Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 791–800
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis of new 2-acetyl and 2-benzoyl quinoxaline 1,4-di-*N*-oxide derivatives as anti-*Mycobacterium tuberculosis* agents

Andrés Jaso, Belén Zarranz, Ignacio Aldana *, Antonio Monge

Unidad en Investigación y Desarrollo de Medicamentos, Centro de Investigación en Farmacobiología Aplicada (CIFA), Universidad de Navarra, c/Irunlarrea s/n, 31080 Pamplona, Spain

Received 6 March 2003; received in revised form 3 June 2003; accepted 6 June 2003

Abstract

A series of 2-acetyl and 2-benzoyl-6(7)-substituted quinoxaline 1,4-di-*N*-oxide derivatives were synthesized and evaluated for in vitro antituberculosis activity. The results show that 2-acetyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives with chlorine, methyl or methoxy group in position 7 of the benzene moiety (compounds **2**, **4** and **6**, respectively) and unsubstituted (**3**) have good antitubercular activity, exhibiting EC_{90}/MIC values between 0.80 and 4.29. In conclusion, the potency, selectivity and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.

Keywords: Quinoxaline-1,4-dioxide; Tuberculosis; Anti-*Mycobacterium*

1. Introduction

Tuberculosis is a contagious disease with high mortality worldwide. 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]. In addition, about a third of the world's population harbors a dormant *Mycobacterium tuberculosis* infection, representing a significant reservoir of disease for the future.

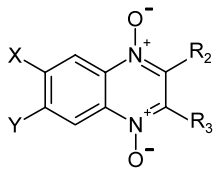
Current frontline therapy consists of administering one of three drugs (isoniazid, rifampin or pyrazinamide) for 2 months followed by 4 months of follow-up therapy with isoniazid and rifampin [4]. Thus, the problem arising due to MDR-TB requires the development of new therapeutic agents that have a unique mechanism of action, different from the currently used antitubercular drugs, in order to treat drug-resistant forms of the disease [5].

The quinoxaline derivatives show very interesting biological properties (antibacterial, antiviral, anticancer, antifungal, antihelmintic, insecticidal) [6,7]. Over the last two decades, many mono- and di-*N*-oxides and 2-oxo derivatives of this heterocyclic system have appeared on the scene and their biological activities have been reported. Some quinoxalin-2-ones have evidenced antifungal activity [8,9], whereas the quinoxaline 1-oxides have shown antibacterial activity [10]. Oxidation of both nitrogens of the quinoxaline ring dramatically increases the diversity of biological properties, such as antibacterial activity [11–14], promotion of animal growth in feed additives [15–17], hypoxia-selective activity [18], etc.

Several papers have been published, in which both synthesis and biological activity assessments of a large amount of quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives have been described. 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% [19] 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% [20,21]. On the other hand, we observed that the lack of the two *N*-oxide groups generally led to the loss of the antimycobacterial activity [21,22].

* Corresponding author.
E-mail address: ialdana@unav.es (I. Aldana).

0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00137-5



I: R_2=CN R_3=NH-C_6H_5
II: R_2=COOEt R_3=CH_3
III: R_2=COR R_3=CH_2Z

Fig. 1. General structure of quinoxaline 1,4-di-*N*-oxide.

As a result of this research, and for the purpose of obtaining new and more potent antitubercular compounds which can improve the current chemotherapeutic antituberculosis treatments, we have synthesized and evaluated 28 new 2-carbonylquinoxaline 1,4-di-*N*-oxide derivatives possessing acetyl or benzoyl groups in position 2 and different substituents, such as methyl, (4-phenylpiperazin-1-yl)methyl, or phenoxymethyl derivatives in position 3 (**III**, Fig. 1).

2. Chemistry

The aforementioned compounds were prepared according to the synthetic sequences illustrated in Fig. 2. The starting compounds, benzofuroxane, 5-substituted or 5,6-disubstituted benzofuroxane, were obtained by previously described methods [23].

The synthesis of compounds **1**–**12** was carried out by the reaction of appropriate benzofuroxane with the corresponding acetoacetone, using triethylamine as the catalyst. Formation of isomeric quinoxaline 1,4-di-*N*-oxides was observed in the case of monosubstituted benzofuroxanes. According to the previous reports [6], 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 methoxy substituent. In practice, the workup and purification allow isolation of the major isomer [24].

The bromation of 2-acetyl-3-methylquinoxaline derivatives was carried out by the reaction with *N*-bromosuccinimide and peroxy benzoic acid as the catalyst (**13**–**15**). The intermediate 2-acetyl-3-bromomethyl-6,7-dimethylquinoxaline 1,4-di-*N*-oxide used for the purpose of obtaining the compounds **17**, **20** and **22** could not be totally purified. Compounds **16**–**28** were obtained by the reaction of appropriate 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide and the corresponding phenol or 1-phenylpiperazine derivative.

All the compounds were chemically characterized by thin-layer chromatography (TLC), melting point, infra-

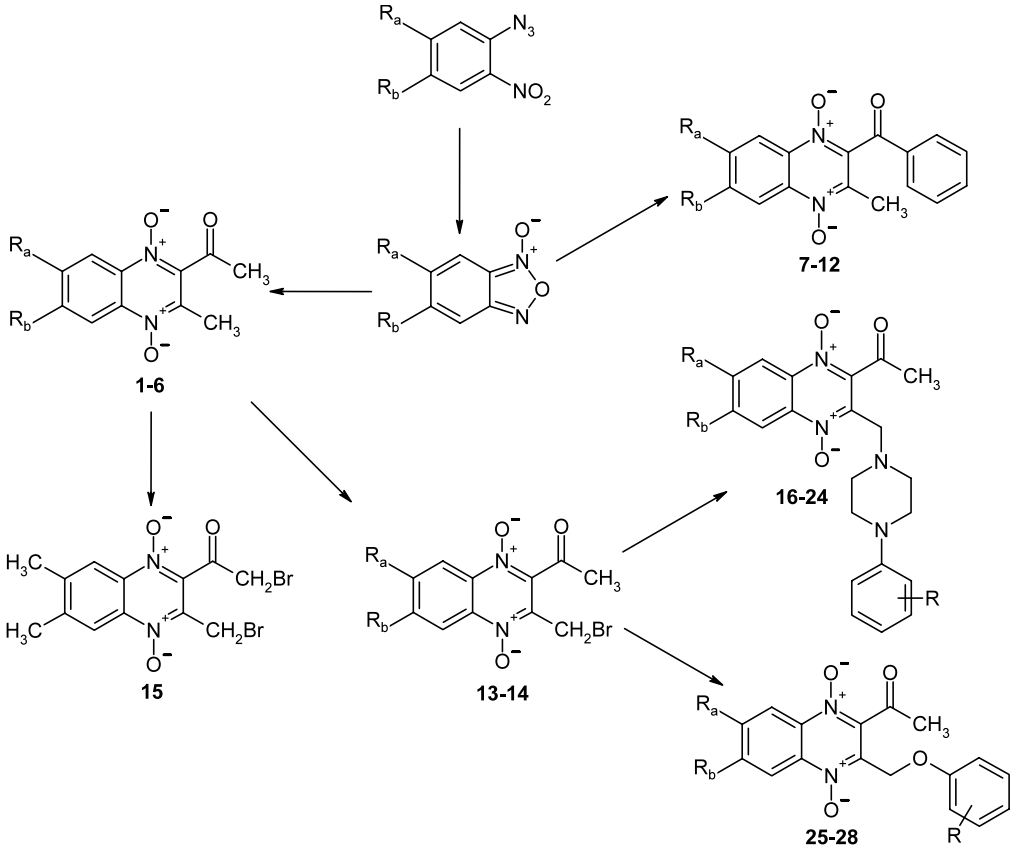


Fig. 2. Synthetic route for compounds **1**–**28**.

red (IR) and nuclear magnetic resonance (^{1}H-NMR) spectra, as well as elemental microanalysis.

Elemental microanalysis

Compound	Calculated	Found
1	C: 45.99, H: 2.79, N: 9.76	C: 45.58, H: 2.76, N: 9.51
2	C: 52.29, H: 3.58, N: 11.08	C: 52.36, H: 3.52, N: 11.04
3	C: 60.55, H: 4.58, N: 12.84	C: 60.24, H: 4.38, N: 12.91
4	C: 62.07, H: 5.17, N: 12.07	C: 62.05, H: 5.17, N: 11.97
5	C: 63.41, H: 5.69, N: 11.38	C: 62.80, H: 5.65, N: 11.11
6	C: 58.06, H: 4.84, N: 11.29	C: 58.26, H: 4.96, N: 11.24
7	C: 55.01, H: 2.86, N: 8.02	C: 54.59, H: 2.86, N: 8.03
8	C: 61.05, H: 3.50, N: 8.90	C: 60.80, H: 3.65, N: 8.77
9	C: 68.57, H: 4.28, N: 10.00	C: 68.52, H: 4.47, N: 9.99
10	C: 68.34, H: 4.85, N: 9.38	C: 68.04, H: 4.87, N: 9.38
11	C: 69.12, H: 5.28, N: 8.96	C: 69.08, H: 5.15, N: 9.07
12	C: 65.80, H: 4.52, N: 9.03	C: 65.38, H: 4.29, N: 8.69
13	C: 44.44, H: 3.03, N: 9.42	C: 44.36, H: 3.07, N: 9.40
14	C: 36.07, H: 1.91, N: 7.65	C: 36.59, H: 1.94, N: 7.59
15	C: 38.63, H: 2.97, N: 6.93	C: 38.91, H: 3.02, N: 6.68
16	C: 58.33, H: 5.09, N: 16.20	C: 58.10, H: 5.06, N: 16.38
17	C: 60.32, H: 5.57, N: 15.30	C: 60.01, H: 5.50, N: 15.15
18	C: 52.33, H: 3.94, N: 11.63	C: 52.26, H: 3.76, N: 11.34
19	C: 60.43, H: 5.16, N: 13.43	C: 60.13, H: 5.21, N: 13.42
20	C: 59.04, H: 5.98, N: 11.97	C: 59.30, H: 5.63, N: 11.58
21	C: 64.00, H: 5.94, N: 13.58	C: 63.66, H: 6.08, N: 13.75
22	C: 65.38, H: 6.47, N: 12.71	C: 65.52, H: 6.63, N: 12.25
23	C: 62.63, H: 6.04, N: 13.28	C: 62.83, H: 6.14, N: 13.15
24	C: 67.34, H: 6.12, N: 14.29	C: 66.89, H: 6.05, N: 14.27
25	C: 65.81, H: 4.52, N: 9.03	C: 65.62, H: 4.50, N: 8.92
26	C: 58.46, H: 3.87, N: 8.02	C: 58.66, H: 4.05, N: 7.91
27	C: 61.10, H: 4.95, N: 7.92	C: 61.17, H: 4.93, N: 8.08
28	C: 57.46, H: 3.66, N: 11.83	C: 57.49, H: 3.91, N: 11.59

3. Pharmacology

In vitro evaluation of the antituberculosis activity was carried out at the GWL Hansen's Disease Centre within the Tuberculosis Antimicrobial Acquisition and 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), 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 capacity to inhibit the growth of virulent *M. tuberculosis*. Biological tests have been performed according to the previously described method [25,26].

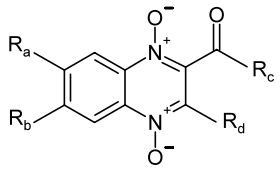
4. Results and discussion

The results of the in vitro evaluation of antituberculosis activity are reported in Tables 1–3. As described in the previous papers, the presence of 1,4-di-*N*-oxides is essential for the activity of the compounds [21,22]. In general, most compounds with an acetyl or benzoyl group in position 2, **1**–**12**, have been shown to possess good antimycobacterial activity in the first level screening, with growth inhibition values ranging from 96 to 100%. Only compounds **11** and **12** showed low activity (44 and 68% growth inhibition values, respectively). Similar values have been obtained for compounds **25**–**28** (96, 99, 100 and 97%, respectively). On the contrary, 2-acetyl-3-(4-phenylpiperazin-1-yl)methylquinoxaline derivatives, **16**–**24**, showed no activity (Table 1).

Second level and cytotoxicity results are summarized in Table 2. All the compounds that were active in the first level screening were then tested to determine the actual minimum inhibitory concentration (MIC). MIC is defined as the lowest concentration causing a 90% reduction in fluorescence with regard to controls. Therefore, compounds **1**, **2** and **6** have been proven to be the most active, with MIC values ranging from 0.39 to 1.56 μM. Concurrent with the determination of MICs, 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

Table 1
Results of the first antituberculosis screening



Compound	R_a	R_b	R_c	R_d	MIC (μg mL^{-1}) [a]	GI (%) [b]
1	Cl	Cl	CH_3	CH_3	< 6.25	100
2	Cl	H	CH_3	CH_3	< 6.25	100
3	H	H	CH_3	CH_3	< 6.25	100
4	CH_3	H	CH_3	CH_3	< 6.25	96
5	CH_3	CH_3	CH_3	CH_3	< 6.25	100
6	OCH_3	H	CH_3	CH_3	< 6.25	100
7	Cl	Cl	C_6H_5	CH_3	< 6.25	100
8	Cl	H	C_6H_5	CH_3	< 6.25	100
9	H	H	C_6H_5	CH_3	< 6.25	100
10	CH_3	H	C_6H_5	CH_3	< 6.25	98
11	CH_3	CH_3	C_6H_5	CH_3	> 6.25	44
12	OCH_3	H	C_6H_5	CH_3	> 6.25	68
13	H	H	CH_3	CH_2Br	> 6.25	32
14	Cl	Cl	CH_3	CH_2Br	> 6.25	27
15	CH_3	CH_3	CH_2Br	CH_2Br	> 6.25	0
16	H	H	CH_3	[4-(4-nitrophenyl) piperazyn-1-yl]methyl	> 6.25	0
17	CH_3	CH_3	CH_3	[4-(4-nitrophenyl) piperazyn-1-yl]methyl	> 6.25	0
18	Cl	Cl	CH_3	[4-(4-chlorophenyl)piperazyn-1-yl]methyl	> 6.25	0
19	H	H	CH_3	[4-(4-chlorophenyl)piperazyn-1-yl]methyl	> 6.25	0
20	CH_3	CH_3	CH_3	[4-(4-chlorophenyl)piperazyn-1-yl]methyl	> 6.25	0
21	H	H	CH_3	[4-(4-methoxyphenyl)piperazyn-1-yl]methyl	> 6.25	0
22	CH_3	CH_3	CH_3	[4-(4-methoxyphenyl)piperazyn-1-yl]methyl	> 6.25	0
23	H	H	CH_3	[4-(2-methoxyphenyl)piperazyn-1-yl]methyl	> 6.25	0
24	H	H	CH_3	[4-(2-methylphenyl)piperazyn-1-yl]methyl	> 6.25	0
25	H	H	CH_3	(1-phenoxy)methyl	< 6.25	96
26	H	H	CH_3	[1-(4-chloro)phenoxy]methyl	< 6.25	99
27	H	H	CH_3	[1-(4-methoxy)phenoxy]methyl	< 6.25	100
28	H	H	CH_3	[1-(4-nitro)phenoxy]methyl	< 6.25	97

[a] MIC of rifampin: 0.015–0.125 μg mL^{-1} versus *M. tuberculosis* $H_{37}Rv$ (97% inhibition).
[b] Growth inhibition of virulent $H_{37}Rv$ strain of *M. tuberculosis*. According to the TAACF program, compounds effecting less than 90% inhibition are considered to be inactive.

presence of an electron-withdrawing group in position 7 or in positions 6 and 7 of the benzene moiety reduces not only the MIC values, but also the IC_{50} values. On the contrary, an electron-releasing group increases these values. The best SI values have been obtained from unsubstituted compounds or from compounds having just one substituent in position 7. When the methyl of the acetyl group is replaced by a phenyl group, the activity is totally lost, except in the case of compound **9**. With regard to compounds **25**–**28**, the obtainment of the IC_{50} and SI values is expected soon. All these compounds have an MIC < 6.25 μM.

Six compounds, **2**–**6** and **9**, each with a high SI value (> 20.13, > 20, > 20, > 10, > 37.82 and > 10, respectively), were then tested for efficacy in vitro in a TB-infected macrophage model, showing good values of EC_{90} and EC_{99}. Macrophage assay results are reported in Table 3. Based on the data obtained, four compounds stand out in the macrophage assay, e.g. **3** and **4** (EC_{90}/MIC = 0.87 and 0.80, respectively). With regard to compounds **5** and **9**, the macrophage assay has been scheduled.

5. Conclusions

Screening of the in vitro antimycobacterial activity of these novel series, 2-acetyl and 2-benzoyl-6(7)-substituted quinoxaline 1,4-di-*N*-oxides, has evidenced that the 2-acetyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives with chlorine, methyl and methoxy group in position 7 of the benzene moiety (compounds **2**, **4** and **6**, respectively) and unsubstituted (**3**) have emerged as new compounds endowed with antitubercular activity,

Table 2
Results of second level and cytotoxicity antituberculosis assays

Compound	MIC (μM) [a]	IC_{50} (μM) [b]	SI (IC_{50}/MIC) [c]
1	0.39	3.3	8.46
2	0.78	15.7	20.13
3	3.13	> 62.5	> 20
4	3.13	> 62.5	> 20
5	6.25	> 62.5	> 10
6	1.56	4.0	37.82
7	6.25	3.3	0.53
8	3.13	14.68	4.7
9	6.25	> 62.5	> 10
10	6.25	> 10	> 1.6
25	6.25	not available	not available
26	3.13	not available	not available
27	3.13	not available	not available
28	6.25	not available	not available

[a] Actual minimum inhibitory concentration (MABA assay).
[b] Measurement of cytotoxicity in VERO cells.
[c] Selectivity index.

Table 3
Results of macrophage assay

Compound	EC_{90} [a]	EC_{99} [a]	EC_{90}/MIC [b]
2	2.44	8.34	3.13
3	2.72	7.16	0.87
4	2.50	7.83	0.80
6	6.7	> 12.5	4.29

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

exhibiting EC_{90}/MIC values between 0.80 and 4.29. 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.

6. Experimental protocols

6.1. Chemistry

6.1.1. General

Melting points were determined with a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The ^{1}H-NMR spectra were recorded on a Bruker AC-200E instrument (Rheinstetten, Germany), using TMS as the internal standard and with DMSO-d_6 as the solvent; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and br s (broad singlet). The IR spectra were performed on a Perkin–Elmer 1600 FTIR (Norwalk, CT) in KBr pellets; the frequencies are expressed in cm^{-1}. Elemental microanalyses 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, Düren, Germany) was used for TLC and silica gel 60 (0.040–0.063 mm) for column chromatography (Merck). HPLC conditions: Column Nova Pack C18 60 A 4 μm (3.9×150 mm^2); mobile phase: acetonitrile/propan-2-ol, 50/50; flux: 1 mL min^{-1}.

Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (FEROSA, 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, België) and Lancaster (Bischheim-Strasbourg, France).

6.1.2. General procedure for compounds *1–12*

The corresponding acetyl acetone or 1-benzoylacetone (10.6 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. Then, triethylamine was added drop by drop (0.1 mL) and the reaction mixture was stirred at room temperature in darkness for 24 h. After evaporating to dryness under pressure, a crude solid was obtained which was precipitated and washed by adding diethyl ether (or *n*-hexane), affording the target compound. The obtained yellow precipitate was purified by recrystallization using methanol; yields: 19–76%.

*6.1.2.1. 2-Acetyl-6,7-dichloro-3-methylquinoxaline 1,4-di-N-oxide (**1**).* This compound was obtained in 71% yield from 5,6-dichlorobenzofuroxane (1.00 g, 3.5 mmol) and acetyl acetone (1.7 g, 16.9 mmol) after 24 h stirred; m.p., 152–153 °C; IR (KBr): ν 1716, 1324, 1021 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.35 (s, 3H, C_3–CH_3), 2.64 (s, 3H, $COCH_3$), 8.62 (s, 1H, H_5), 8.64 (s, 1H, H_8) ppm; Anal. $C_{11}H_8Cl_2N_2O_3$ (C, H, N).

*6.1.2.2. 2-Acetyl-7-chloro-3-methylquinoxaline 1,4-di-N-oxide (**2**).* This compound was obtained in 71% yield from 5-chlorobenzofuroxane (0.30 g, 1.4 mmol) and acetyl acetone (0.60 g, 6.0 mmol) after 24 h under stirring; m.p., 148–149 °C. IR (KBr): ν 1712, 1322 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 2.70 (s, 3H, $COCH_3$), 7.79 (dd, 1H, H_6, $J_{6-5} = 9.0$ Hz, $J_{6-8} =$ 2.1 Hz), 8.53 (s, 1H, H_8), 8.55 (d, 1H, H_5) ppm; Anal. $C_{11}H_9ClN_2O_3$ (C, H, N).

*6.1.2.3. 2-Acetyl-3-methylquinoxaline 1,4-di-N-oxide (**3**).* This compound was obtained in 73% yield from

benzofuroxane (0.32 g, 2.4 mmol) and acetyl acetone (1.06 g, 10.6 mmol) after 24 h under stirring; m.p., 152–153 °C. IR (KBr) ν 1719, 1523, 1322 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 2.63 (s, 3H, $COCH_3$), 7.93–7.99 (m, 2H, H_6+H_7), 8.39–8.49 (m, 2H, H_5+H_8) ppm; Anal. $C_{11}H_{10}N_2O_3$ (C, H, N). HPLC: $R_t = 2.52$ min.

*6.1.2.4. 2-Acetyl-3,7-dimethylquinoxaline 1,4-di-N-oxide (**4**).* This compound was obtained in 32% yield from 5-methylbenzofuroxane (0.36 g, 2.4 mmol) and acetyl acetone (1.06 g, 10.6 mmol) after 24 h under stirring; m.p., 163–164 °C; IR (KBr) ν 1719, 1523, 1322 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.34 (s, 3H, C_3–CH_3), 2.57 (s, 3H, CH_3–Ar), 2.64 (s, 3H, $COCH_3$), 7.81 (d, 1H, H_6, $J_{6-5} = 8.6$ Hz), 8.23 (s, 1H, H_8), 8.36 (d, 1H, H_5) ppm; Anal. $C_{12}H_{12}N_2O_3$ (C, H, N).

*6.1.2.5. 2-Acetyl-3,6,7-trimethylquinoxaline 1,4-di-N-oxide (**5**).* This compound was obtained in 66% yield from 5,6-dimethylbenzofuroxane (1.00 g, 6.1 mmol) and acetyl acetone (5.40 g, 27.0 mmol) after 24 h under stirring; m.p., 205–206 °C; IR (KBr) ν 1719, 1510, 1421, 1324 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.34 (s, 3H, C_3–CH_3), 2.48 (s, 6H, $2CH_3$–Ar), 2.64 (s, 3H, $COCH_3$), 8.17 (s, 1H, H_5), 8.21 (s, 1H, H_8) ppm; Anal. $C_{13}H_{14}N_2O_3$ (C, H, N); HPLC: $R_t = 2.63$ min.

*6.1.2.6. 2-Acetyl-3-methyl-7-methoxyquinoxaline 1,4-di-N-oxide (**6**).* This compound was obtained in 57% yield from 5-methoxybenzofuroxane (0.40 g, 2.4 mmol) and acetyl acetone (1.06 g, 10.6 mmol) after 24 h under stirring; m.p., 150–151 °C. IR (KBr): ν 1716, 1524, 1324, 1237 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 2.65 (s, 3H, $COCH_3$), 3.99 (s, 3H, OCH_3), 7.60 (dd, 1H, H_6, $J_{6-5} = 9.8$ Hz, $J_{6-8} = 2.0$ Hz), 7.74 (ds, 1H, H_8), 8.40 (d, 1H, H_5) ppm; Anal. $C_{12}H_{12}N_2O_4$ (C, H, N); HPLC: $R_t = 2.27$ min.

*6.1.2.7. 2-Benzoyl-6,7-dichloro-3-methylquinoxaline 1,4-di-N-oxide (**7**).* This compound was obtained in 35% yield from 5,6-dichlorobenzofuroxane (0.30 g, 1.4 mmol) and 1-benzoylacetone (1.04 g, 6.4 mmol) after 24 h under stirring; m.p., 195–196 °C; IR (KBr): ν 1687, 1323, 1247, 1074 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.27 (s, 3H, C_3–CH_3), 7.56 (t, 2H, $H_{3'}+H_{5'}$), 7.74 (t, 1H, $H_{4'}$, $J_{4'-3'} = 6.3$ Hz), 8.04 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 7.7$ Hz), 8.56 (s, 1H, H_5), 8.69 (s, 1H, H_8) ppm; Anal. $C_{16}H_{10}Cl_2N_2O_3$ (C, H, N).

*6.1.2.8. 2-Benzoyl-7-chloro-3-methylquinoxaline 1,4-di-N-oxide (**8**).* This compound was obtained in 35% yield from 5-chlorobenzofuroxane (0.50 g, 2.9 mmol) and 1-benzoylacetone (2.10 g, 12.9 mmol) after 24 h under stirring; m.p., 175–176 °C. IR (KBr): ν 1674, 1328, 1268 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H, C_3–CH_3), 7.58 (t, 2H, $H_{3'}+H_{5'}$), 7.77 (t, 1H, $H_{4'}$, $J_{4'-3'} = 5.6$ Hz), 8.02–8.09 (m, 3H, $H_6+H_{2'}+H_{6'}$), 8.37 (s, 1H, H_8), 8.53 (d, 1H, H_5, $J_{5-6} = 9.1$ Hz) ppm; Anal. $C_{16}H_{11}ClN_2O_3$ (C, H, N).

*6.1.2.9. 2-Benzoyl-3-methylquinoxaline 1,4-di-N-oxide (**9**).* This compound was obtained in 76% yield from benzofuroxane (0.25 g, 1.8 mmol) and 1-benzoylacetone (1.29 g, 7.95 mmol) after 72 h under stirring; m.p., 214–215 °C. IR (KBr): ν 1674, 1334, 1251, 1075 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H, C_3–CH_3), 7.55 (t, 2H, $H_{3'}+H_{5'}$), 7.73 (t, 1H, $H_{4'}$, $J_{4'-3'} = 7.4$ Hz), 8.00–8.05 (m, 2H, H_6+H_7), 8.07 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 8.0$ Hz), 8.40 (d, 1H, H_5, $J_{5-6} = 8.4$ Hz), 8.55 (d, 1H, H_8, $J_{8-7} = 8.6$ Hz) ppm; Anal. $C_{16}H_{12}N_2O_3$ (C, H, N).

*6.1.2.10. 2-Benzoyl-3,7-dimethylquinoxaline 1,4-di-N-oxide (**10**).* This compound was obtained in 19% yield from 5-methylbenzofuroxane (0.36 g, 2.4 mmol) and 1-benzoylacetone (1.72 g, 10.6 mmol) after 24 h under stirring; m.p., 209–210 °C. IR (KBr) ν 1678, 1329, 816 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H, C_3–CH_3), 2.57 (s, 3H, CH_3–Ar), 7.57 (t, 2H, $H_{3'}+H_{5'}$), 7.76 (t, 1H, $H_{4'}$, $J_{4'-3'} = 8.1$ Hz), 7.84 (d, 1H, H_6, $J_{6-5} = 9.0$ Hz), 8.04 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 7.6$ Hz), 8.18 (s, 1H, H_8), 8.42 (d, 1H, H_5) ppm; Anal. $C_{17}H_{14}N_2O_3 \cdot \frac{1}{4}H_2O$ (C, H, N).

*6.1.2.11. 2-Benzoyl-3,6,7-trimethylquinoxaline 1,4-di-N-oxide (**11**).* This compound was obtained in 50% yield from 5,6-dimethylbenzofuroxane (0.39 g, 2.4 mmol) and 1-benzoylacetone (1.57 g, 10.6 mmol) after 24 h under stirring; m.p., 227–228 °C. IR (KBr) ν 1677, 1596, 1451, 1329 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.28 (s, 3H, C_3–CH_3), 2.48 (s, 3H, C_6–CH_3), 2.51 (s, 3H, C_7–CH_3), 7.57 (t, 2H, $H_{3'}+H_{5'}$), 7.76 (t, 1H, $H_{4'}$, $J_{4'-3'} = 7.5$ Hz), 8.02 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 7.6$ Hz), 8.14 (s, 1H, H_5), 8.30 (s, 1H, H_8) ppm; Anal. $C_{18}H_{16}N_2O_3 \cdot \frac{1}{4}H_2O$ (C, H, N); HPLC: $R_t = 2.53$ min.

*6.1.2.12. 2-Benzoyl-3-methyl-7-methoxyquinoxaline 1,4-di-N-oxide (**12**).* This compound was obtained in 27% yield from 5-methoxybenzofuroxane (0.40 g, 2.4 mmol) and 1-benzoylacetone (1.70 g, 10.6 mmol) after 48 h under stirring; m.p., 215–216 °C. IR (KBr) ν 1678, 1524, 1327, 1240, 1075 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 3.98 (s, 3H, OCH_3), 7.52–7.78 (m, 5H, $H_{3'}+H_{4'}+H_{5'}+H_6+H_8$), 8.05 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 7.6$ Hz), 8.46 (d, 1H, H_5, $J_{5-6} = 9.4$ Hz) ppm; Anal. $C_{17}H_{14}N_2O_4$ (C, H, N); HPLC: $R_t = 2.39$ min.

*6.1.3. General procedure for compounds **13–15***

The bromation of 2-acetyl-3-methylquinoxaline 1,4-di-*N*-oxide derivatives was carried out by reaction with *N*-bromosuccinimide, and with peroxide benzoic as the

catalyst. First, a solution of the appropriate 2-acetyl-3-methylquinoxaline 1,4-di-*N*-oxide derivative (7.5 mmol) in carbon tetrachloride (35 mL) and chloroform (35 mL) was prepared. Next, *N*-bromosuccinimide (7.5 mmol) and peroxide benzoic (0.20 g) were added. The solution was stirred and heated under reflux for 24 h. The solvent was eliminated under pressure, and the resulting solid was washed with diethyl ether and methanol. Finally, the obtained yellow precipitate was purified by recrystallization using methanol. Column chromatography on silica gel (flash chromatography) was applied when necessary; yields: 81–84%.

*6.1.3.1. 2-Acetyl-3-bromomethylquinoxaline 1,4-di-N-oxide (**13**).* This compound was obtained in 81% yield from 2-acetyl-3-methylquinoxaline 1,4-di-*N*-oxide (3.00 g, 13.7 mmol) and *N*-bromosuccinimide (2.66 g, 13.7 mmol) after 24 h under stirring, using benzoyl peroxide as the catalyst (0.40 g). It was then purified by flash chromatography (eluent DCM/MeOH); m.p., 142–143 °C. IR (KBr): *v* 1701, 1346, 1273 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.65 (s, 3H, $COCH_3$), 4.66 (s, 2H, C_3–CH_2Br), 8.01–8.06 (m, 2H, H_6+H_7), 8.45–8.53 (m, 2H, H_5+H_8) ppm; Anal. $C_{11}H_9BrN_2O_3$ (C, H, N).

*6.1.3.2. 2-Acetyl-3-bromomethyl-6,7-dichloroquinoxaline 1,4-di-N-oxide (**14**).* This compound was obtained in 84% yield from 2-acetyl-6,7-dichloro-3-methylquinoxaline 1,4-di-*N*-oxide (1.00 g, 2.7 mmol) and *N*-bromosuccinimide (0.70 g, 4.0 mmol) after 24 h under stirring, using benzoyl peroxide as the catalyst (0.20 g); m.p., 195–196 °C. IR (KBr): *v* 1717, 1332, 1029 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.71 (s, 3H, $COCH_3$), 4.68 (s, 2H, C_3–CH_2Br), 8.71 (s, 1H, H_5), 8.76 (s, 1H, H_8) ppm; Anal. $C_{11}H_7BrCl_2N_2O_3$ (C, H, N).

*6.1.3.3. 2-Bromoacetyl-3-bromomethyl-6,7-dimethylquinoxaline 1,4-di-N-oxide (**15**).* This compound was obtained in 2% yield from 2-acetyl-3,6,7-trimethylquinoxaline 1,4-di-*N*-oxide (1.01 g, 4.1 mmol) and *N*-bromosuccinimide (0.72 g, 4.1 mmol) after 24 h under stirring, using benzoyl peroxide as the catalyst (0.20 g). It was then purified by flash chromatography, eluting with a DCM/MeOH gradient; m.p. 219–220 °C. IR (KBr): *v* 1719, 1544, 1334 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.65 (s, 3H, C_6–CH_3), 2.67 (s, 3H, C_7–CH_3), 4.63 (s, 2H, C_3–CH_2Br), 5.02 (s, 2H, $COCH_2Br$), 8.35 (s, 1H, H_5), 8.58 (s, 1H, H_8) ppm; Anal. $C_{13}H_{12}Br_2N_2O_3$ (C, H, N); HPLC: $R_t = 1.63$ min.

*6.1.4. General procedure for compounds **16**–**28***

The corresponding phenol or piperazine derivative (4.8 mmol) was added to a solution of the appropriate 2-acetyl-3-bromomethyl quinoxaline 1,4-di-*N*-oxide (2.4 mmol) in dry tetrahydrofuran (35 mL). Next, potassium carbonate was added as the catalyst (0.40 g). The solution was stirred at room temperature in darkness for 24–72 h. The solvent was eliminated under pressure. The obtained yellow precipitate was purified by recrystallization, using methanol. Column chromatography on silica gel (flash chromatography) was applied when necessary; yields: 7–76%.

*6.1.4.1. 2-Acetyl-3-[4-(4-nitrophenyl)piperazyn-1-yl]methylquinoxaline 1,4-di-N-oxide (**16**).* This compound was obtained in 39% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.35 g, 1.1 mmol) and 1-(4-nitrophenyl)piperazine (0.25 g, 1.1 mmol) after 24 h under stirring; m.p., 172–173 °C. IR (KBr): *v* 1703, 1328, 1243, 1096 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.65 (s, 3H, $COCH_3$), 3.42 (br s, 8H, piperazine), 3.98 (s, 2H, C_3–CH_2–), 7.04 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 8.8$ Hz), 8.01–8.06 (m, 4H, $H_{3'}+H_{5'}+H_6+H_7$), 8.45–8.49 (m, 2H, H_5+H_8) ppm; Anal. $C_{21}H_{21}N_5O_5\cdot\frac{1}{2}H_2O$ (C, H, N).

*6.1.4.2. 2-Acetyl-3,6,7-[4-(4-nitrophenyl)piperazyn-1-yl]trimethylquinoxaline 1,4-di-N-oxide (**17**).* This compound was obtained in 76% yield from 2-acetyl-3-bromomethyl-6,7-dimethylquinoxaline 1,4-di-*N*-oxide (0.29 g, 0.9 mmol) and 1-(4-nitrophenyl)piperazine (0.20 g, 0.9 mmol) after 48 h under stirring; m.p., 202–203 °C. IR (KBr) *v* 1708, 1596, 1328, 1237, 1029 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.50 (s, 6H, $2CH_3$–Ar), 2.64 (s, 3H, $COCH_3$), 3.34 (br s, 8H, piperazine), 3.97 (s, 2H, C_3–CH_2–), 7.03 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 7.5$ Hz), 8.04 (d, 2H, $H_{3'}+H_{5'}$), 8.23 (s, 1H, H_5), 8.25 (s, 1H, H_8) ppm; Anal. $C_{23}H_{25}N_5O_5\cdot\frac{1}{4}H_2O$ (C, H, N).

*6.1.4.3. 2-Acetyl-6,7-dichloro-3-[4-(4-chlorophenyl)piperazyn-1-yl]methylquinoxaline 1,4-di-N-oxide (**18**).* This compound was obtained in 8% yield from 2-acetyl-3-bromomethyl-6,7-dichloroquinoxaline 1,4-di-*N*-oxide (0.30 g, 1.1 mmol) and 1-(4-chlorophenyl)piperazine dihydrochloride (0.30 g, 1.1 mmol) after 40 h under stirring; m.p., 163–164 °C. IR (KBr): *v* 1682, 1329, 1233 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.61 (br s, 4H, piperazine), 2.62 (s, 3H, $COCH_3$), 3.04 (br s, 4H, piperazine), 3.94 (s, 2H, C_3–CH_2), 6.94 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'} = 7.0$ Hz), 7.22 (d, 2H, $H_{3'}+H_{5'}$), 8.65 (s, 1H, H_5), 8.67 (s, 1H, H_8) ppm; Anal. $C_{21}H_{19}Cl_3N_4O_4$ (C, H, N).

*6.1.4.4. 2-Acetyl-3-[4-(4-chlorophenyl)piperazyn-1-yl]methylquinoxaline 1,4-di-N-oxide (**19**).* This compound was obtained in 25% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.35 g, 1.1 mmol) and 1-(4-chlorophenyl)piperazine dihydrochloride (0.30 g, 1.1 mmol) after 24 h under stirring; m.p., 150–151 °C. IR (KBr): *v* 1709, 1338, 1239, 1039 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.66 (s, 3H, $COCH_3$), 2.68 (br s,

4H, piperazine), 3.14 (br s, 4H, piperazine), 4.00 (s, 2H, C_3–CH_2–), 6.97 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=8.9$ Hz), 7.25 (d, 2H, $H_{3'}+H_{5'}$), 8.00–8.05 (m, 2H, H_6+H_7), 8.47–8.55 (m, 2H, H_5+H_8) ppm; Anal. $C_{21}H_{21}ClN_4O_3\cdot\frac{1}{4}H_2O$ (C, H, N).

*6.1.4.5. 2-Acetyl-3,6,7-[4-(4-chlorophenyl)piperazyn-1-yl]trimethylquinoxaline 1,4-di-N-oxide (**20**).* This compound was obtained in 7% yield from 2-acetyl-3-bromomethyl-5,6-dimethylquinoxaline 1,4-di-*N*-oxide (0.30 g, 0.9 mmol) and 1-(4-chlorophenyl)piperazine dihydrochloride (0.25 g, 0.9 mmol) after 48 h under stirring. It was then purified by flash chromatography (eluent DCM/MeOH, 98:2); m.p., 154–155 °C. IR (KBr) *v* 1708, 1496, 1453, 1331, 1232 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.52 (s, 6H, $2CH_3$–Ar), 2.62 (s, 3H, $COCH_3$), 2.69 (br s, 4H, piperazine), 3.08 (br s, 4H, piperazine), 3.96 (s, 2H, C_3–CH_2–), 6.94 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=8.9$ Hz), 7.22 (d, 2H, $H_{3'}+H_{5'}$), 8.20 (s, 1H, H_5), 8.24 (s, 1H, H_8) ppm; Anal. $C_{23}H_{25}ClN_4O_3\cdot1\frac{1}{2}H_2O$ (C, H, N). HPLC: $R_t=1.70$ min.

*6.1.4.6. 2-Acetyl-3-[4-(4-methoxyphenyl)piperazyn-1-yl]methylquinoxaline 1,4-di-N-oxide (**21**).* This compound was obtained in 33% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.30 g, 1.1 mmol) and 1-(4-methoxyphenyl)piperazine (0.25 g, 1.1 mmol) after 24 h under stirring; m.p., 139–140 °C. IR (KBr): *v* 1696, 1330, 1227, 1031 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.62 (s, 3H, $COCH_3$), 2.64 (br s, 4H, piperazine), 2.95 (br s, 4H, piperazine), 3.66 (s, 3H, $C_{4'}$–OCH_3), 3.95 (s, 2H, C_3–CH_2–), 6.79 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=9.1$ Hz), 6.87 (d, 2H, $H_{3'}+H_{5'}$), 7.92–8.02 (m, 2H, H_6+H_7), 8.42–8.51 (m, 2H, H_5+H_8) ppm; Anal. $C_{22}H_{24}N_4O_4\cdot\frac{1}{4}H_2O$ (C, H, N).

*6.1.4.7. 2-Acetyl-3,6,7-[4-(4-methoxyphenyl)piperazyn-1-yl]trimethylquinoxaline 1,4-di-N-oxide (**22**).* This compound was obtained in 14% yield from 2-acetyl-3-bromomethyl-5,6-dimethylquinoxaline 1,4-di-*N*-oxide (0.11 g, 0.4 mmol) and 1-(4-methoxyphenyl)piperazine (0.09 g, 0.4 mmol) after 48 h under stirring. It was then purified by flash chromatography (eluent DCM/MeOH, 99:1); m.p., 148–149 °C. IR (KBr) *v* 1708, 1511, 1450, 1330, 1239 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.49 (s, 6H, $2CH_3$–Ar), 2.50 (s, 3H, $COCH_3$), 2.62 (br s, 4H, piperazine), 2.96 (br s, 4H, piperazine), 3.67 (s, 3H, $C_{4'}$–OCH_3), 3.95 (s, 2H, C_3–CH_2–), 6.80 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=9.1$ Hz), 6.87 (d, 2H, $H_{3'}+H_{5'}$), 8.22 (s, 1H, H_5), 8.26 (s, 1H, H_8) ppm; Anal. $C_{24}H_{28}N_4O_4\cdot\frac{1}{4}H_2O$ (C, H, N); HPLC: $R_t=1.63$ min.

*6.1.4.8. 2-Acetyl-3-[4-(2-methoxyphenyl)piperazyn-1-yl]methylquinoxaline 1,4-di-N-oxide (**23**).* This compound was obtained in 28% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.30 g, 1.1 mmol) and 1-(2-methoxyphenyl)piperazine (0.25 g, 1.1 mmol) after 24 h under stirring; m.p., 146–147 °C. IR (KBr) *v* 1707, 1323, 1238, 1024 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.62 (br s, 4H, piperazine), 2.64 (s, 3H, $COCH_3$), 2.90 (br s, 4H, piperazine), 3.76 (s, 3H, $C_{4'}$–OCH_3), 3.96 (s, 2H, C_3–CH_2–), 6.87–6.92 (m, 4H, $H_{3'}+H_{4'}+H_{5'}+H_{6'}$), 7.97–7.99 (m, 2H, H_6+H_7), 8.45–8.49 (m, 2H, H_5+H_8) ppm; Anal. $C_{22}H_{24}N_4O_4\cdot\frac{1}{2}H_2O$ (C, H, N).

*6.1.4.9. 2-Acetyl-3-[4-(2-methylphenyl)piperazyn-1-yl]methylquinoxaline 1,4-di-N-oxide (**24**).* This compound was obtained in 7% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.30 g, 1.1 mmol) and 1-(*o*-tolyl)piperazine hydrochloride (0.25 g, 1.1 mmol) after 24 h under stirring; m.p., 142–143 °C. IR (KBr): *v* 1708, 1334, 1224, 1027 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.23 (s, 3H, $C_{2'}$–CH_3), 2.65 (br s, 4H, piperazine), 2.69 (s, 3H, $COCH_3$), 2.78 (br s, 4H, piperazine), 3.98 (s, 2H, C_3–CH_2–), 6.95–7.13 (m, 4H, $H_{3'}+H_{4'}+H_{5'}+H_{6'}$), 7.95–7.99 (m, 2H, H_6+H_7), 8.44–8.49 (m, 2H, H_5+H_8) ppm; Anal. $C_{22}H_{24}N_4O_3$ (C, H, N).

*6.1.4.10. 2-Acetyl-3-(1-phenoxy)methylquinoxaline 1,4-di-N-oxide (**25**).* This compound was obtained in 17% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.50 g, 1.7 mmol) and phenol (0.40 g, 1.1 mmol) after 24 h under stirring; m.p., 180–181 °C. IR (KBr): *v* 1724, 1344, 1325, 1236 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.64 (s, 3H, $COCH_3$), 5.43 (s, 2H, C_3–CH_2–), 7.01–7.05 (m, 3H, $H_{3'}+H_{4'}+H_{5'}$), 7.35 (t, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=5.4$ Hz), 8.02–8.06 (m, 2H, H_6+H_7), 8.47–8.55 (m, 2H, H_5+H_8) ppm; Anal. $C_{17}H_{14}N_2O_4$ (C, H, N).

*6.1.4.11. 2-Acetyl-3-[1-(4-chloro)phenoxy]methylquinoxaline 1,4-di-N-oxide (**26**).* This compound was obtained in 22% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.30 g, 1.7 mmol) and 4-chlorophenol (0.30 g, 1.1 mmol) after 24 h under stirring. It was then purified by flash chromatography (eluent DCM/MeOH, 99:1); m.p., 171–172 °C. IR (KBr): *v* 1725, 1346, 1239 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.63 (s, 3H, $COCH_3$), 5.42 (s, 2H, C_3–CH_2), 7.07 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=8.9$ Hz), 7.19 (d, 2H, $H_{3'}+H_{5'}$), 8.01–8.06 (m, 2H, H_6+H_7), 8.46–8.54 (m, 2H, H_5+H_8) ppm; Anal. $C_{17}H_{13}ClN_2O_4\cdot\frac{1}{4}H_2O$ (C, H, N).

*6.1.4.12. 2-Acetyl-3-[1-(4-methoxy)phenoxy]methylquinoxaline 1,4-di-N-oxide (**27**).* This compound was obtained in 17% yield from

2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.50 g, 1.7 mmol) and 4-methoxyphenol (0.21 g, 1.1 mmol) after 24 h under stirring. It was then purified by flash chromatography (eluent DCM/MeOH, 98:2); m.p., 177–178 °C. IR (KBr): ν 1725, 1339, 1231 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.38 (s, 3H, $COCH_3$), 3.70 (s, 3H, $C_{4'}$–OCH_3), 5.37 (s, 2H, C_3–CH_2–), 6.90 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=9.1$ Hz), 6.97 (d, 2H, $H_{3'}+H_{5'}$), 8.02–8.05 (m, 2H, H_6+H_7), 8.46–8.54 (m, 2H, H_5+H_8) ppm; Anal. $C_{18}H_{16}N_2O_5\cdot\frac{3}{4}H_2O$ (C, H, N).

*6.1.4.13. 2-Acetyl-3-[1-(4-nitro)phenoxy]methylquinoxaline 1,4-di-N-oxide (**28**).* This compound was obtained in 29% yield from 2-acetyl-3-bromomethylquinoxaline 1,4-di-*N*-oxide (0.30 g, 1.7 mmol) and 4-nitrophenol (0.28 g, 1.1 mmol) after 24 h under stirring. It was then purified by flash chromatography (eluent DCM/MeOH, 97:3); m.p., 194–195 °C. IR (KBr): ν 1724, 1338, 1254 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.66 (s, 3H, $COCH_3$), 5.57 (s, 2H, C_3–CH_2), 7.28 (d, 2H, $H_{2'}+H_{6'}$, $J_{2'-3'}=9.3$ Hz), 8.04–8.07 (m, 2H, H_6+H_7), 8.26 (d, 2H, $H_{3'}+H_{5'}$), 8.48–8.56 (m, 2H, H_5+H_8) ppm. Anal. $C_{17}H_{13}N_3O_6$ (C, H, N).

6.2. Biological evaluation

6.2.1. In vitro evaluation of antituberculosis activity

Primary screening was conducted at 6.25 μg mL^{-1} against *M. tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [25]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [27]. Compounds showing $\geq$90% inhibition in the primary screening were considered active and then re-tested at a lower concentrations against *M. tuberculosis* H_{37}Rv in order to determine the actual MIC, using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% with respect to the controls. Rifampin (RMP) was used as the reference compound (RMP MIC = 0.015–0.125 μg mL^{-1}).

Compounds were also tested for cytotoxicity (IC_{50}) in VERO cell line at a concentration equal to and greater than the MIC for *M. tuberculosis* H_{37}Rv. 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 (RMP $IC_{50}>100$ μg mL^{-1}, SI > 800).

6.2.2. Macrophage assay

Compounds with an MIC $\leq$ 6.25 μg mL^{-1} and an SI > 10 were then tested to evaluate efficacy in vitro in a TB-infected macrophage model [26]. The EC_{90} and EC_{99} are defined as the concentrations effecting 90 and 99% reductions in residual mycobacterial growth after 7 days, as compared to untreated controls. Compounds with $EC_{90} > 16 \times$ MIC are considered inactive (RMP EC_{90} = 0.04–0.1 μg mL^{-1}, EC_{99} = 0.5–1.5 μg mL^{-1}).

Acknowledgements

Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the US National Institute of Allergy and Infectious Diseases. We also thank Dr. Joseph A. Maddry (TAACF) and his team for their collaboration. We wish to thank the Gobierno de Navarra for grants given to B. Zarranz and to CYTED (Red Iberoamericana para la Investigación y Descubrimiento de Medicamentos).

References

[1] D.E. Snider, M. Raviglione, A. Kochi, Global burden of tuberculosis, in: B. Bloom (Ed.), Tuberculosis: Pathogenesis, Protection and Control, ASM, Washington, DC, 1994, p. 3.
[2] T.M. Shinnick, C.H. King, F.D. Quinn, Am. J. Med. Sci. 309 (1995) 92–98.
[3] D.E. Snider, Jr., J.R. La Montagne, J. Infect. Dis. 169 (1994) 1189–1196.
[4] J.B. Bass, Jr., L.S. Farer, P.C. Hopewell, R. O'Brien, R.F. Jacobs, F. Ruben, D.E. Snider, Jr., G. Thornton, Am. J. Respir. Crit. Care Med. 149 (1994) 1359–1374.
[5] A.K. Pathak, V. Pathak, L. Seitz, J.A. Maddry, S.S. Gurcha, G.S. Besra, W.J. Suling, R.C. Reynolds, Bioorg. Med. Chem. 9 (2001) 3129–3143.
[6] G.W.H. Cheeseman, R.F. Cookson, in: A. Weissberger, E.C. Taylor (Eds.), The Chemistry of Heterocyclic Compounds, Vol. 35, Wiley, New York, 1979, pp. 1–27, 35–38.
[7] A.E.A. Porter, in: A.R. Katrizky, C.W. Rees (Eds.), Comprehensive Heterocyclic Chemistry, vol. 3, Pergamon Press, New York, 1984, pp. 157–197.
[8] G.A. Carter, T. Clark, C.S. James, R.S.T. Loeffler, Pestic. Sci. 14 (1983) 135–144.
[9] P. Sanna, A. Carta, M. Loriga, S. Zanetti, L. Sechi, Il Fármaco 54 (1999) 169–177.
[10] J.P. Dirlam, J.E. Presslitz, B.J. Williams, J. Med. Chem. 26 (1983) 1122–1126.
[11] I.S. Musatova, A.S. Elina, E.N. Padeiskaya, L.D. Shipilova, G.G. Yakobson, G.G. Furin, Khim. Farm. Zh. 16 (1982) 934–938.
[12] I.S. Musatova, A.S. Elina, E.N. Padeiskaya, Khim. Farm. Zh. 16 (1982) 1063–1067.
[13] A. Monge, M.J. Gil, M.A. Pascual, M.A. Gastelurrutia, Ann. R. Acad. Farm. 49 (1983) 37–47 [Chem. Abst. 99 (1983) 194924s].
[14] A. Monge, M.J. Gil, M.A. Pascual, Ann. R. Acad. Farm. 49 (1983) 199–209.
[15] J.D. Johnston, US Patent 3,344,022 (1967).
[16] R.H.B. Galt, US Patent 3,479,354 (1969).
[17] W. Schmid, Switz. Patent CH 630,908 (1982).
[18] A. Monge, J.A. Palop, A. López de Cerain, V. Senador, F.J. Martínez-Crespo, Y. Sainz, S. Narro, E. García, C. de Miguel, M.

González, E. Hamilton, A.J. Barker, E.D. Clarke, D.T. Greenhow, J. Med. Chem. 38 (1998) 1786–1792.
[19] M.A. Ortega, Y. Sainz, M.E. Montoya, et al., Die Pharm. 54 (1999) 24–25.
[20] Y. Sainz, M.E. Montoya, F.J. Martínez-Crespo, et al., Arzneim.-Forsch./Drug Res. 49 (1999) 55–59.
[21] M.E. Montoya, Y. Sainz, M.A. Ortega, et al., Organización Farmacéutica Ibero-Latinoamericana (OFIL) 8 (1998) 36–41.
[22] M.E. Montoya, Y. Sainz, M.A. Ortega, et al., Il Farmaco 53 (1998) 570–573.
[23] M.A. Ortega, Y. Sainz, M.E. Montoya, A. Jaso, B. Zarranz, I. Aldana, A. Monge, Arzneim. Forsch./Drug Res. 52 (2002) 113–119.
[24] M.A. Ortega, M.J. Morancho, F.J. Martínez-Crespo, et al., Eur. J. Med. Chem. 35 (2000) 21–30.
[25] L.A. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.
[26] P.S. Skinner, S.K. Furney, M.R. Jacobs, et al., Antimicrob. Agents Chemother. 38 (1994) 2557–2563.
[27] S.G. Franzblau, M.B. Cook, V.K. Quenzer, R.M. Ferguson, R.H. Gilman, J. Clin. Microbiol. 36 (1998) 362.