year, 95% of which occur in developing countries.[3] 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.[4] Because of the fact that the current frontline therapy for TB consists of administering three different drugs (isoniazid, rifampin, and pyrazinamide) during an extended period of time[5] as well as the problems that arise due to MDR-TB (multidrug-resistant tuberculosis), it is necessary to develop new therapeutic agents that have a unique mechanism of action, different from those of the currently used antitubercular drugs, to treat drug-resistant forms of the disease.[6]

The quinoxaline derivatives show very interesting biological properties (antibacterial, antiviral, anticancer, antifungal, antihelmintic, insecticidal).[7,8] Over the past 2 decades, many mono- and di-*N*-oxides and 2-oxo derivatives of this heterocyclic system have been prepared and their biological activities have been reported. Some quinoxalin-2-ones have evidenced antifungal activity,[9,10] whereas the quinoxaline 1-oxides have shown antibacterial activity.[11] Oxidation of both nitrogens of the quinoxaline ring dramatically increases the diversity of biological properties such as antibacterial activity,[12−15] promotion of animal growth in feed additives,[16−18] hypoxia-selective activity,[19] etc.



I: R_2=alkyl or aryl R_3=CH_3

II: R_2=NHAr R_3=CH_3

III: R_2=OR R_3=CH_2R

Figure 1. General structure of 3-methyl-2-carbonylquinoxaline 1,4-dioxide.

Several papers have been published in which both synthesis and biological activity assessments of a large number of quinoxaline and quinoxaline 1,4-dioxide derivatives have been described. Different 2-acetyl and 2-benzoyl-3-methylquinoxaline 1,4-dioxide[20] (**I**, Figure 1) and *N*-aryl-3-methylquinoxaline-2-carboxamide 1,4-dioxides (**II**, Figure 1) derivatives showed high lead activity.[21] On the other hand, we observed that the lack of the two *N*-oxide groups generally led to the loss of the antimycobacterial activity.[22,23]

As a result of this research and for the purpose of obtaining new and more potent antitubercular compounds that can improve the current chemotherapeutic antituberculosis treatments, we have synthesized and evaluated 29 new quinoxaline-2-carboxylate 1,4-dioxide derivatives possessing methyl, ethyl, benzyl, and *tert*-butyl groups in position 2 and substituents such as methyl or 2-ethoxy-2-oxoethyl in position 3 (**III**, Figure 1).

Chemistry

The aforementioned compounds were prepared according to the synthetic sequences illustrated in Figure

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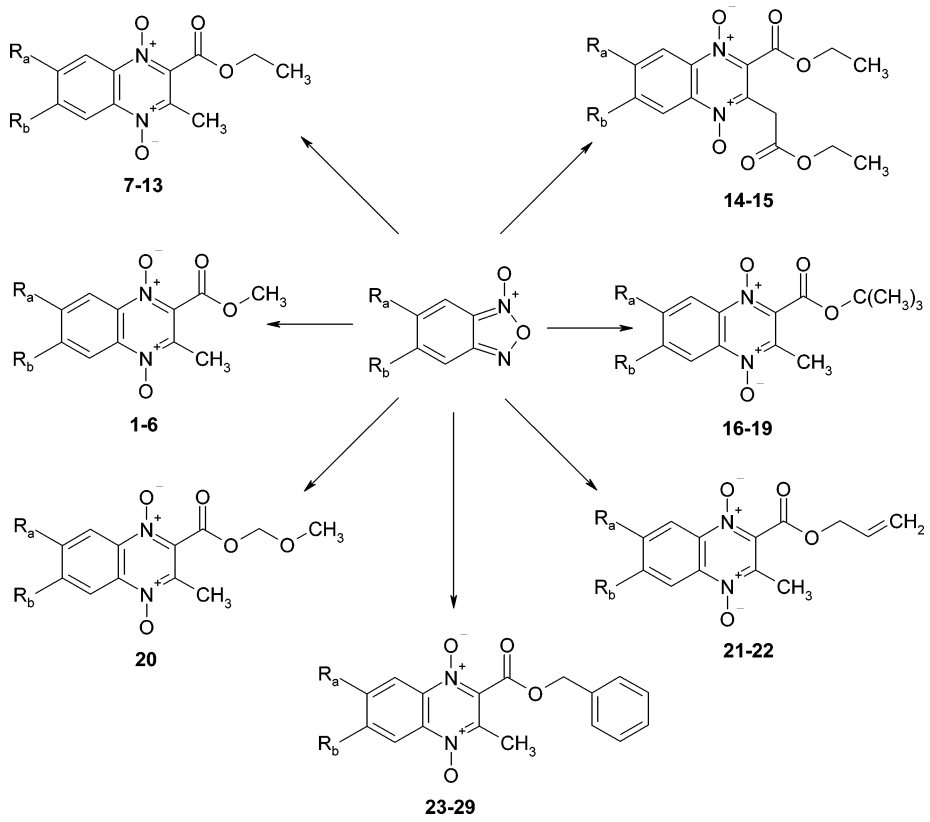


Figure 2. Synthetic route for compounds **1**−**29**.

2. The starting compound (benzofuroxane, 5-substituted or 5,6-disubstituted benzofuroxane) was obtained by previously described methods.[23]

The synthesis of compounds **1**−**13**, **15**, **16**, and **18**−**29** was carried out by reaction of the appropriate benzofuroxane with the corresponding β-ketoester, using triethylamine as the catalyst. Formation of isomeric quinoxaline 1,4-di-*N*-oxides was observed in the case of monosubstituted benzofuroxanes. According to previous reports,[7] we have observed that 7-substituted quinoxaline 1,4-di-*N*-oxides prevailed over the 6-isomer or only the 7-isomer formed in the case of a methoxy substituent. In practice, the workup and purification allow isolation of the major isomer.[24] Another synthetic route was carried out with a molecular sieve as the catalyst in order to obtain compounds **14** and **17**.[25]

All of the compounds were chemically characterized by thin-layer chromatography (TLC), melting point, infrared (IR), and nuclear magnetic resonance (^{1}H 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 U.S. National Institute of Allergy and Infectious Disease (NIAID), the 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 capability to inhibit the growth of virulent *M. tuberculosis*. Biological tests have been performed according to the previously described method.[26,27]

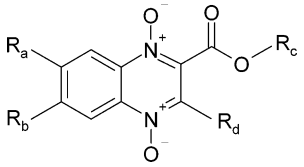
Results and Discussion

The results of the in vitro evaluation of antituberculosis activity are reported in Tables 1−3. In general, quinoxaline-2-carboxylate 1,4-dioxide derivatives have been shown to possess good antimycobacterial activity at the first level screening, with growth inhibition values ranging from 92% to 100%. Only compounds **16**−**19** (*tert*-butylquinoxalin-2-carboxylate 1,4-dioxide derivatives) failed to show activity. In the case of compound **21**, the presence of 6,7-dimethyl groups showed low activity (32% growth inhibition value).

Second level and cytotoxicity results are summarized in Table 1. All of the compounds that were active at the first level screening were then tested to determine the actual MIC. MIC is defined as the lowest concentration causing a 90% reduction in fluorescence with regard to controls. A significant number of samples (**5**, **11**, **12**, **15**, and **26**−**29**) showed high activity comparable to RMP with MICs ranging from 0.05 to 0.20 μg/mL.

Concurrent with the determination of MIC's, compounds were tested for cytotoxicity (IC_{50}) in VERO cells, and the selectivity index (SI = IC_{50}/MIC) was calculated. The activity is significantly affected by substituents on the quinoxaline nucleus. It has been observed that the presence of a chloro, methyl, or methoxy group in position 7 of the benzene moiety reduces both the MIC and IC_{50} values. The results have shown that in general antituberculosis activity depends on the substituents in the carboxylate group, improving in the following order: benzyl > ethyl > 2-methoxyethyl > allyl > *tert*-butyl.

Table 1. Results of Second Level and Cytotoxicity Antituberculosis Assays



compd	R_a	R_b	R_c	R_d	MIC (μg/mL)[a]	IC_{50} (μg/mL)[b]	SI (IC_{50}/MIC)[c]
1	CH_3	CH_3	CH_3	CH_3	3.13	>62.5	>20
2	CH_3	H	CH_3	CH_3	1.56	>62.5	>40.06
3	OCH_3	H	CH_3	CH_3	1.56	52.58	33.7
4	H	H	CH_3	CH_3	1.56	>10	>6.41
5	Cl	Cl	CH_3	CH_3	0.20	1.68	8.4
6	F	F	CH_3	CH_3	0.39	1.48	3.8
7	CH_3	CH_3	CH_3	CH_2CH_3	6.25	>62.5	>10
8	CH_3	H	CH_3	CH_2CH_3	1.56	>62.5	>40.06
9	OCH_3	H	CH_3	CH_2CH_3	6.25	39.95	6.4
10	H	H	CH_3	CH_2CH_3	1.56	47	30.13
11	Cl	H	CH_3	CH_2CH_3	0.20	>10	>50
12	Cl	Cl	CH_3	CH_2CH_3	<0.20	4	>20
13	F	F	CH_3	CH_2CH_3	1.56	1.2	0.77
14	Cl	H	$CH_2COOCH_2CH_3$	CH_2CH_3	1.56	1.89	1.2
15	Cl	Cl	$CH_2COOCH_2CH_3$	CH_2CH_3	0.20	1.22	6.1
16	CH_3	CH_3	CH_3	$C(CH_3)_3$	>6.25	ND	ND
17	H	H	CH_3	$C(CH_3)_3$	>6.25	ND	ND
18	Cl	H	CH_3	$C(CH_3)_3$	>6.25	ND	ND
19	Cl	Cl	CH_3	$C(CH_3)_3$	>6.25	ND	ND
20	Cl	Cl	CH_3	$CH_3CH_2OCH_3$	0.39	4.5	11.54
21	CH_3	CH_3	CH_3	$CH_2CH{=}CH_2$	>6.25	ND	ND
22	Cl	Cl	CH_3	$CH_2CH{=}CH_2$	6.25	5.8	0.93
23	CH_3	CH_3	CH_3	$CH_2C_6H_5$	3.13	insoluble	
24	CH_3	H	CH_3	$CH_2C_6H_5$	0.39	56.05	143.7
25	OCH_3	H	CH_3	$CH_2C_6H_5$	0.78	>62.5	>80.1
26	H	H	CH_3	$CH_2C_6H_5$	0.10	47	470
27	Cl	H	CH_3	$CH_2C_6H_5$	0.10	7.6	76
28	Cl	Cl	CH_3	$CH_2C_6H_5$	>6.25	ND	ND
29	F	F	CH_3	$CH_2C_6H_5$	>6.25	ND	ND

[a] Actual minimum inhibitory concentration (MABA assay). MIC of rifampin: 0.015−0.125 μg/mL versus *M. tuberculosis* $H_{37}Rv$. [b] Measurement of cytotoxicity in VERO cells. [c] Selectivity index.

Table 2. Results of Macrophage Assay

compd	EC_{90} (μg/mL)[a]	EC_{99} (μg/mL)[a]	EC_{90}/MIC[b]
1	1.64	6.15	0.52
2	1.94	5.83	1.24
10	0.88	3.32	0.56
11	0.46	1.14	2.30
12	0.63	5.99	>3.15
20	>1.56	>1.56	>4.00
24	1.13	3.79	2.90
26	0.15	>2.00	1.50
27	0.0005	0.08	0.01

[a] The EC_{90} and EC_{99} are defined as the concentrations causing 90% and 99% reductions in residual mycobacterial growth after 7 days, compared to untreated controls. [b] Compounds with EC_{90} > 16 MIC are considered inactive.

The best SI values have been obtained from unsubstituted compounds or from compounds having just one substituent in position 7. The benzyl quinoxaline-2-carboxylate 1,4-dioxide derivatives (**24**−**27**) have been proven to be the most active, with SI values ranging from 76 to 470.

Fourteen compounds (Tables 2 and 3) with a high SI value were then selected for testing for in vitro efficacy in a TB-infected macrophage model and for single-drug-resistant minimun inhibitory concentration (SDRMIC) assay. Macrophage assay results are reported in Table 2. On the basis of the data obtained, five compounds stand out in the macrophage assay, e.g., **10**, **26**, and **27** (EC_{90}/MIC = 0.56, 1.50, and 0.01, respectively). They are 7-chloroquinoxaline-2-carboxylate derivatives and their unsubstituted analogues, with a benzyl or ethyl group in the carboxylate. All of the compounds tested were active at this level and have been selected for in vivo assays.

SDRMIC assay results are reported in Table 3. Compounds **7** and **28** were evaluated against seven drug-resistant strains of *M. tuberculosis* $H_{37}Rv$. They were active in all of the resistant strains.

The compounds that were active in Tables 2 and 3 were selected for in vivo assay.

Conclusions

Screening of the in vitro antimycobacterial activity of these novel series, quinoxaline-2-carboxylate 1,4-dioxides, has indicated that the ethyl and benzyl 3-methylquinoxaline-2-carboxylate 1,4-dioxide derivatives with a chlorine group in position 7 of the benzene moiety (compounds **11** and **27**) and the unsubstituted derivatives (compounds **10** and **26**) have emerged as new compounds endowed with antitubercular activity, exhibiting EC_{90}/MIC values between 0.01 and 2.30. In conclusion, it has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.

Experimental Section

General. Melting points were determined using a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The ^1H NMR spectra were recorded on a

Table 3. Results of SDRMIC Assay

		compound **7**		compound **28**	
assay	strain[a]	MIC (μg/mL)	(resistant strain MIC)/(H_{37}Rv MIC)[b]	MIC (μg/mL)	(resistant strain MIC)/(H_{37}Rv MIC)
bactec	H37Rv	0.20		6.25	
alamar	H37Rv	0.39		≤3.13	
alamar	erdman	0.39		≤3.13	
alamar	EMB-R	0.78	2.00	≤3.13	1.00
alamar	INH-R	1.56	4.00	6.25	≥2.00
alamar	RMP-R	0.39	1.00	≤3.13	1.00
alamar	ETA-R	0.78	2.00	6.25	≥2.00
alamar	TAC-R	0.78	2.00	≤3.13	1.00
alamar	CIP-R	0.39	1.00	12.5	≥3.99
alamar	KM-R	0.39	1.00	≤3.13	1.00

[a] Strains resistant to the following drugs: EMB = ethambutol; INH =i soniazid; RMP = rifampin; ETA = ethionamide; TAC = thiacetazone; CIP = ciprofloxacin; KM = kanamycin. [b] In order for a compound to be considered active against a strain, the relationship (resistant strain MIC)/(H_{37}Rv MIC) should be less than 8.

Bruker AC-200E instrument (200 MHz) and Bruker 400 Ultrashield (400 MHz), using TMS as the internal standard and with DMSO-d_6 as the solvent. The chemical shifts are reported in ppm (δ), and coupling constant (J) values are given in hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), c (quadruplet), dd (double doublet), and m (multiplet). The IR spectra were obtained from a Perkin-Elmer 1600 FTIR (Norwalk, CT) in KBr pellets. The frequencies are expressed in cm^{-1}. Elemental microanalysis results were obtained on an elemental analyzer (Carlo Erba 1106, Milan, Italy) from vacuum-dried samples. The analytical results for C, H, and N were within ±0.4 of the theoretical values.

Alugram SIL G/UV_{254} (layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG, Postfach 101352, D-52313 Düren, Germany) was used for thin layer chromatography, and silica gel 60 (0.040−0.063 mm) was used for column chromatography (Merck). HPLC conditions were the following: column Nova Pack C18 60 A 4 μm (3.9 mm × 150 mm); mobile phase, acetonitrile/propan-2-ol (50:50) or acetonitrile/water (60:40); 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 Pharmaceuticalaan 3a, 2440 Geel, Belgium), and Lancaster (Bischheim-Strasbourg, France).

General Procedure for Compounds 1−29. Method A. The corresponding β-ketoester (10.6 mmol) was added to a solution of the appropriate benzofuroxane (2.4 mmol). The mixture was allowed to stand at 0 °C. Triethylamine was added dropwise (1 mL), and the reaction mixture was stirred at room temperature in darkness for 1−3 days. After evaporation to dryness under pressure, either a crude solid or a brown oil was obtained. It was then precipitated and washed by adding diethyl ether (or n-hexane), affording the target compound. The obtained yellow precipitate was purified by recrystallization from methanol. Flash column chromatography on silica gel (flash chromatography) was applied when necessary. Yields: 2−44%.

Method B. Molecular sieves [4Å (powder), 3 mmol] were added to a solution of the corresponding β-ketoester (2.4 mmol) and the appropriate benzofuroxane (2.4 mmol) in methanol (100 mL). The solvent (methanol) was evaporated in an evaporator at 20 °C. The molecular sieve containing the adsorbed reagents was allowed to stand for 0.5−1 h without drying, at 90 °C. The product was purified by means of flash column chromatography on silica gel, using a gradient of dichloromethane/methanol as the mobile phase. Yield: 3%.

Methyl 3,6,7-Trimethylquinoxaline-2-carboxylate 1,4-Dioxide (1). This compound was obtained in 20% yield from 5,6-dimethylbenzofuroxane (1.00 g, 6.10 mmol) and methyl acetoacetate (5.90 g, 50.83 mmol) after 24 h under stirring (method A): mp 200−201 °C; IR (KBr) ν 1747, 1508, 1438, 1326, 1265 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.40 (s, 3H, C_3-CH_3), 2.46 (s, 6H, $2CH_3-Ar$), 4.02 (s, 3H, $COOCH_3$), 8.11 (s, 1H, H_5), 8.15 (s, 1H, H_8) ppm. Anal. $C_{13}H_{14}N_2O_4$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 2.64 min.

Methyl 3,7-Dimethylquinoxaline-2-carboxylate 1,4-Dioxide (2). This compound was obtained in 5% yield from 5-methylbenzofuroxane (0.36 g, 2.4 mmol) and methyl acetoacetate (1.23 g, 10.6 mmol) after 24 h under stirring (method A): mp 125−126 °C; IR (KBr) ν 1743, 1330, 1285, cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.41 (s, 3H, C_3-CH_3), 2.57 (s, 3H, CH_3-Ar), 4.01 (s, 3H, $COOCH_3$), 7.82 (d, 1H, H_6, J_{6-5} = 8.1 Hz), 8.28 (s, 1H, H_8), 8.35 (d, 1H, H_5, J_{5-6} = 9 Hz) ppm. Anal. $C_{12}H_{12}N_2O_4 \cdot {}^1/_4H_2O$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 2.64 min.

Methyl 7-Methoxy-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (3). This compound was obtained in 2% yield from 5-methoxybenzofuroxane (0.40 g, 2.4 mmol) and methyl acetoacetate (1.23 g, 10.6 mmol) after 24 h under stirring. It was purified by recrystallization from methanol/n-hexane (method A): mp 146−147 °C; IR (KBr) ν 1749, 1330, 1253, 1177 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.37 (s, 3H, CH_3), 3.95 (s, 3H, $COOCH_3$), 3.99 (s, 3H, OCH_3), 7.58 (dd, 1H, H_6, J_{6-5} = 9.2 Hz, J_{6-8} = 2.6 Hz), 7.68 (ds, 1H, H_8, J_{8-6} = 2.6 Hz), 8.36 (d, 1H, H_5, J_{5-6} = 9.2 Hz) ppm. Anal. $C_{12}H_{12}N_2O_5 \cdot {}^1/_4H_2O$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 2.36 min.

Methyl 3-Methylquinoxaline-2-carboxylate 1,4-Dioxide (4). This compound was obtained in 11% yield from benzofuroxane (0.33 g, 2.4 mmol) and methyl acetoacetate (1.23 g, 10.6 mmol) after 24 h under stirring. It was purified by recrystallization from methanol/n-hexane (method A): mp 155−156 °C; IR (KBr) ν 1746, 1524, 1454, 1341, 1245 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.41 (s, 3H, CH_3), 4.00 (s, 3H, $COOCH_3$), 7.91−8.02 (m, 2H, $H_6 + H_7$), 8.38−8.48 (m, 2H, $H_5 + H_8$) ppm. Anal. $C_{11}H_{10}N_2O_4 \cdot {}^1/_2H_2O$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 2.48 min.

Methyl 6,7-Dichloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (5). This compound was obtained in 26% yield from 5,6-dichlorobenzofuroxane (0.46 g, 2.24 mmol) and methyl acetoacetate (2.47 g, 19.12 mmol) after 24 h under stirring (method A): mp 201−202 °C; IR (KBr) ν 1749, 1327, 1260 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.42 (s, 3H, C_3-CH_3), 4.03 (s, 3H, $COOCH_3$), 8.60 (s, 1H, H_5), 8.64 (s, 1H, H_8) ppm. Anal. $C_{11}H_8Cl_2N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 3.80 min.

Methyl 6,7-Difluoro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (6). This compound was obtained in 3% yield from 5,6-difluorobenzofuroxane (0.40 g, 2.33 mmol) and methyl acetoacetate (0.54 g, 4.18 mmol) after 24 h under stirring (method A): mp 148−149 °C; IR (KBr) ν 1735, 1333, 1260, 1176 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, C_3-CH_3), 4.02 (s, 3H, $COOCH_3$), 8.47 (dd, 1H, H_5, J_{5-6} = 8.5 Hz), 8.51 (dd, 1H, H_8, J_{8-7} = 8.5 Hz) ppm. Anal. $C_{11}H_8F_2N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 2.00 min.

Ethyl 3,6,7-Trimethylquinoxaline-2-carboxylate 1,4-Dioxide (7). This compound was obtained in 30% yield from 5,6-dimethylbenzofuroxane (0.40 g, 2.4 mmol) and ethyl ace-

toacetate (1.38 g, 10.6 mmol) after 24 h under stirring (method A): mp 183−184 °C; IR (KBr) ν 2988, 1748, 1518, 1328, 1255 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.36 (t, 3H, CH_2CH_3, J = 7.2 Hz), 2.41 (s, 3H, C_3-CH_3), 2.48 (s, 6H, $2CH_3-Ar$), 4.50 (c, 2H, CH_2CH_3, J = 7.1 Hz), 8.15 (s, 1H, H_5), 8.20 (s, 1H, H_8) ppm. Anal. $C_{14}H_{16}N_2O_4$ (C, H, N).

Ethyl 3,7-Dimethylquinoxaline-2-carboxylate 1,4-Dioxide (8). This compound was obtained in 5% yield from 5-methylbenzofuroxane (0.36 g, 2.4 mmol) and ethyl acetoacetate (1.38 g, 10.6 mmol) after 24 h under stirring. It was purified by recrystallization from methanol/*n*-hexane (method A): mp 159−160 °C; IR (KBr) ν 2980, 1740, 1525, 1331, 1244 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (t, 3H, CH_2CH_3, J = 7.1 Hz), 2.42 (s, 3H, C_3-CH_3), 2.58 (s, 3H, CH_3-Ar), 4.50 (c, 2H, CH_2CH_3, J = 7.1 Hz), 7.83 (d, 1H, H_6, J_{6-5} = 8.8 Hz), 8.22 (s, 1H, H_8), 8.36 (d, 1H, H_5, J_{5-6} = 8.8 Hz) ppm. Anal. $C_{13}H_{14}N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 2.03 min.

Ethyl 7-Methoxy-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (9). This compound was obtained in 11% yield from 5-methoxybenzofuroxane (0.20 g, 1.2 mmol) and ethyl acetoacetate (0.69 g, 5.3 mmol) after 24 h under stirring (method A): mp 157−158 °C; IR (KBr) ν 1732, 1334, 1250 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.30 (t, 3H, CH_2CH_3, J = 7.0 Hz), 2.36 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 4.45 (c, 2H, CH_2CH_3, J = 7.0 Hz), 7.55 (dd, 1H, H_6, J_{6-5} = 9.4 Hz, J_{6-8} = 2.7 Hz), 7.67 (ds, 1H, H_8, J_{8-6} = 2.6 Hz), 8.34 (d, 1H, H_5, J_{5-6} = 9.4 Hz) ppm. Anal. $C_{13}H_{14}N_2O_5 \cdot {}^1/_2H_2O$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 1.96 min.

Ethyl 3-Methylquinoxaline-2-carboxylate 1,4-Dioxide (10). This compound was obtained in 30% yield from benzofuroxane (2.50 g, 18.38 mmol) and ethyl acetoacetate (9.23 g, 70.91 mmol) after 24 h under stirring (method A): mp 135−136 °C; IR (KBr) ν 1739, 1332, 1288 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (t, 3H, $COOCH_2CH_3$, J = 6.9 Hz), 2.44 (s, 3H, C_3-CH_3), 4.50 (c, 2H, $COOCH_2CH_3$, J = 7.0 Hz), 7.95−8.01 (m, 2H, $H_6 + H_7$), 8.40−8.49 (m, 2H, $H_5 + H_8$) ppm. Anal. $C_{12}H_{12}N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 2.21 min.

Ethyl 7-Chloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (11). This compound was obtained in 26% yield from 5-chlorobenzofuroxane (1.50 g, 8.80 mmol) and ethyl acetoacetate (5.14 g 39.49 mmol) after 24 h under stirring (method A): mp 173−174 °C; IR (KBr) ν 1744, 1326, 1277 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (t, 3H, $COOCH_2CH_3$, J = 7.0 Hz), 2.42 (s, 3H, C_3-CH_3), 4.51 (c, 2H, $COOCH_2CH_3$, J = 7.0 Hz), 8.02 (dd, 1H, H_6, J_{6-5} = 9.1 Hz, J_{6-8} = 1.6 Hz), 8.40 (s, 1H, H_8), 8.46 (d, 1H, H_5, J_{5-6} = 9.1 Hz) ppm. Anal. $C_{12}H_{11}ClN_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 3.23 min.

Ethyl 6,7-Dichloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (12). This compound was obtained in 25% yield from 5,6-dichlorobenzofuroxane (0.30 g, 1.50 mmol) and ethyl acetoacetate (0.80 g, 6.15 mmol) after 24 h under stirring (method A): mp 197−198 °C; IR (KBr) ν 1748, 1326, 1264 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.44 (t, 3H, $COOCH_2CH_3$, J = 7.1 Hz), 2.52 (s, 3H, C_3-CH_3), 4.59 (c, 2H, $COOCH_2CH_3$, J = 7.1 Hz), 8.69 (s, 1H, H_5), 8.73 (s, 1H, H_8) ppm. Anal. $C_{12}H_{10}Cl_2N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 5.12 min.

Ethyl 6,7-Difluoro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (13). This compound was obtained in 11% yield from 5,6-difluorobenzofuroxane (0.40 g, 2.33 mmol) and ethyl acetoacetate (2.06 g, 15.60 mmol) after 24 h under stirring (method A): mp 114−115 °C; IR (KBr) ν 1738, 1331, 1257, 1171 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (t, 3H, $COOCH_2CH_3$, J = 7.1 Hz), 2.43 (s, 3H, C_3-CH_3), 4.50 (c, 2H, $COOCH_2CH_3$, J = 7.1 Hz), 8.47 (dd, 1H, H_5, J_{5-6} = 7.3 Hz), 8.51 (dd, 1H, H_8, J_{8-7} = 7.3 Hz) ppm. Anal. $C_{12}H_{10}F_2N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 2.77 min.

Ethyl 7-Chloro-3-(2-ethoxy-2-oxoethyl)quinoxaline-2-carboxylate 1,4-Dioxide (14). This compound was obtained in 3% yield from 5-chlorobenzofuroxane (0.40 g, 2.35 mmol) and diethyl 3-oxoglutarate (2.22 g, 10.98 mmol) after 0.5 h of heating (method B): mp 102−103 °C; IR (KBr) ν 1739, 1331, 1283 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.15 (t, 3H, $CH_2COOCH_2CH_3$, J = 7.0 Hz), 1.33 (t, 3H, $COOCH_2CH_3$, J = 7.0 Hz), 3.93 (s, 2H, $CH_2COOCH_2CH_3$), 4.11 (c, 2H, $CH_2COOCH_2CH_3$, J = 7.1 Hz), 4.48 (c, 2H, $COOCH_2CH_3$, J = 7.0 Hz), 8.06 (dd, 1H, H_6, J_{6-5} = 9.2 Hz, J_{6-8} = 1.6 Hz), 8.44 (s, 1H, H_8), 8.47 (d, 1H, H_5, J_{5-6} = 9.2 Hz) ppm. Anal. $C_{15}H_{15}ClN_2O_6$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 4.76 min.

Ethyl 6,7-Dichloro-3-(2-ethoxy-2-oxoethyl)quinoxaline-2-carboxylate 1,4-Dioxide (15). This compound was obtained in 9% yield from 5,6-dichlorobenzofuroxane (0.50 g, 2.44 mmol) and diethyl 3-oxoglutarate (4.33 g, 21.41 mmol) after 24 h under stirring (method A): mp 136−137 °C; IR (KBr) ν 1736, 1329, 1262, 1023 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.18 (t, 3H, $CH_2COOCH_2CH_3$, J = 7.1 Hz), 1.32 (t, 3H, $COOCH_2CH_3$, J = 7.0 Hz), 3.94 (s, 2H, $CH_2COOCH_2CH_3$), 4.11 (c, 2H, $CH_2COOCH_2CH_3$, J = 7.1 Hz), 4.49 (c, 2H, $COOCH_2CH_3$, J = 7.0 Hz), 8.65 (s, 1H, H_5), 8.67 (s, 1H, H_8) ppm. Anal. $C_{15}H_{14}Cl_2N_2O_6$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 8.32 min.

***tert*-Butyl 3,6,7-Trimethylquinoxaline-2-carboxylate 1,4-Dioxide (16).** This compound was obtained in 3% yield from 5,6-dimethylbenzofuroxane (0.40 g, 2.4 mmol) and *tert*-butyl acetoacetate (1.67 g, 10.6 mmol) after 24 h under stirring (method A): mp 191−192 °C; IR (KBr) ν 2932, 1742, 1458, 1330, 1154 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60 (s, 9H, $C(CH_3)_3$), 2.43 (s, 3H, C_3-CH_3), 2.49 (s, 6H, $2CH_3-Ar$), 8.16 (s, 1H, H_5), 8.22 (s, 1H, H_8) ppm. Anal. $C_{16}H_{20}N_2O_4$ (C, H, N).

***tert*-Butyl 3-Methylquinoxaline-2-carboxylate 1,4-Dioxide (17).** This compound was obtained in 3% yield from benzofuroxane (1.00 g, 7.4 mmol) and *tert*-butyl acetoacetate (1.17 g, 7.4 mmol) after 1 h of heating (method B). After column cromatography, the product was washed with diethyl ether and recrystallized from methanol: mp 128−129 °C; IR (KBr) ν 1734, 1341, 1259, 1163 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.59 (s, 9H, $C(CH_3)_3$), 2.44 (s,3H, CH_3), 7.94−7.99 (m, 2H, $H_6 + H_7$), 8.43 (m, 2H, $H_5 + H_8$) ppm. Anal. $C_{14}H_{16}N_2O_4 \cdot {}^1/_4H_2O$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 3.06 min.

***tert*-Butyl 7-Chloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (18).** This compound was obtained in 3% yield from 5-chlorobenzofuroxane (0.30 g, 1.76 mmol) and *tert*-butyl acetoacetate (1.36 g, 8.60 mmol) after 24 h under stirring (method A): mp 143−144 °C; IR (KBr) ν 1736, 1329, 1278 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60 (s, 9H, $COOC(CH_3)_3$), 2.43 (s, 3H, C_3-CH_3), 8.03 (dd, 1H, H_6, J_{6-5} = 9.1 Hz, J_{6-8} = 1.9 Hz), 8.41 (s, 1H, H_8), 8.46 (d, 1H, H_5, J_{5-6} = 9.1 Hz) ppm. Anal. $C_{14}H_{15}ClN_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 5.26 min.

***tert*-Butyl 6,7-Dichloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (19).** This compound was obtained in 9% yield from 5,6-dichlorobenzofuroxane (0.20 g, 0.98 mmol) and *tert*-butyl acetoacetate (0.67 g, 4.24 mmol) after 24 h under stirring (method A): mp 174−175 °C; IR (KBr) ν 1734, 1326 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.58 (s, 9H, $COOC(CH_3)_3$), 2.41 (s, 3H, C_3-CH_3), 8.58 (s, 1H, H_5), 8.60 (s, 1H, H_8) ppm. Anal. $C_{14}H_{14}Cl_2N_2O_4$ (C, H, N).

2-Methoxyethyl 6,7-Dichloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (20). This compound was obtained in 11% yield from 5,6-dichlorobenzofuroxane (0.30 g, 1.46 mmol) and 2-methoxyethyl acetoacetate (1.06 g, 6.62 mmol) after 24 h under stirring (method A): mp 150−151 °C; IR (KBr) ν 1738, 1328, 1277, 1131 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (s, 3H, C_3-CH_3), 3.29 (s, 3H, $COOCH_2CH_2OCH_3$), 3.66 (t, 2H, $COOCH_2CH_2OCH_3$, J = 4.5 Hz), 4.60 (t, 2H, $COOCH_2CH_2OCH_3$, J = 4.5 Hz), 8.61 (s, 1H, H_5), 8.65 (s, 1H, H_8) ppm. Anal. $C_{13}H_{12}Cl_2N_2O_5$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 4.09 min.

Allyl 3,6,7-Trimethylquinoxaline-2-carboxylate 1,4-Dioxide (21). This compound was obtained in 10% yield from 5,6-dimethylbenzofuroxane (0.50 g, 3.0 mmol) and allyl acetoactate (1.88 g, 13.3 mmol) after 24 h under stirring (method A): mp 139−140 °C; IR (KBr) ν 1741, 1443, 1328, 1273, 1221 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.50 (s, 6H, $2CH_3-Ar$), 2.55 (s, 3H, C_3-CH_3), 4.97 (d, 2H, $COOCH_2CH$, J = 5.9 Hz), 5.38 (d, 1H, $CH=CH_2$, H_{cis}, J = 10.2 Hz), 5.52 (d, 1H, $CH=CH_2$, H_{trans}, J = 17.1 Hz), 5.98−6.06 (m, 1H, $CH=CH_2$, J = 6.0 Hz), 8.29 (s, 1H, H_5), 8.34 (s, 1H, H_8) ppm. Anal.

$C_{15}H_{16}N_2O_4 \cdot {}^1/_4H_2O$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 3.05 min.

Allyl 6,7-Dichloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (22). This compound was obtained in 7% yield from 5,6-dichlorobenzofuroxane (0.30 g, 1.46 mmol) and allyl acetoacetate (0.93 g, 6.54 mmol) after 24 h under stirring (method A): mp 141−142 °C; IR (KBr) ν 1686, 1637, 1329, 1241 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.42 (s, 3H, C_3-CH_3), 4.98 (d, 2H, COOC**H**$_2$CH=CH$_2$, J = 5.4 Hz), 5.35 (d, 1H, COOCH$_2$CH=C**H**$_2$, H$_{cis}$, J = 10.5 Hz), 5.51 (d, 1H, COOCH$_2$-CH=C**H**$_2$, H$_{trans}$, J = 17.8 Hz), 6.01 (m, 1H, COOC**H**=CH$_2$), 8.61 (s, 1H, H$_5$), 8.63 (s, 1H, H$_8$) ppm. Anal. $C_{13}H_{10}Cl_2N_2O_4$ (C, H, N).

Benzyl 3,6,7-Trimethylquinoxaline-2-carboxylate 1,4-Dioxide (23). This compound was obtained in 21% yield from 5,6-dimethylbenzofuroxane (0.50 g, 3.0 mmol) and benzyl acetoacetate (2.50 g, 13.3 mmol) after 24 h under stirring (method A): mp 159−160 °C; IR (KBr) ν 3065, 2936, 1740, 1514, 1328 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.36 (s, 3H, C_3-CH_3), 2.47 (s, 6H, 2CH_3−Ar), 5.52 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.37−7.54 (m, 5H, H$_{2'}$ + H$_{3'}$ + H$_{4'}$ + H$_{5'}$ + H$_{6'}$), 8.17 (s, 1H, H$_5$), 8.20 (s, 1H, H$_8$) ppm. Anal. $C_{19}H_{18}N_2O_4 \cdot {}^1/_4H_2O$ (C, H, N). HPLC: ACN/propan-2-ol (50:50), t_R = 3.05 min.

Benzyl 3,7-Dimethylquinoxaline-2-carboxylate 1,4-Dioxide (24). This compound was obtained in 17% yield from 5-methylbenzofuroxane (0.36 g, 2.4 mmol) and benzyl acetoacetate (2.04 g, 10.6 mmol) after 24 h under stirring and purified by flash chromatography, eluting with DCM/MeOH 97.5:2.5 (method A): mp 111−112 °C; IR (KBr) ν 1741, 1329, 1236 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.40 (s, 3H, C_3-CH_3), 2.54 (s, 3H, CH_3−Ar), 5.46 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.32−7.41 (m, 5H, H$_{2'}$ + H$_{3'}$ + H$_{4'}$ + H$_{5'}$ + H$_{6'}$), 7.62 (d, 1H, H$_6$), 8.29 (s, 1H, H$_8$), 8.41 d, 1H, H$_5$) ppm. Anal. $C_{18}H_{16}N_2O_4 \cdot {}^1/_2H_2O$ (C, H, N).

Benzyl 7-Methoxy-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (25). This compound was obtained in 17% yield from 5-methoxybenzofuroxane (0.20 g, 1.2 mmol) and benzyl acetoacetate (1.02 g, 5.3 mmol) after 24 h under stirring. It was purified by flash chromatography, eluting with DCM/MeOH 98:2. The obtained oil was precipitated by adding cold diethyl ether (method A): mp 129−130 °C; IR (KBr) ν 1739, 1331, 1237 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.39 (s, 3H, C_3-CH_3), 3.98 (s, 3H, OCH_3), 5.46 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.39−7.51 (m, 5H, H$_{2'}$ + H$_{3'}$ + H$_{4'}$ + H$_{5'}$ + H$_{6'}$), 7.59 (d, 1H, H$_6$), 8.29 (s, 1H, H$_8$), 8.41 d, 1H, H$_5$) ppm. Anal. $C_{18}H_{16}N_2O_5$ (C, H, N).

Benzyl 3-Methylquinoxaline-2-carboxylate 1,4-Dioxide (26). This compound was obtained in 41% yield from benzofuroxane (0.40 g, 2.4 mmol) and benzyl acetoacetate (1.70 g, 10.6 mmol) after 24 h under stirring (method A): mp 102−103 °C; IR (KBr) ν 3087, 1752, 1519, 1330, 1233 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 5.54 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.95−8.01 (m, 2H, H$_6$ + H$_7$), 8.41−8.44 (m, 2H, H$_5$ + H$_8$) ppm. Anal. $C_{17}H_{14}N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 2.59 min.

Benzyl 7-Chloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (27). This compound was obtained in 14% yield from 5-chlorobenzofuroxane (1.50 g, 8.80 mmol) and benzyl acetoacetate (5.56 g, 28.93 mmol) after 24 h under stirring (method A): mp 148−149 °C; IR (KBr) ν 1749, 1322, 1287 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, C_3-CH_3), 5.54 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.40−7.45 (m, 3H, H$_{3'}$ + H$_{4'}$ + H$_{5'}$), 7.52 (d, 2H, H$_{2'}$ + H$_{6'}$, $J_{2'-3'}$ = 8.1 Hz), 8.02 (dd, 1H, H$_6$, J_{6-5} = 9.2 Hz, J_{6-8} = 2.3 Hz), 8.42 (s, 1H, H$_8$), 8.45 (d, 1H, H$_5$, J_{5-6} = 9.2 Hz) ppm. Anal. $C_{17}H_{13}ClN_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 7.02 min.

Benzyl 6,7-Dichloro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (28). This compound was obtained in 44% yield from 5,6-dichlorobenzofuroxane (0.50 g, 2.44 mmol) and benzyl acetoacetate (2.40 g, 12.49 mmol) after 24 h under stirring (method A): mp 147−148 °C; IR (KBr) ν 1749, 1327, 1252 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, C_3-CH_3), 5.54 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.39−7.45 (m, 3H, H$_{3'}$ + H$_{4'}$ + H$_{5'}$), 7.52 (d, 2H, H$_{2'}$ + H$_{6'}$, $J_{2'-3'}$ = 8.0 Hz), 8.62 (s, 1H, H$_5$), 8.64 (s, 1H, H$_8$) ppm. Anal. $C_{17}H_{12}Cl_2N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 12.79 min.

Benzyl 6,7-Difluoro-3-methylquinoxaline-2-carboxylate 1,4-Dioxide (29). This compound was obtained in 20% yield from 5,6-difluorobenzofuroxane (0.40 g, 2.33 mmol) and benzyl acetoacetate (0.55 g, 2.86 mmol) after 24 h under stirring (method A): mp 148−149 °C; IR (KBr) ν 1687, 1323, 1249, 1074 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, C_3-CH_3), 5.54 (s, 2H, COOC**H**$_2$C$_6$H$_5$), 7.39−7.45 (m, 3H, H$_{3'}$ + H$_{4'}$ + H$_{5'}$), 7.52 (d, 2H, H$_{2'}$ + H$_{6'}$, $J_{2'-3'}$ = 7.6 Hz), 8.47−8.52 (m, 2H, H$_5$ + H$_8$) ppm. Anal. $C_{17}H_{12}F_2N_2O_4$ (C, H, N). HPLC: ACN/H_2O (60:40), t_R = 3.47 min.

Biological Evaluation. In Vitro Evaluation of Antituberculosis Activity. Primary Screen (Level 1). The primary screen is conducted at 6.25 μg/mL against *Mycobacterium tuberculosis* H$_{37}$Rv (ATCC 27294) in BACTEC 12B medium, using the microplate alamar blue assay (MABA).[26] Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system.[28] Generally, compounds effecting <90% inhibition in the primary screen (MIC > 6.25 μg/mL) are not further evaluated.

Determination of Minimum Inhibitory Concentration (MIC) (Level 2). Compounds demonstrating at least 90% inhibition in the primary screen are retested at lower concentrations against *M. tuberculosis* H$_{37}$Rv in order to determine the actual MIC in the MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampin (RMP) was used as the reference compound (RMP MIC = 0.015−0.125 μg/mL).

Determination of 50% Inhibitory Concentrations (IC_{50}) (Level 2). Concurrent with the determination of MIC values, compounds are tested for cytotoxicity (IC_{50}) in VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for *M. tuberculosis* H$_{37}$Rv. After 72 h of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 nonradioactive cell proliferation assay. The selectivity index (SI = IC_{50}/MIC) was also determined; it is considered significant when greater than 10 (RMP IC_{50} > 100 μg/mL, SI > 800).

Macrophage Assay. Determination of 90% and 99% Effective Concentration (EC_{90} and EC_{99}) (Level 3). Compounds with MIC ≤ 6.25 μg/mL and SI > 10 were then tested to evaluate in vitro efficacy in a TB-infected macrophage model.[26] Compounds are tested for the killing of *M. tuberculosis* Erdman (ATCC 35801) in monolayers of mouse bone marrow macrophages (EC_{90} and EC_{99}; lowest concentration effecting a 90% and 99% reduction, respectively, in colony forming units at 7 days compared to drugfree controls) at 4-fold concentrations equivalent to 0.25, 1, 4, and 16 times the MIC. Compounds with EC_{90} > 16 MIC are considered inactive in the model (RMP EC_{90} = 0.04−0.1 μg/mL, EC_{99} = 0.5−1.5 μg/mL).

Determination of MIC against Three Strains of Single-Drug-Resistant *M. tuberculosis* (SDRMIC) (Level 3). Concurrent with the testing of compounds in macrophages, MIC values are determined in the MABA for a minimum of three strains of drug-resistant *M. tuberculosis* (each strain resistant to a single TB drug). Typically, all compounds progressing to this stage of screening will be tested against *M. tuberculosis* strains resistant to isoniazid (ATCC 35822), rifampin (ATCC 35838), and other drug-resistant strains (these latter strains determined by the compound type) as well as the drug-sensitive strains H$_{37}$Rv and Erdman. Generally, MIC's for SDR strains should not be greater than 10 MIC for nonresistant strains in order for compound evaluation to continue.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Snider, D. E.; Raviglione, M.; Kochi, A. In *Global Burden of Tuberculosis. Tuberculosis: Patogenesis, Protection and Control*; Bloom, B., Eds; ASM: Washington, DC, 1994; pp 3−4.

(2) Enarson, D. A. Global disease and the role of international collaboration. *Can. Med. Assoc. J*. **2000**, *162*, 57−61.

(3) Bishai, W. R.; Graham, N. M. H.; Harrington, S.; Pope, D. S.; Hooper, N.; Astemborski, J. Molecular and geographic patterns of tuberculosis transmission after 15 years of directly observed therapy. *JAMA, J. Am. Med. Assoc.* **1998**, *280*, 1679−1684.

(4) OMS. WHO annual report on global TB control summary. *Weekly Epidemiol. Rec.* **2003**, *78* (15), 121−128.

(5) Bass, J. B., Jr.; Farer, L. S.; Hopewell, P. C.; O'Brien, R.; Jacobs, R. F.; Ruben, F.; Snider, D. E., Jr.; Thornton, G. Treatment of tuberculosis and tuberculosis infection in adults and children. American Thoracic Society and The Centers for Disease Control and Prevention. *Am. J. Respir. Crit. Care Med.* **1994**, *149*, 1359−1374.

(6) Pathak, A. K.; Pathak, V.; Seitz, L.; Maddry, J. A.; Gurcha, S. S.; Besra, G. S.; Suling, W. J.; Reynolds, R. C. Studies on (β,1-5) and (β,1-6) Linked Octyl Galf Disaccharides as Substrates for Mycobacterial Galactosyltransferase Activity. *Bioorg. Med. Chem.* **2001**, *9*, 3129−3143.

(7) Cheeseman, G. W. H.; Cookson, R. F. In *The Chemistry of Heterocyclic Compounds*, 2nd ed.; Weissberger, A., Taylor, E. C., Eds.; Wiley: New York, 1979; pp 1−27, 35−38.

(8) Porter, A. E. A. In *Comprehensive Heterocyclic Chemistry*; Katrizky, A. R., Rees, C. W., Eds.; Pergamon Press: New York, 1984; Vol. 3, pp 157−197.

(9) Carta, A.; Paglietti, G.; Rhbarnikookar, M. E.; Sanna, P.; Sechi, L.; Zanetti, S. Novel substituted quinoxaline 1,4-dioxides with in vitro antimycobacterial and anticandida activity. *Eur. J. Med. Chem*. **2002**, *37*, 355−366.

(10) Sanna, P.; Carta, A.; Loriga, M.; Zanetti, S.; Sechi, L. Synthesis of 3,6,7-substituted-quinoxalin-2-ones for evaluation of antimicrobial and anticancer activity. Part 2. *Farmaco* **1999**, *54*, 169−177.

(11) Dirlam, J. P.; Presslitz, J. E.; Williams, B. J. Synthesis and antibacterial activity of some 3-[(alkylthio)methyl]quinoxaline 1-oxide derivatives. *J. Med. Chem.* **1983**, *26*, 1122−1126.

(12) Takabatake, T.; Takabatake, Y.; Miyazawa, T.; Hasegawa, M. Synthesis and antibacterial properties of quinoxaline 1,4-dioxide derivatives. *Yakugaku Zasshi* **1996**, *116*, 491−496.

(13) Novacek, L. The antibacterial, quinoxaline 1,4-dioxide. *Cesk. Farm.* **1981**, *30*, 26−32.

(14) el-Hawash, S. A.; Habib, N. S.; Fanaki, N. H. Quinoxaline derivatives. Part II: Synthesis and antimicrobial testing of 1,2,4-triazolo[4,3-*a*]quinoxaline 1,2,4-triazino[4,3-*a*]quinoxalines and 2-pyrazolylquinoxalines. *Pharmazie* **1999**, *54*, 808−813.

(15) Badran, M. M.; Abouzid, K. A.; Hussein, M. H. Synthesis of certain substituted quinoxalines as antimicrobial agents (Part II). *Arch Pharmacal. Res.* **2003**, *26*, 107−113.

(16) Hutchinson, M. J.; Young, P. Y.; Hewitt, S. A.; Faulkner, D.; Kennedy, D. G. Development and validation of an improved method for confirmation of the carbadox metabolite, quinoxaline-2-carboxylic acid, in porcine liver using LC−electrospray MS−MS according to revised EU criteria for veterinary drug residue analysis. *Analyst* **2002**, *127*, 342−346.

(17) Dunlop, S. H.; McEwen, S. A.; Meek, A. H.; Friendship, R. A.; Clarke, R. C.; Black, W. D. Antimicrobial drug use and related management practices among Ontario swine producers. *Can. Vet. J.* **1998**, *39*, 87−96.

(18) Yen, T. J.; Pnd, W. G. Effects of carbadox, copper, or Yucca shidigera extract on growth performance and visceral weight of young pigs. *J Anim. Sci*. **1993**, *71*, 2140−2146.

(19) Monge, A.; Palop, J. A.; López de Cerain, A.; Senador, V.; Martínez-Crespo, F. J.; Sainz, Y.; Narro, S.; García, E.; de Miguel, C.; González, M.; Hamilton, E.; Barker, A. J.; Clarke, E. D.; Greenhow, D. T. Hypoxia-selective agents derived from 2-quinoxalinecarbonitrile 1,4-di-*N*-oxides. *J. Med. Chem.* **1995**, *38*, 1786−1792.

(20) Zarranz, B.; Jaso, A.; Aldana, I.; Monge, A. Synthesis and antimycobacterial activity of new quinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives. *Bioorg. Med. Chem.* **2003**, *11*, 2149−2156.

(21) Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. Synthesis of new 2-acetyl and 2-benzoyl quinoxaline 1,4-di-*N*-oxide derivatives as anti-*Mycobacterium tuberculosis* agents. *Eur. J. Med. Chem.* **2003**, *38*, 791−800.

(22) Montoya, M. E.; Sainz, Y.; Ortega, M. A.; Lopez de Cerain, A.; Monge, A. Synthesis and antituberculosis activity of some new 2-quinoxalinecarbonitriles. *Farmaco* **1998**, *53*, 570−573.

(23) Ortega, M. A.; Sainz, Y.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. Anti-*Mycobacterium tuberculosis* agents derived from quinoxaline-2-carbonitrile and quinoxaline-2-carbonitrile 1,4-di-*N*-oxide. *Arzneim.-Forsch.* **2002**, *52*, 113−119.

(24) Ortega, M. A.; Morancho, M. J.; Martínez-Crespo, F. J.; Sainz, Y.; Montoya, M. E.; Lopez de Cerain, A.; Monge, A. New quinoxalinecarbonitrile 1,4-di-*N*-oxide derivatives as hypoxic-cytotoxic agents. *Eur. J. Med. Chem.* **2000**, *35*, 21−30.

(25) Takabatake, T.; Hasegawa, Y.; Hasegawa, H. The reactions of benzofuroxan with carbonyl compounds on the surface of solid catalyst. *J. Heterocycl. Chem*. **1993**, *30*, 1477−1479.

(26) Collins, L. A.; Franzblau, S. G. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.

(27) Skinner, P. S.; Furney, S. K.; Jacobs, M. R.; Klopman, G.; Ellner, J. J.; Orme, I. M. A bone marrow-derived murine macrophage model for evaluating efficacy of antimycobacterial drugs under relevant physiological conditions. *Antimicrob. Agents Chemother.* **1994**, *38*, 2557−2563.

(28) Kelly, B. P.; Furney, S. K.; Jessen, M. T.; Orme, I. M. Low-dose aerosol infection model for testing drugs for efficacy against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother*. **1996**, *40*, 2809−2812.

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