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Synthesis and in vitro evaluation of new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide against *Entamoeba histolytica*

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ABSTRACT

In our search for new antiamoebic agents, a new series of ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives have been synthesized using the Beirut reaction. All compounds were characterized by spectroscopic techniques and elemental analysis. Antiamoebic activity was evaluated in vitro against *Entamoeba histolytica* strain HM1:IMSS by the microdilution method, and the structure–activity relationship was analyzed. We found that eleven quinoxaline derivatives showed greater activity than metronidazole and nitazoxanide with IC_{50} values in the range 1.99–0.35 μM. Compounds **T-001** and **T-016** shows IC_{50} values of 1.41 and 1.47 μM, respectively, with a value of selectivity index >60.

1. Introduction

Amoebiasis is a parasitic infection caused by the microaerophilic protozoa *Entamoeba histolytica*.[1,2] This disease is considered a public health problem in endemic countries where it frequently affects children, young adults, and immunocompromised patients.[3,4] Worldwide, amoebiasis is the second leading cause of death among parasitic diseases.[5] According to the World Health Organization (WHO), this disease causes about 110,000 deaths, and more than 500 million people are infected annually.[6] In Mexico it is considered an endemic disease. Antiamoebic drugs, such as metronidazole with a 5-nitroimidazole group, and nitazoxanide with a 5-nitrothiazole group (Fig. 1), among others, have been used to treat infection. However, these drugs have several gastrointestinal side effects.[7] Furthermore, in the last two decades, the literature has reported that metronidazole has mutagenic and carcinogenic properties in murine models[8–10] and protozoan resistance against conventional drugs.[11–14] Based on these considerations, it is important to search for new antiamoebic compounds for use as potential antiparasitic agents with greater potency, efficacy, and safety for patients.

On the other hand, quinoxalines are heterocyclic compounds that contain both benzene and pyrazine rings and whose biological properties have been known for a century.[15] They are considered isomeric compounds of quinozaline, phtalazine and cinnoline, and isosteric compounds of pyrazinamide, isoniazide and quinoline (Fig. 2).[16]

Currently, the synthesis and development of various quinoxalines and their derivatives (Fig. 3) focus on different biological targets. Some of these compounds are antitumoural agents, which act as DNA intercalators, and inhibitors of enzymes involved in nucleic acid biosynthesis; for example, dyhidrofolate reductase (DHFR) and human thymidylate synthase (TS).[17,18] Quinoxaline derivatives has demonstrated anti-inflammatory and analgesic activity by acting on prostaglandin E2 inhibition (PGE2) and nitric acid production, respectively.[19] Another series of quinoxaline derivatives have antiviral activity, inhibiting viral replication of herpes

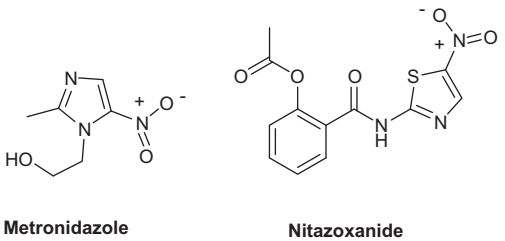

Figure 1. Chemical structure of antiamoebic drugs.

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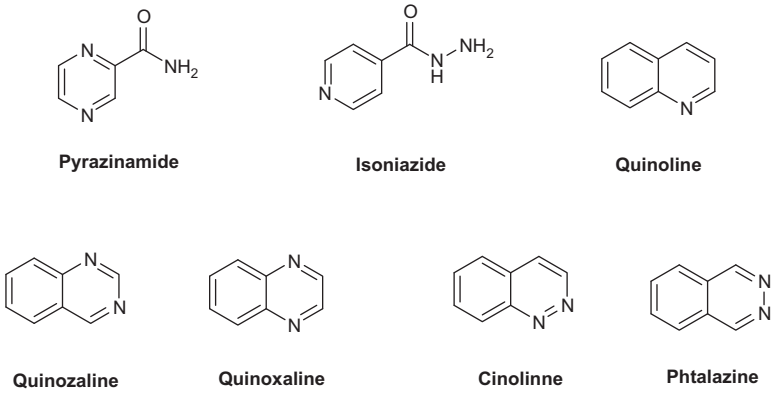


Figure 2. Structure of *N*-heterocyclic isomeric and isosteric compounds of the ring of quinoxaline.

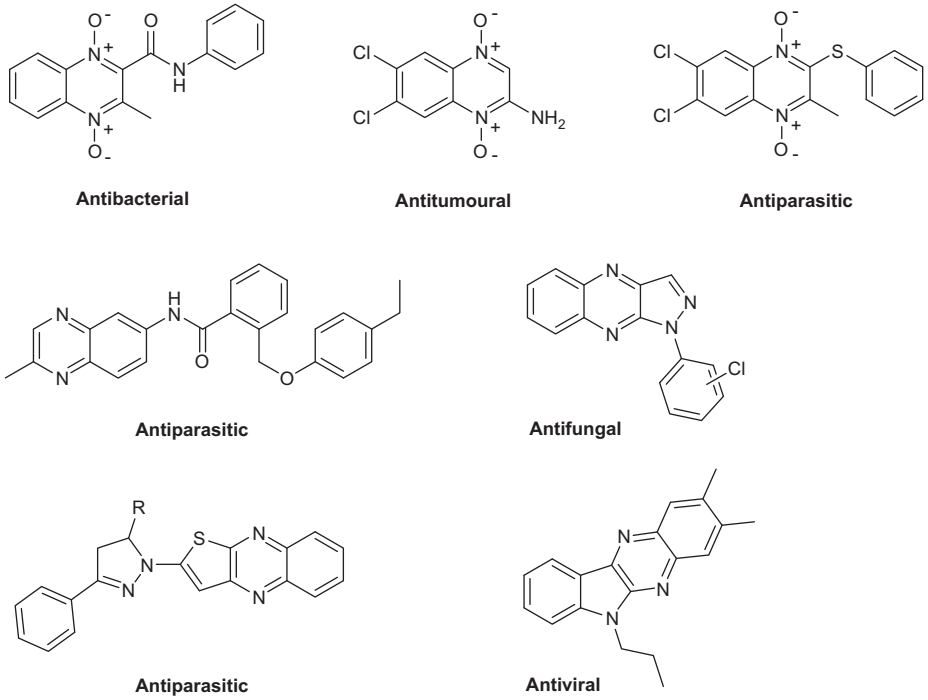


Figure 3. Structure of quinoxaline derivatives with biological activity.

simplex type 1, cytomegalovirus, and varicella-zoster virus.[20] Particularly, quinoxaline 1,4-di-*N*-oxide derivatives are versatile compounds that have been studied as antitumoural, antibacterial, and antiparasitic agents.[15,21–26] Interestingly, its activity is significantly affected by substituents on quinoxaline nucleus. It has been observed that the presence of different group at 7-position on quinoxaline reduces or enhance biological activity. However, Jaso et al., suggest that activity principally depends on the substituents in the carboxylate group in position 2.[27]

In the last century, quinoxaline 1,4-dioxide derivatives were found to exert a therapeutic effect against amoebiasis in dogs, rats, and cats models, but were also toxic.[28] Recently, Budakoti et al., obtained quinoxaline derivatives with better antiamoebic activity and less toxicity than metronidazole. Interestingly, modification of the compounds from chalcones, to pyrazolines, and further to quinoxalines results in an increase in antiamoebic activity.[29] Following with the use of quinoxaline as a scaffold in drug antiamoebic design, in a first approximation, we have initiated an exploration on quinoxalines 1,4-di-*N*-oxide with a new methyl carboxylate group at 7-position on quinoxaline nucleus that has not been reported, in an effort to analysis how a carboxylate group acts in new derivatives containing both carbonyl and carboxylate group at 2- and 7-position on the quinoxaline nucleus, respectively, which could change solubility properties and also to correlate the structure with biological activity. Additionally, we include an ethyl carboxylate at 7-position to show potential steric and metabolic effects by esterase enzyme on our compounds. Therefore, our present work focuses on the in vitro evaluation of the effect of a new series of ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives on *Entamoeba histolytica*.

2. Material and methods

2.1. Synthetic procedure

The Beirut reaction was the general procedure used for synthesis of quinoxaline1,4-di-*N*-oxide derivatives, as described in methods A–C.[30]

2.1.1. Method A

Compound synthesis was achieved by the reaction of the corresponding diketone derivative (10.6 mmol) with the appropriate

benzofuroxane (2.4 mmol) in dry chloroform (35 mL).[31,32] Triethylamine (TEA) was added (1 mL), and the reaction mixture was stirred at room temperature for 3–7 days. After evaporation to dryness at low pressure, a crude solid or brown oil was obtained. This was then precipitated and washed by adding diethyl ether, affording the target compound. The residue was purified by column chromatography on silica gel, when necessary using dichloromethane:methanol (95:5).

2.1.2. Method B

Compound synthesis was achieved with a variation of the Beirut reaction. The corresponding diketone (10 mmol) was added to a solution of the appropriate benzofuroxane (5 mmol) dissolved in a minimum amount of methanol (20–35 mL) in the presence of calcium chloride (0.1 mmol) and ethanolamine (1 mmol) as catalysts.[33,34] The reaction was stirred at room temperature for 6–24 h. The solvent was removed under vacuum; the quinoxaline 1,4-di-*N*-oxide derivative was isolated by addition of 50 mL of water followed by extraction with dichloromethane (5×40 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness, and further purified by column chromatography on silica gel when necessary using dichloromethane:methanol (95:5). The mixture was then precipitated and washed with cold diethyl ether in order to obtain a yellow solid.

2.1.3. Method C

Acetoacetate (2 mmol) and potassium carbonate (1 mmol) were added to a solution of 1 mmol of the appropriate benzofuroxane in 50 mL of acetone.[26,35] The suspension was stirred at room temperature for 2–24 h. The quinoxaline 1,4-di-*N*-oxide derivatives were isolated by the addition of 50 mL of water followed by extraction with dichloromethane (5×40 mL). The organic layer was then dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel when necessary using dichloromethane:methanol (95:5).

According to previous reports,[36] we have observed that 7-substituted quinoxaline 1,4-di-*N*-oxide derivatives were prevailing over the 6-isomer. In practice, the workup and purification permitted the isolation of the 7-isomer and reagents. All quinoxaline 1,4-di-*N*-oxide derivatives were characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. NMR was recorded on a Bruker Ultrashield 400 MHz device, using Tetramethylsilane (TMS) as the internal standard, and dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent. The chemical shifts are reported in ppm (), and coupling constant (J) values are given in hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). IR spectroscopy was performed on a Perkin–Elmer 1600 FTIR in KBr Pellets. The frequencies are expressed in cm^{-1}. Alugram1 SIL G/UV254 (layer: 0.2 mm) was used for thin layer chromatography (TLC).

2.2. In vitro evaluation

2.2.1. Stock solutions

Stock solutions of new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives were prepared by dissolving in 0.1% (vol/vol) dimethyl sulfoxide (DMSO, Sigma–Aldrich) at a level at which no inhibition of trophozoites occurs.[37] The solution was further diluted to 1 mL by adding freshly prepared culture medium to reach a concentration of 1 mg/mL. Two fold serial dilutions were made in each well of a 96-well microtiter plate (Corning Costar) in 100 μL of culture medium. Each test included metronidazole and nitazoxanide as reference amoebicidal drugs, growth control wells (culture medium with trophozoites only), and a blank well (culture medium only).

2.2.2. Culture conditions of *Entamoeba histolytica*

Entamoeba histolytica trophozoite strain HM1:IMSS in log-phase were axenically grown in BI-S-33 medium[38] supplemented with 20% (vol/vol) calf serum (Microlab, S.A de C.V). The number of trophozoites per mL was estimated by trypan blue exclusion (In Vitro, S.A de C.V) in a hematocytometer to confirm viability. The trophozoite suspension used was diluted to 10^5 trophozoites per mL by adding fresh medium and 100 μL of this suspension was added to the test and control wells in the plate.

2.2.3. Antiamoebic assay

All ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives were screened in vitro for antiamoebic activity against Entamoeba histolytica by the microdilution method.[39] A 100 μL suspension with *Entamoeba histolytica* trophozoites was added to each test and control well, and afterwards the plates were incubated at 37 °C for 48 h.

2.2.4. Evaluation of antiamoebic activity

After incubation, trophozoite growth on the plate was checked with an inverted microscope (ZEISS Allegra™ 2IR). After that, 10 μL of WST-1 reagent (Millipore, S.A de C.V) was added to each test and control well and plates were incubated again at 37 °C for 4 h. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader (Biorad Ultramark). The percentage (%) of growth inhibition of trophozoites was determined from the optical densities of control and test wells. From the % of inhibition, the half maximal inhibitory concentration (IC_{50}) was obtained by linear regression between the concentration of compounds used and for the % of inhibition. The assay was performed in duplicate, and mean and standard deviation (SD) were determined. The reference used to select compounds with antiamoebic activity was based on an IC_{50} <10 μM.

2.3. Cytotoxicity evaluation

2.3.1. Cell culture

Green monkey kidney cell line (VERO) was cultured in minimal essential medium MEM (In Vitro, S.A de C.V) with nonessential aminoacid 0.1 mM (In Vitro, S.A de C.V) and 1 mM pyruvate (In Vitro, S.A de C.V) and 10% fetal bovine serum (Microlab, S.A de C.V), at 37 °C in 5% CO_2 humidified incubator. The viability and number of cell per milliliter were determined with trypan blue (In Vitro, S.A de C.V) in a hematocytometer. Prepared a cell suspension at density of 10^5 cell/mL and 100 μL of this suspension were added to each of the wells of the plate.

2.3.2. Cell proliferation assay (WST-1)

The cell proliferation assay is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Expansion in the number of viable cells results in an increase in the overall activity of mitochondrial dehydrogenases in viable cells only.[40]

2.3.3. Cytotoxic assay

Exponentially growing cells were plated at 10^4 cells per well into 96-well plates (Costar, Corning) and incubated for 24 h before the addition of compounds to achieve the maximum confluence of the cells. Stock solutions were prepared and dissolved in 0.1% (v/v) dimethyl sulfoxide (DMSO, Sigma–Aldrich) and further diluted with fresh complete medium to achieve 0.1–100 μM concentration. Cells were treated with different concentrations of test compounds for 48 h at 37 °C in 5% CO_2 humidified incubator with untreated control sample. The toxicity effect was determined using 10 μL WST-1 (Millipore, Inc.) and incubated for 4 h at 37 °C. The metabolized WST-1 product was quantified by measuring the

absorbance at 490 nm on a Microplate reader (Biorad Ultramark) with a reference wavelength of 630 nm. All assays were performed in quadruplicate and repeated twice. The results were determined the mean and standard deviation, and statistical analysis of the results was assessed by analysis of variance (ANOVA). The reference used to select compounds to determine toxicity profile was based on an IC_{50} ⩽reference amoebicidal drugs (metronidazole and nitazoxanide) in antiamoebic activity assay.

2.3.4. Selectivity index (SI)

Selectivity index (SI) was calculated and defined as: Toxicity IC_{50} value for the VERO cells/IC_{50} values for the *Entamoeba histolytica* under study; where toxicity IC_{50} is defined as the concentration of compound that kills 50% of the kidney epithelial cells (VERO) and protozoal IC_{50} is the concentration that kills 50% of amoeba.

3. Results and discussion

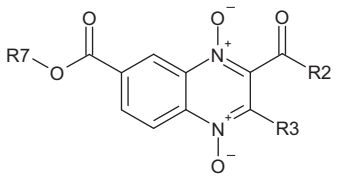
In this work, 25 new quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives were synthesized as shown in Figure 4, following the methods previously described. The synthesized compounds were divided in two series of 12 and 13 compounds by substitution at the 7-position on the quinoxaline ring: ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide.

The next step was an in vitro evaluation of all new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives to determine their antiamoebic activity. These results are shown in Table 1.

Quinoxaline 1,4-di-*N*-oxide derivatives have been studied as interesting compounds in organic and medicinal chemistry; from their molecular structure, a series of new compounds with biological activity[3,11,15,21,22,27,41] have been designed, synthesized, and evaluated. In the present work, we report in vitro screening of new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives on *Entamoeba histolytica* strain HM1:IMSS. The new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives showed an IC_{50} in the range of 18.07–0.35 μM. Structure–activity relationship (SAR) analysis of new methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives showed that incorporation of aliphatic substitutions with one carbon atom at the 2- and 3-position on the quinoxaline 1,4-di-*N*-oxide ring (**T-001**) had better activity than both metronidazole and nitazoxanide drugs. However, incorporation of an electron-donating (OCH_3) group at 2-position (**T-002**) decreased biological activity, although potency values were similar to the reference drugs. Subsequently, incorporation of more carbon atoms in the lineal chain (OCH_2CH_3) at 2-position (**T-003**) enhanced amoebicidal potency. These results show that a steric effect is very important in biological activity. Although, addition of a branched aliphatic chain [$OC(CH_3)_3$] (**T-004**) showed solubility problems in antiamoebic assays.

On the other hand, incorporation of an aromatic group, phenyl and phenylamide at 2-position in compounds **T-005** and **T-006**, respectively, showed an activity similar to that of both reference drugs. Surprisingly, when we considered incorporation of an

Table 1
Antiamoebic activity of new quinoxaline 1,4-di-*N*-oxide derivatives



Compound	R_2	R_3	R_7	IC_{50} (μM)	±SD
T-001	CH_3	CH_3	CH_3	1.41	0.012
T-002	OCH_3	CH_3	CH_3	4.17	0.007
T-003	OCH_2CH_3	CH_3	CH_3	1.99	0.022
T-004	$OC(CH_3)_3$	CH_3	CH_3	ND[a]	ND[a]
T-005	C_6H_5	CH_3	CH_3	3.43	0.017
T-006	NHC_6H_5	CH_3	CH_3	4.14	0.020
T-007	OCH_2CH_3	C_6H_5	CH_3	12.25	0.026
T-008	CH_3	CF_3	CH_3	1.53	0.000
T-009	$CH(CH_3)_2$	CF_3	CH_3	1.88	0.024
T-010	C_6H_5	CF_3	CH_3	6.07	0.001
T-011	C_4H_3S	CF_3	CH_3	0.35	0.026
T-012	$C_{10}H_7$	CF_3	CH_3	5.29	0.004
T-013	OCH_2CH_3	$CH_3CH_2OCOCH_2$	CH_3	1.91	0.021
T-014	OCH_3	CH_3	CH_3CH_2	1.88	0.007
T-015	OCH_2CH_3	CH_3	CH_3CH_2	4.18	0.093
T-016	NH_2	CH_3	CH_3CH_2	1.47	0.004
T-017	C_6H_5	CH_3	CH_3CH_2	1.93	0.024
T-018	NHC_6H_5	CH_3	CH_3CH_2	ND[a]	ND[a]
T-019	$OCH_2C_6H_5$	CH_3	CH_3CH_2	ND[a]	ND[a]
T-020	CH_3	CF_3	CH_3CH_2	1.98	0.050
T-021	CH_2CH_3	CF_3	CH_3CH_2	18.07	0.038
T-022	C_4H_3S	CF_3	CH_3CH_2	2.5	0.001
T-023	$C_{10}H_7$	CF_3	CH_3CH_2	ND[a]	ND[a]
T-024	C_4H_3O	CHF_2	CH_3CH_2	3.81	0.002
T-025	OCH_2CH_3	C_6H_5	CH_3CH_2	7.13	0.017
MTZ	—	—	—	4.5	0.020
NTZ	—	—	—	3.9	0.024

MTZ: metronidazole.
NTZ: nitazoxanide.
IC_{50}: half maximal inhibitory concentration.
±SD: standard deviation.
μM: micromolar.
[a] ND: not determined by solubility problems.

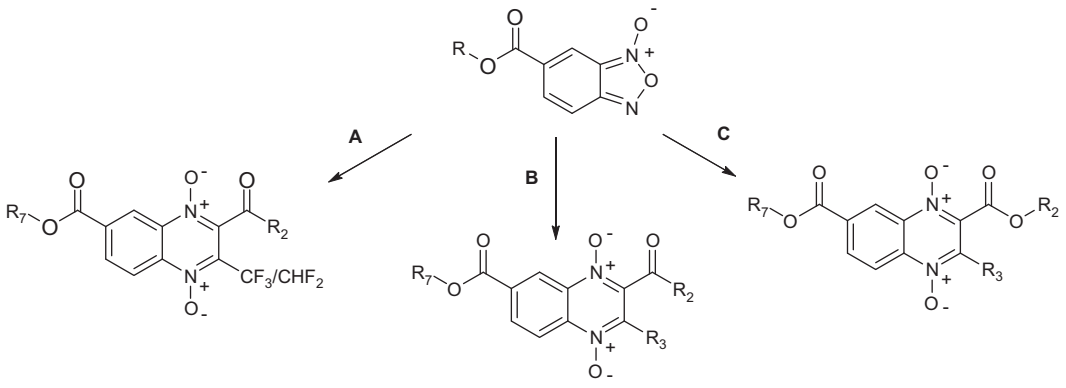


Figure 4. Synthesis of quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives.

aromatic group at 3-position on the quinoxaline 1,4-di-*N*-oxide ring (**T-007**), this produced a drastically low biological activity (IC_{50} = 12.25 μM). In previous research,[42] incorporation of an electron-withdrawing group enhanced antiprotozoal activity; therefore we decided to incorporate a trifluoromethyl group at 3-position (**T-008**) on the quinoxaline nucleus, but results showed an unmodifiable activity in an analogue compound (**T-001**) with the methyl group at 2-position. Addition of a branched aliphatic chain at 2-position (**T-009**) on 3-trifluoromethyl quinoxaline 1,4-di-*N*-oxide compounds did not enhance biological activity, and the addition of an aromatic group, such as phenyl and napthyl, in compound **T-010** and **T-012**, respectively, produced drastically low activity. Interestingly, the incorporation of a thiophene ring (**T-011**) enhanced biological activity 11-fold with IC_{50} values of 0.35 μM. Even though phenyl and thiophene rings are considered bioisosteric, we suggest that this biological activity may be due to the polarity of the sulfur atom. Finally, addition of an ester aliphatic group at 3-position (**T-013**, IC_{50} = 1.91 μM) maintained biological activity below that of both reference drugs.

According to the rules of drug design, if an enhanced number of carbon atoms (less than 8 or 9) are placed in an aliphatic chain, there is enhanced biological activity. For analysis of the steric effect, we incorporate an ethyl ester group at 7-position on the quinoxaline 1,4-di-*N*-oxide ring. Additionally, incorporation of ethyl ester could show a potential metabolic effect of esterase enzyme on our compounds. Compounds **T-014** and **T-017** showed enhanced activity in comparison to their counterparts **T-002** and **T-005**, respectively, but, compounds **T-015**, **T-020**, and **T-022** showed decreased antiamoebic activity, and compounds **T-018**, **T-019** and **T-023** showed solubility problems. Interestingly, an amide free group (**T-016**) at 2-position produced better activity than both reference drugs. These results show an important steric effect at 7-posiiton on the quinoxaline nucleus in antiamoebic activity, and nule or low metabolic effect.

Later, quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives (**T-001**, **T-003**, **T-008**, **T-009**, **T-011**, **T-014**, **T-016**, **T-017**, **T-020** and **T-022**) which showed the better antiamoebic results than references drugs were assessed for toxicity profile (Table 2).

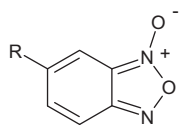
Data in table 2 show that the tested compounds **T-001**, **T-003**, **T-014**, **T-016** and **T-017** have a value of IC_{50} >100 μM on VERO cells. However, metronidazole has a same value of IC_{50} on VERO cells. Compounds **T-008**, **T-009**, **T-011**, **T-020** and **T-022** showed a value of IC_{50} <15 μM, similar to nitazoxanide drug. Therefore selectivity index (SI) is a good parameter to determinate that the toxic activity of quinoxaline 1,4-di-*N*-oxide derivatives is selective on *Entamoeba histolytica*. Compounds **T-001**, and **T-016** showed SI values three fold that metronidazole. **T-003**, **T-014** and **T-017** show a value of SI >50, which is better than both reference drugs. Compounds **T-008** (SI = 2.14), **T-009** (SI = 3.70), **T-011** (SI = 16.74), **T-020** (SI = 3.63) and **T-022** (SI = 2.83) showed less SI value than metronidazole (SI >22.22) and similar to nitazoxanide (SI = 2.75).

Table 2
Toxicity profile in vitro on kidney epithelial cells (VERO) of quinoxaline-7-carboxilate-1,4-di-*N*-oxide derivatives most active against *Entamoeba histolytica* strain

Compound	Cytotoxic activity		SI
	IC_{50} (μM)	±SD	
T-001	>100	—	>70.92
T-003	>100	—	>50.25
T-008	3.28	2.12	2.14
T-009	6.97	1.65	3.70
T-011	5.86	1.97	16.74
T-014	>100	—	>53.19
T-016	>100	—	>68.02
T-017	>100	—	>51.81
T-020	13.08	4.96	6.60
T-022	6.74	3.68	2.69
MTZ	>100	—	>22.22
NTZ	10.74	5.66	2.75

HM1: IMSS.
MTZ: metronidazole.
NTZ: nitazoxanide.
SI: selectivity index.
IC_{50}: half maximal inhibitory concentration.
±SD: standard deviation.
μM: micromolar.

Table 3
Antiamoebic activity of benzofuroxane derivatives precursors



Compound	R	IC_{50} (μM)	±SD
A	HOOC	[a]ND	[a]ND
B	CH_3CH_2OOC	221.25	0.009
C	CH_3OOC	74.48	0.046
D	H	11.98	0.025
MTZ	—	4.5	0.020
NTZ	—	3.9	0.024

MTZ: metronidazole.
NTZ: nitazoxanide.
IC_{50}: half maximal inhibitory concentration.
±SD: standard deviation.
μM: micromolar.
[a] ND: not determined by solubility problems.

In summary, we showed that the quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives **T-001**, **T-003**, **T-014**, **T-016**, and **T-017** have better antiamoebic activity and SI value than the reference drugs, and that these could be considered lead compounds for designing potential antiamoebic agents[43,44].

Finally, on the other hand, several studies have shown that the benzofuroxane ring has various biological activities as anti-*Trypanosoma cruzi* and -*Mycobacterium tuberculosis* agents, among others.[45–47] Additionally, is very common in medicinal chemistry to evaluate the starting compounds or intermediates to discard or confirm their potential activity, therefore we evaluated three benzofuroxane derivative precursors as possible antiamoebic agents. These results are shown in Table 3.

Benzofuroxane derivative precursors B–D showed IC_{50} values in the range of 221.25–11.98 μM. The incorporation of an ethyl and methyl ester group at 5-position on the benzofuroxane ring decreases antiamoebic activity, while benzofuroxane without the addition of substituent groups at the 5-position showed better activity against this protozoal parasite. These results lead us to propose the development of quinoxaline derivatives without a substituent in position 7, to determinate if the behavior of antiamoebic activity in both benzofuroxane and quinoxaline derivatives is identical or different.

4. Conclusion

We examined the biological activities of 21 new ethyl and methyl quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives synthesized by the Beirut reaction. Five quinoxaline-7-carboxylate 1,4-di-*N*-oxide derivatives showed better antiamoebic activity and SI value than the reference drugs, metronidazole and nitazoxanide, therefore these should be considered lead compounds for the development of new antiamoebic agents. The potency of the new compounds was two to eleven times greater than the reference compounds. Further optimization and stability in physiological conditions of this series are in progress in our research group.

5. Experimental data

5.1. T-001

Methyl 2-acetyl-3-methyl-quinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 32.6% yield from methyl benzofuroxane-5-carboxylate and 2,4-pentanedione using method B. IR (KBr): 2955 (ArC–H), 1726 (C=O), 1331 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 2.38 (s, 3H, CH_3), 2.66 (s, 3H, COCH_3), 3.98 (s, 3H, CH_3OOC), 8.37 (d, J = 8.95 Hz, 1H, H5), 8.54 (d, J = 8.90 Hz, 1H, H6), 8.94 (s, 1H, H8). Calculated analysis for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14. Found: C, 56.35; H, 4.13; N, 10.05.

5.2. T-002

Dimethyl 3-methylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 12% yield from methyl benzofuroxane-5-carboxylate and methyl acetoacetate using method C. IR (KBr): 1715.30 (C=O), 1333.57 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 2.44 (s, 3H, CH_3), 3.97 (s, 3H, COOCH_3), 4.03 (s, 3H, CH_3OOC), 8.38 (d, J = 8.93 Hz, 1H, H5), 8.56 (d, J = 8.89 Hz, 1H, H6), 8.85 (s, 1H, H8). Calculated analysis for $C_{13}H_{12}N_2O_6$: C, 53.43; H, 4.14; N, 9.59. Found: C, 53.29; H, 3.97; N, 9.45.

5.3. T-003

Ethyl methyl-3-methylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 17% yield from methyl benzofuroxane-5-carboxylate and ethyl acetoacetate using method C. IR (KBr): 1719.20 and 1741.12 (C=O), 1328.02 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.36 (t, 3H, $COOCH_2CH_3$), 2.45 (s, 3H, CH_3), 3.97 (s, 3H, CH_3OOC), 4.5 (q, J_1 = 7.10 Hz, J_2 = 7.12 Hz, and J_3 = 7.10 Hz, 2H, $COOCH_2CH_3$), 8.39 (d, J = 8.98 Hz, 1H, H5), 8.56 (d, J = 8.99 Hz, 1H, H6), 8.85 (s, 1H, H8). Calculated analysis for $C_{14}H_{14}N_2O_6$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.56; H, 4.38; N, 9.35.

5.4. T-004

Tert-butyl methyl-3-methylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 7% yield from methyl benzofuroxane-5-carboxylate and *tert*-butyl acetoacetate using method C. IR (KBr): 1719.78 and 1738.16 (C=O), 1324.32 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.60 (s, 9H, $COOC(CH_3)_3$), 2.45 (s, 3H, CH_3), 3.96 (s, 3H, CH_3OOC), 8.36 (t, J = 9.39 Hz, 1H, H5), 8.49–8.55 (m, 1H, H6), 8.87 (s, 1H, H8). Calculated analysis for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.31; H, 5.12; N, 8.21.

5.5. T-005

Methyl 2-benzoyl-3-methylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 10.9% yield from methyl benzofuroxane-5-carboxylate and 1-phenyl-1,3-butanedione using method B. IR (KBr): 2949 (ArC-H), 1726 and 1674 (C=O), 1328 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 2.54 (s, 3H, CH_3), 4.04 (s, 3H, CH_3OOC), 7.56 (t, J = 7.75 Hz, 2H, H3 and H5, C_6H_5), 7.72 (t, J = 8.08 Hz, 1H, H4, C_6H_5), 7.90–7.93 (m, 2H, H2 and H6, C_6H_5), 8.52 (d, J = 9.0 Hz, 1H, H5), 8.76 (d, J = 9.01 Hz, 1H, H6), 9.22 (s, 1H, H8). Calculated analysis for $C_{18}H_{11}F_3N_2O_5$: C, 55.11; H, 2.83; N, 7.14. Found: C, 54.89; H, 2.75; N, 7.58.

5.6. T-006

Methyl 2-phenylamide-3-methylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 32.5% yield from methyl benzofuroxane-5-carboxylate and 3-oxo-*N*-phenylbutanamide using method B. IR (KBr): 3077 (N–H), 2949 (ArC–H), 1728 and 1682 (C=O), 1328 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 2.52 (s, 3H, CH_3), 3.99 (s, 3H, CH_3OOC), 7.20 (t, J = 7.38 Hz, 1H, H4, NHC_6H_5), 7.42 (t, J = 7.78 Hz, 2H, H3 and H5, NHC_6H_5), 7.67 (d, J = 7.92 Hz, H2 and H6, NHC_6H_5), 8.41 (d, J = 8.84 Hz, H5), 8.62 (d, J = 8.93 Hz, 1H, H6), 9.0 (s, 1H, H8), 11.01 (s, 1H, NH). Calculated analysis for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.05; H, 4.12; N, 11.46.

5.7. T-007

Ethyl methyl-3-phehylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 17% yield from methyl benzofuroxane-5-carboxylate and ethyl benzoylacetate using method C. IR (KBr): 1715.30 and 1726.88 (C=O), 1327.65 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 0.96 (t, 3H, $COOCH_2CH_3$), 4.16 (s, 3H, CH_3OOC), 4.45 (q, J_1 = 6.67 Hz, J_2 = 6.40 Hz, 2H, $COOCH_2CH_3$), 7.57 (s, 5H, C_6H_5), 8.44 (d, J = 8.59 Hz, 1H, H5), 8.61 (t, J = 7.98 Hz, 1H, H6), 8.95 (d, J = 8.99 Hz, 1H, H8). Calculated analysis for $C_{19}H_{16}N_2O_6$: C, 61.96; H, 4.38; N, 7.61. Found: C, 61.82; H, 4.15; N, 7.53.

5.8. T-008

Methyl 2-acetyl-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 17.7% yield from methyl benzofuroxane-5-carboxylate and 1,1,1-trifluoro-2,4-pentanedione using method A. IR (KBr): 2962 (ArC–H), 1732 (C=O), 1337 (*N*-oxide), 1236 and 1173 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 2.62 (s, 3H, COCH_3), 3.99 (s, 3H, CH_3OOC), 8.51 (d, J = 8.95, Hz, 1H, H5), 8.58 (d, J = 8.94, 1H, H6), 8.92 (s, 1H, H8). Calculated analysis for $C_{13}H_9F_3N_2O_5$: C, 47.28; H, 2.75; N, 8.48. Found: C, 47.01; H, 2.56; N 8.45.

5.9. T-009

Methyl 2-isobutyryl-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 23.9% yield from methyl benzofuroxane-5-carboxylate and 1,1,1-trifluoro-methyl-5-methyl-2,4-hexanedione using method A. IR (KBr): 2923 (ArC–H), 1730 (C=O), 1356 (*N*-oxide), 1293 and 1187 (Ar-CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.27 (s, 6H, $CH(CH_3)_2$), 3.17 (q, J_1 = 14.09 Hz, J_2 = 7.05 Hz, 1H, $CH(CH_3)_2$), 4.07 (s, 3H, CH_3OOC), 8.57 (d, J = 8.98 Hz, 1H, H5), 8.64 (d, J = 8.95 Hz, 1H, H6), 9.27 (s, 1H, H8). Calculated analysis for $C_{15}H_{13}F_3N_2O_5$: C, 50.29; H, 3.66; N, 7.82. Found: C, 49.89; H, 3.47; N, 7.35.

5.10. T-010

Methyl 2-benzoyl-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 10.9% yield from methyl benzofuroxane-5-carboxylate and 4,4,4-trifluoro-1-phenyl-1,3-butanedione using method A. IR (KBr): 2957 (ArC–H), 1732 and 1689 (C=O), 1337 (*N*-oxide), 1255 and 1164 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 4.01 (s, 3H, CH_3OOC), 7.61 (t, J = 7.09 Hz, 2H, H3 and H5, C_6H_5), 7.79 (t, J = 7.24 Hz, 1H, H4, C_6H_5), 8.15 (d, J = 7.82 Hz, 2H, H2 and H6, C_6H_5), 8.52–8.54 (m, 2H, H5 and H6), 8.97 (s, 1H, H8). Calculated analysis for $C_{18}H_{11}F_3N_2O_5$: C, 55.11; H, 2.83; N, 7.14. Found: C, 54.98; H, 2.63; N, 6.85.

5.11. T-011

Methyl 2-(thiophene-2-carbonyl)-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 5.6% yield from methyl benzofuroxane-5-carboxylate and 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione using method A. IR (KBr): 2955 (ArC–H), 1732 and 1664 (C=O), 1360 (*N*-oxide), 1264 and 1162 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 4.09 (s, CH_3OOC), 7.22 (t, J = 4.41 Hz, 1H, H4, C_4H_3S), 7.63 (d, J = 3.77 Hz, H5, C_4H_3S), 7.9 (d, J = 4.85 Hz, H3, C_4H_3S), 8.58 (d, J = 8.86 Hz, 1H, H5), 8.67 (d, J = 8.89 Hz, 1H, H6), 9.31 (s, 1H, H8). Calculated analysis for $C_{16}H_9F_3N_2O_5S$: C, 48.25; H, 2.28; N, 7.03. Found: C, 48.01; H, 1.96; N, 6.76.

5.12. T-012

Methyl 2-(naphthyl-2-carbonyl)-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 11.2% yield from methyl benzofuroxane-5-carboxylate and 4,4,4-trifluoromethyl-1-(2-naphthyl)-1,3-butanedione using method A. IR (KBr): 2962 (ArC–H), 1729 and 1685 (C=O), 1349 (*N*-oxide), 1287 and 1174 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 4.43–4.50 (m, 3H, CH_3OOC), 7.65 (t, J = 7.50 Hz, 1H, H3, $C_{10}H_7$), 7.75 (t, J = 7.85 Hz, 1H, H6, $C_{10}H_7$), 8.01 (d, J = 8.12 Hz, 1H, H7, $C_{10}H_7$), 8.07 (d, J = 8.14 Hz, 1H, H5, $C_{10}H_7$), 8.14 (s, 2H, H2 and H4, $C_{10}H_7$), 8.52–8.55 (m, 1H, H8, $C_{10}H_7$), 8.56 (d, J = 8.87 Hz, 1H, H5), 8.88 (s, 1H, H6), 9.02 (s, 1H, H8). Calculated analysis for $C_{22}H_{13}F_3N_2O_5$: C, 59.74; H, 2.96; N, 6.33. Found: C, 59.48; H, 2.57; N, 6.05.

5.13. T-013

Ethyl methyl-3-(2-ethoxy-2-oxoethyl)quinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 6% yield from methyl benzofuroxane-5-carboxylate and diethyl 3-oxoglutarate using method C. IR (KBr): 1716.11 and 1737.12 (C=O), 1326.59 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.17 (s, 3H, $COCH_2CH_3$), 1.32 (s, 3H, $COOCH_2CH_3$), 3.95 (s, 2H, $CH_2COOCH_2CH_3$), 3.97 (s, 3H, CH_3OOC), 4.12 (q, J_1 = 6.25 Hz, J_2 = 13.27 Hz, 2H, $COOCH_2CH_3$), 4.50 (q, J_1 = 6.50 Hz, J_2 = 13.43 Hz, 2H, $CH_2COOCH_2CH_3$), 8.43 (d, J = 8.26 Hz, 1H, H5), 8.57 (d, J = 8.78 Hz, 1H, H6), 8.89 (s, 1H, H8). Calculated analysis for $C_{16}H_{16}N_2O_8$: C, 52.75; H, 4.43; N, 7.69. Found: C, 52.41; H, 4.25; N, 7.46.

5.14. T-014

Ethyl methyl-3-methyl-quinoxaline-2,7-dicarboxilate 1,4-di-*N*-oxide. This compound was obtained in 10% yield from ethyl benzofuroxane-5-carboxylate and methyl acetoacetate using method C. IR (KBr): 1715.30 and 1726.88 (C=O), 1327.65 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.35 (s, 3H, CH_3CH_2OOC), 2.44 (s, 3H, CH_3), 4.03 (s, 3H, $COOCH_3$), 4.48 (q, J_1 = 7.08 Hz, J_2 = 7.07 Hz, 2H, CH_3CH_2OOC), 8.39 (d, J = 8.99 Hz, 1H, H5), 8.56 (d, J = 8.99 Hz, 1H, H6), 8.84 (s, 1H, H8). Calculated analysis for $C_{14}H_{14}N_2O_6$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.76; H, 4.27; N, 9.13

5.15. T-015

Diethyl 3-methylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 13% yield from ethyl benzofuroxane-5-carboxylate and ethyl acetoacetate using method C. IR (KBr): 1715.30 and 1740.12 (C=O), 1333.57 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.34–1.40 (m, 6H, CH_3CH_2OOC and $COOCH_2CH_3$), 2.45 (s, 3H, CH_3), 4.4 (q, J_1 = 7.10 Hz, J_2 = 7.09 HZ, 2H, CH_3CH_2OOC), 4.5 (q, J_1 = 7.08 Hz, J_2 = 7.07 Hz, 2H, $COOCH_2CH_3$), 8.39 (d, J = 8.99 Hz, 1H, H5), 8.56 (d, J = 8.98 Hz, 1H, H6), 8.90 (s, 1H, H8). Calculated analysis for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04; N, 8.75. Found: C, 56.09; H, 4.96; N, 8.56.

5.16. T-016

Ethyl 2-amide-3-methylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 11.7% yield from ethyl benzofuroxane-5-carboxylate and acetoacetamide using method B. IR (KBr): 3300 (NH), 2990 (ArC–H), 1692 (C=O), 1321 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.38 (t, 3H, CH_3CH_2OOC), 2.48 (s, 3H, CH_3), 4.4 (q, J_1 = 7.33 Hz, J_2 = 14.57 Hz, 2H, CH_3CH_2OOC), 8.25 (s, 2H, NH_2), 8.38–8.40 (m, 1H, H5), 8.56–8.61 (m, 1H, H6), 8.93 (s, 1H, H8). Calculated analysis for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.21; H, 4.35; N, 14.16.

5.17. T-017

Ethyl 2-benzoyl-3-methylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 12.3% yield from ethyl benzofuroxane-5-carboxylate and 1-phenyl-1,3-butanedione using method B. IR (KBr): 2979 (ArC–H), 1720 and 1684 (C=O), 1331 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.41 (t, J = 7.11 Hz, 3H, CH_3CH_2OOC), 2.32 (s, 3H, CH_3), 4.46 (q, J_1 = 7.10 Hz, J_2 = 7.13 Hz, 2H, CH_3CH_2OOC), 7.6 (t, J = 7.8 Hz, 2H, H3 and H5, C_6H_5), 7.79 (t, J = 7.43 Hz, 1H, H4, C_6H_5), 8.1 (d, J = 7.34 Hz, 2H, H2 and H6, C_6H_5), 8.38 (d, J = 8.9 Hz, 1H, H5), 8.5 (d, J = 8.94 Hz, 1H, H6), 8.99 (s, 1H, H8). Calculated analysis for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95. Found: C, 64.58; H, 4.32; N, 7.67.

5.18. T-018

Ethyl 2-phenylamide-3-methylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 22.4% yield from ethyl benzofuroxane-5-carboxylate and 3-oxo-*N*-phenylbutanamide using method B. IR (KBr): 2981 (ArC–H), 1714 and 1661 (C=O), 1369 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.36 (m, 3H, CH_3CH_2OOC), 2.51 (s, 1H, CH_3), 4.39–4.47 (q, J_1 = 7.10 Hz, J_2 = 7.22 Hz, 2H, CH_3CH_2O), 7.20 (t, J = 7.34 Hz, 1H, H4-NHC_6H_5), 7.41 (t, J = 7.16 Hz, 2H, H3 and H5, NHC_6H_5), 7.65 (d, J = 7.84 Hz, H2 and H6, NHC_6H_5), 8.39 (d, J = 8.98 Hz, 1H, H5), 8.59 (d, J = 8.97 Hz, 1H, H6), 8.96 (s, 1H, H8), 11.10 (s, 1H, NH). Calculated analysis for $C_{19}H_{17}N_3O_5$: C, 62.12; H, 4.66; N, 11.44. Found: C. 61.98; H, 4.49; N, 11.23.

5.19. T-019

Benzyl ethyl-3-methylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 3% yield from ethyl benzofuroxane-5-carboxylate and benzyl acetoacetate using method C. IR (KBr): 1715.30 and 1726.88 (C=O), 1327.65 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.35 (t, 3H, CH_3CH_2OOC), 2.51 (s, 3H, CH_3), 4.35 (q, J_1 = 7.08 Hz, J_2 = 7.15 Hz, 2H, CH_3CH_2OOC), 5.47 (s, 2H: $COOCH_2C_6H_5$), 7.39–7.46 (m, 3H, C_6H_5), 7.53 (d, J = 7.69 Hz, 2H, C_6H_5), 7.70 (d, J = 8.64 Hz,1H, H5), 8.03, (d, J = 8.63 Hz, 1H, H6), 8.35 (s, 1H, H8). Calculated analysis for $C_{20}H_{18}N_2O_6$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.95; H, 4.71; N, 7.42.

5.21. T-020

Ethyl 2-acetyl-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 11.9% yield from ethyl benzofuroxane-5-carboxylate and 1,1,1-trifluoro-2,4-pentanedi-

one using method A. IR (KBr): 2978 (ArC–H), 1746 (C═O), 1355 (*N*-oxide), 1271 and 1158 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.40 (t, J = 7.11 Hz, 3H, CH_3CH_2OOC), 2.62 (s, 3H, $COCH_3$), 4.45 (q, J_1 = 7.12 Hz, J_2 = 7.13 Hz, 2H, CH_3CH_2OOC), 8.52 (d, J = 8.95 Hz, 1H, H5), 8.58 (d, J = 8.96 Hz, 1H, H6), 8.92 (s, 1H, H8). Calculated analysis for $C_{14}H_{11}F_3N_2O_5$: C, 48.85; H, 3.22; N, 8.14. Found: C, 48.58; H, 3.15; N, 8.23.

5.22. T-021

Ethyl 2-propionyl-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 5.15% yield from ethyl benzofuroxane-5-carboxylate and 1,1,1-trifluoro-2,4-hexanedione using method A. IR (KBr): 2981 (ArC–H), 1728 (C═O), 1355 (*N*-oxide), 1258 and 1169 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.35 (t, 3H, $COCH_2CH_3$), 1.39 (t, 3H, CH_3CH_2OOC), 4.45 (q, J_1 = 6.99 Hz, J_2 = 14.10 Hz, 2H, $COCH_2CH_3$), 4.5 (q, J_1 = 7.05 Hz, J_2 = 14.17 Hz, 2H, CH_3CH_2OOC), 8.50 (d, J = 8.96 Hz, 1H, H5), 8.56 (d, J = 8.94 Hz, 1H, H6), 8.88 (s, 1H, H8). Calculated analysis for $C_{15}H_{13}F_3N_2O_5$: C, 50.29; H, 3.66; N, 7.82. Found: C, 50.12; H, 3.36; N, 7.56.

5.23. T-022

Ethyl 2-(thiophene-2-carbonyl)-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 23.2% yield from ethyl benzofuroxane-5-carboxylate and 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione using method A. IR (KBr): 2987 (ArC–H), 1726 and 1665 (C═O), 1336 (*N*-oxide), 1286 and 1161 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.41 (t, J = 7.1 Hz, 3H, CH_3CH_2OOC), 4.47 (q, J_1 = 7.07 Hz, J_2 = 7.12 Hz, 2H, CH_3CH_2OOC), 7.32 (d, J = 4.7 Hz, 1H, H4, C_4H_3S), 8.24 (d, J = 4.59 Hz, H5, C_4H_3S), 8.3 (d, J = 4.8 Hz, H3, C_4H_3S), 8.51 (d, J = 8.95 Hz, 1H, H5), 8.56 (d, J = 8.96 Hz, 1H, H6), 8.96 (s, 1H, H8). Calculated analysis for $C_{17}H_{11}F_3N_2O_5S$: C, 49.52; H, 2.69; N, 6.79. Found: C, 49.36; H, 2.45; N, 6.43.

5.23. T-023

Ethyl 2-(naphthyl-2-carbonyl)-3-trifluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 11.3% yield from ethyl benzofuroxane-5-carboxylate and 4,4,4-trifluoromethyl-1-(2-naphthyl)-1,3-butanedione using method A. IR (KBr): 2979 (ArC–H), 1725 and 1687 (C═O), 1349 (*N*-óxido), 1285 and 1174 (Ar–CF_3) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.42 (t, J = 7.10 Hz, 3H, CH_3CH_2OOC), 4.48 (q, J_1 = 7.08 Hz, J_2 = 7.11 Hz, 2H, CH_3CH_2OOC), 7.65 (t, J = 7.5 Hz, 1H, H3, $C_{10}H_7$), 7.75 (t, J = 7.2 Hz, 1H, H6, $C_{10}H_7$), 8.01 (d, J = 8.12 Hz, 1H, H7, $C_{10}H_7$), 8.07 (d, J = 8.14 Hz, 1H, H5, $C_{10}H_7$), 8.14 (s, 2H, H2, and H4 $C_{10}H_7$), 8.52–8.58 (m, 2H, H5 and H6), 8.88 (s, 1H, H8), 9.02 (s, 1H, H8, $C_{10}H_7$). Calculated analysis for $C_{23}H_{15}F_3N_2O_5$: C, 60.53; H, 3.31; N, 6.14. Found: C, 60.23; H, 3.15; N, 5.89.

5.24. T-024

Ethyl 2-(furyl-2-carbonyl)-3-difluoromethylquinoxaline-7-carboxylate 1,4-di-*N*-oxide. This compound was obtained in 9.3% yield from ethyl benzofuroxane-5-carboxylate and 4,4,4-trifluoro-1-(2-furyl)-1,3-butanedione using method A. IR (KBr): 2972 (ArC–H), 1724 and 1667 (C═O), 1352 (*N*-oxide), 1257 and 1169 (Ar–CHF_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.40 (t, 3H, CH_3CH_2OOC), 4.46 (q, J_1 = 7.09 Hz, J_2 = 7.11 Hz, 2H, CH_3CH_2OOC), 6.82 (s, 1H, CHF_2), 7.48 (s, 1H, H4, C_4H_3O), 7.78 (d, J = 3.67 Hz, 1H, H5, C_4H_3O), 8.21 (s, 1H, H3, C_4H_3O), 8.48 (d, J = 8.96 Hz, 1H, H5) 8.56 (d, J = 8.96 Hz, 1H, H6), 8.96 (s, 1H, H8). Calculated analysis for $C_{17}H_{12}F_2N_2O_6$: C, 53.9; H, 3.17; N, 7.40. Found: C, 53.59; H, 2.85; N, 7.21.

5.25. T-025

Diethyl 3-phenylquinoxaline-2,7-dicarboxylate 1,4-di-*N*-oxide. This compound was obtained in 10% yield from ethyl benzofuroxane-5-carboxylate and ethyl benzoylacetate using method C. IR (KBr): 1715.30 and 1726.88 (C═O), 1327.65 (*N*-oxide) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) ppm: 1.10 (s, 3H, CH_3CH_2OOC), 1.25 (t, 3H, $COOCH_2CH_3$), 4.18 (q, J_1 = 6.60 Hz, J_2 = 6.50 Hz, 2H, $COOCH_2CH_3$), 4.22 (q, J_1 = 6.60 Hz, J_2 = 6.50 Hz, 2H, CH_3CH_2OOC), 7.56 (s, 5H, C_6H_5), 8.49–8.51 (m, 1H, H5), 8.60–8.63 (m, 1H, H6), 8.93 (s, 1H, H8). Calculated analysis for $C_{20}H_{18}N_2O_6$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.71; H, 4.52; N, 7.13.

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