Moroccan Journal of Chemistry

# Quinoxaline 1,4-di-*n*-oxides are the potential for treating tuberculosis

***Mohammad Asif****

*Department of Pharmacy, Guru Ram Das (PG) Institute of Management and Technology, Dehradun, (UK), 248009, India*

**Corresponding Author: Email address:* **aasif321@gmail.com**
Received 08 July 2014, Revised 20 September 2014, Accepted 28 September 2014.

**Abstract**

New drugs active against drug-resistant tuberculosis are urgently needed to extend the range of tuberculosis (TB) treatment options to cover drug resistant infections. New anti-TB agents can improve the current chemotherapeutic anti-TB treatment. The properties and the recent developments of quinoxaline 1,4-di-*N*oxide derivatives as potential anti-TB agents. Some specific agents have excellent anti-TB drug properties, are active on drug resistant strains and non replicating mycobacteria. Various quinoxaline-2-carboxamide 1,4-di-N-oxide derivatives were evaluated as *in-vitro* anti-TB activity against *M. tuberculosis* strain. Compounds with a methyl moiety substituted in position 3 and unsubstituted benzyl substituted on the carboxamide group provide an efficient approach for further development of anti-TB agents. The 3-phenylquinoxaline 1,4-di-N-oxide with selective activity against *M. tuberculosis* have been evaluated. Some compounds showed an MIC value less than 0.2µg/mL, a value on the order of the MIC of rifampicin. The most active and selective compounds carry a fluorine atom in the quinoxaline 7-position or in the phenyl substituent para-position. The potency, low cytotoxicity and selectivity of these compounds make them valid lead compounds for synthesizing new analogues, particularly 7-methyl-3-(4'-fluoro) phenyl quinoxaline-2-carbonitrile 1,4-di-N-oxide compound (MIC <0.2 µg/mL and SI >500).

**Keywords:** Quinoxaline, N-oxides; Anti-tuberculosis agents, *Mycobacterium tuberculosis*

**Abbreviations:** AIDS = Acquired immunodeficiency syndrome; CFU=Colony-forming unit; CIP=**Ciprofloxacin**; EC=Effective concentration; EMB=Ethambutol; GI=Growth inhibition;

HIV =Human immunodeficiency virus; INH=Isoniazid; MBC= Minimum bactericidal concentration; MDR= Multiple drug resistant; MIC = Minimum inhibitory concentration; MTD= Maximum tolerated dose; NIAID = National Institute of Allergy and Infectious Diseases; NRP= Non-replicating persistent; PAS= *p*-aminosalicylic acid; RIF= Rifampin; SDR= Single drug resistant; SI = Selectivity index; TAACF=Tuberculosis Antimicrobial Acquisition and Coordinating Facility; TAC= Thiacetazone; TB= Tuberculosis

## 1. Introduction

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis* (*Mtb*), still remains the leading cause of worldwide death among infectious diseases. The statistics indicate that 1.6 million people throughout the world die from TB. The WHO report published in 2009 established that there were an estimated 9.27 million incident cases of TB in 2007. This means an increase from the 9.24 million cases in 2006, the 8.3 million cases in 2000 and the 6.6 million cases in 1990. Although the total number of incident cases of TB is increasing, it must be said that the number of cases per capita is slowly decreasing. In addition, the statistics showed that an estimated 8.8 million new cases emerged in 2005; 34% of these cases occurred in the South-East Asia region [1-3]. One-third of the population is infected with *Mtb* and the World Health Organization (WHO) estimates that within the next 20 years approximately 30 million people will be infected with the TB. The current frontline therapy for TB consists of administering three or more different drugs (usually Isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB)) over an extended period of time [4-8]. Consequently, problems due to multidrug-resistant (MDR) TB arise and it is necessary to develop new therapeutic agents in order to treat drug resistant forms of the disease. Nevertheless, the continuing emergence of MDR-TB will inevitably make it more difficult in the future to control TB. The global epidemiology of drug-resistant TB, particularly extremely drug-resistant TB (XDR-TB), is unknown and the true magnitude of the problem is probably quite underestimated. MDR-TB, which is defined as TB caused by organisms that are resistant to INH and RIF, continues to threaten the progress being made in controlling the disease. The emergence of XDR-TB, defined as a less common form of MDR-TB that is resistant not only to INH and RIF but also to any one of the fluoroquinolones (FQLs) and to at least one of the second-line drugs (amikacin, capreomycin or kanamycin), has heightened this threat [8,9]. The recent influx of immigrants from countries endemic for disease and co-infection with human immunodeficiency virus (HIV) turns TB into a serious problem in developed countries [10]. The development of HIV co-infection with MDR-TB and XDR-TB highlights the urgent need for new drugs to extend the range of effective TB treatment options.

The quinoxaline derivatives are a class of compounds that show very interesting biological properties and there is active interest in their medicinal chemistry. The oxidation of both nitrogens of this heterocyclic system, in order to obtain quinoxaline 1,4-di-*N*-oxide derivatives,

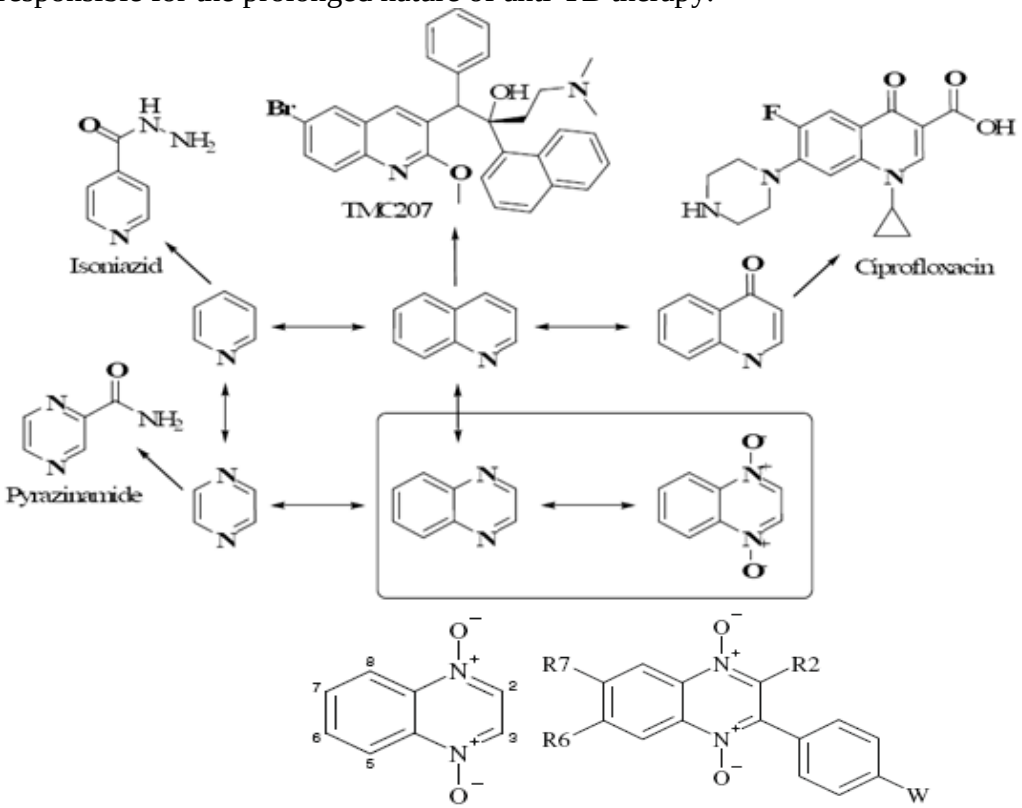
increases the number of biological properties enormously [11]. In fact, the quinoxaline 1,4-di-*N*-oxides were known as potent antibacterial agents since the 1940s, and subtherapeutic levels have been used as animal growth promotors in feed additives [12]. Specific derivatives also show selective cytotoxicity against hypoxic cells present in solid tumors [13]. The recent studies have demonstrated that quinoxaline 1,4-di-*N*-oxides are endowed with anti-TB, antiprotozoal [14,15], anticandida [16,17] activities and mutagenic properties [18-20], depending on specific chemical features. In the last two decades many mono- and di- *N*-oxides of the quinoxaline ring subunit were reported [21].

## 2. Quinoxaline 1,4-di-*n*-oxide as potential antitubercular agents

The quinoxaline 1,4-di-*N*-oxide derivatives as anti-TB drug candidates has shown that specific derivatives show hold promise in the treatment of TB. Quinoxalines and their mono- and di-N-oxide derivatives display a broad range of biological activities and quinoxaline di-N-oxides are known to undergo bioreductions under hypoxia causing DNA damage [18-20,22]. Given the known activity of other classes of bioreductive agents (e.g. metronidazole, PA-824 and OPC-67683), it appeared logical to evaluate of structures against mycobacteria. Over 87,000 compounds have been supplied to TAACF, of which only 0.9% have shown a good in vitro activity/toxicity ratio [23]. One of five lead compound series which were identified and are currently been pursued under the TAACF program is the series of quinoxaline 1,4-di-N-oxide derivatives. Many of these compounds possess excellent anti-TB activity, and the range of possible substituents affords an opportunity to tailor both the pharmacokinetic and activity profiles. As a result of the anti-TB research, large amount of quinoxaline and quinoxaline 1,4-di-N-oxide derivatives have been described [24-29]. Isoniazid (INH) and pyrazinamide (PZA) are based on pyridine and pyrazine rings respectively, both classical bioisosters. The fusion of these aromatic heterocycles with a benzene moiety leads to benzo[*b*]pyridine and benzo[*b*]pyrazine, also know as quinoline and quinoxaline rings. An example of quinoline derivatives is compound TMC207 which is in clinical trials for treating TB [30]. Quinolone derivatives arise from oxidation of quinoline moiety; this heterocycle is the main scaffold of well-know FQLs such as ciprofloxacin (CIP) [31]. The design of new anti-TB drug is based on chemical structural resemblance. In addition, quinoxalines and their mono- and di-*N*-oxide derivatives display a broad range of biological activities [11] and quinoxaline di-*N*-oxides are known to undergo bioreductions under hypoxia causing DNA damage and likely other cellular damage. Given the known activity of other classes of bioreductive agents (e.g. metronidazole, PA-824 and OPC-67683), it appeared logical to us to evaluate our group of structures against mycobacteria [32].

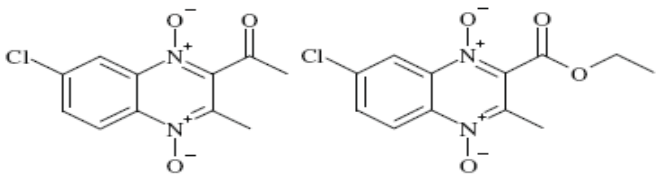
These studies have facilitated a wide-ranging structure-activity relationship (SAR) analysis with diverse functionality incorporated primarily at the 2-, 3-, 6-, and 7-positions (**Fig. 1**). In addition, the lack of the two N-oxide groups generally led to the loss of the anti-TB activity [33,34].

Subsequently, an extended evaluation of the anti-TB activity of most interesting quinoxaline 1,4-di-Noxide derivatives was performed [35,36]. Almost all of these derivatives displayed good inhibitory activity against resistant strains. The susceptibilities of these strains to certain compounds was comparable to that of H37Rv, supporting the theory that 1,4-di-Noxide quinoxaline derivatives have a novel mode of action unrelated to the currently used anti-TB drugs. In addition, specific derivatives were further evaluated in a series of in vivo assays and two of them (lead compounds **I** and **II, Fig. 2**) were found to be active in reducing CFU counts in both the lung and spleen of infected mice following oral administration. This in vivo efficacy is comparable to clinically used TB drugs, although a relatively high dose of compounds was required in order to obtain equivalent reductions in lung CFU. Thus, quinoxaline 1,4-di-N-oxides represent a new class of orally active anti-TB drugs. They are most likely bioreduced to an active metabolite and are active on PA-824 resistant *M. bovis*, thereby indicating that the pathway of bioreduction/activation was different from PA-824, a bioreducible nitroimidazole in clinical trials. In addition, all of the analogues which were tested against non-replicating bacteria (NRP) adapted to low oxygen showed very good activity, indicating that activation occurred in both growing and non-replicating bacteria leading to cell death [35,36]. If the bactericidal activity and activity on NRP bacteria in vitro translate to in vivo conditions, quinoxaline 1,4-di-N-oxides may lead to shortened therapy, because the presence of NRP bacteria is believed to be a major factor responsible for the prolonged nature of anti-TB therapy.




**(a)** **(b)**

**Figure 1**. Chemical structural resemblance of **antitubercular** drugs, (a) Numbered quinoxaline 1,4-di-N-oxide ring; and (b) general structure for all 3- phenylquinoxaline 1,4-di-N-oxide derivatives.



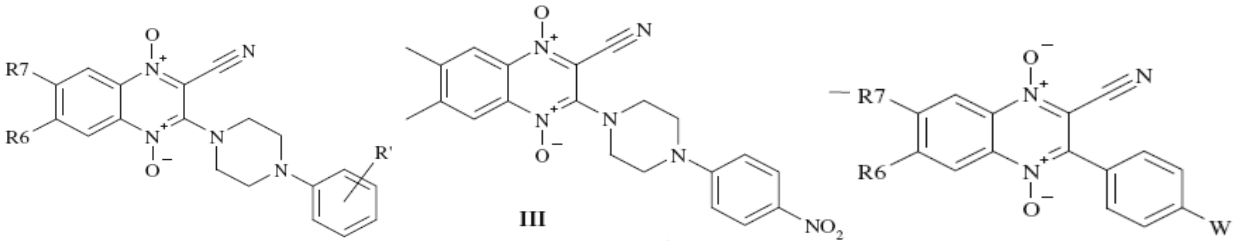
**I** MIC=0.78 μg/mL; SI=20 **II** MIC=0.20 μg/mL; SI>50

**Figure 2**. Structure of the in vivo lead compounds, I and II.

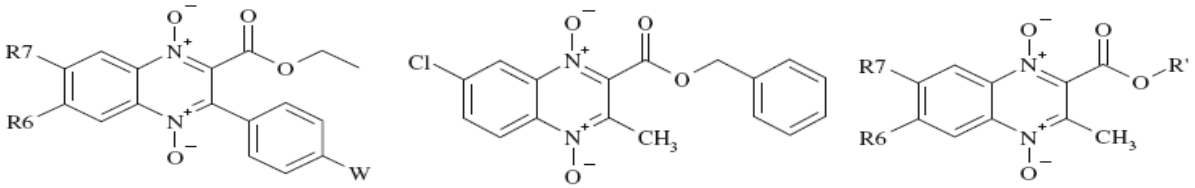
The data indicated 1,4-di-N-oxide quinoxalines hold promise for the treatment of TB. Therefore, the anti-TB activity of a series of 3-phenylquinoxaline 1,4-di-N-oxide derivatives with different patterns of substituents at quinoxaline nucleus (**1-70**, **Fig. 1**). The compounds were also tested against VERO cells in order to obtain parameters of cytotoxicity and selectivity.

Anti-TB activity of a series of 3-(4-phenylpiperazin- 1-yl) quinoxaline-2-carbonitrile and another series of 3-methylquinoxaline-2-carboxylate 1,4-di-N-oxide derivatives was previously determined (**Fig. 3**). The first series demonstrated very good anti-TB activity but almost all of the tested derivatives were insoluble or nonselective [33]. On the other hand, the second series showed very interesting results (lead compounds **II** and **IV**, **Figs. 2** and **3**) [29,37]. Several structural modifications were carried out on the lead compounds of both series by applying the isosteric and homologous strategies (**Fig. 3**), with the aim of improving their anti-TB activity, solubility and selectivity. The replacement of carbonitrile moiety by an ester group was performed in order to study the structural contribution of the carbonitrile group to the anti-TB activity. These modifications were proposed in an attempt to study the importance of the molecular volume and hybridization of C-atom (from sp to sp2) in position 2 of the 1,4-di-N-oxide quinoxaline scaffold within the optimization process of previous prototypes. Homologation of the substituents linked at C-3 of quinoxaline scaffold was obtained by elimination of the piperazinyl ring (series of carbonitrile derivatives) or by replacement of a methyl with a phenyl group (series of carboxylate derivatives). Variations of the electronic profile of group Wlinked to the para position of the phenyl moiety were carried out. All of the modifications were conducted in order to establish the contributions of electronic and steric parameters for the optimization of the previous lead compounds. The compounds **1–70** were prepared [38]. The formation of isomeric quinoxaline 1,4-di-N-oxide was observed in the case of monosubstituted benzofuroxans. Coinciding with previous reports, wehave observed that 7-substituted quinoxaline 1,4-di-N-oxide derivatives were prevailing over the 6-isomer, or in the

case of the methoxy substituent, only the 7-isomerwas formed. In practice,workup and purification permitted the isolation of the 7 isomer [39].

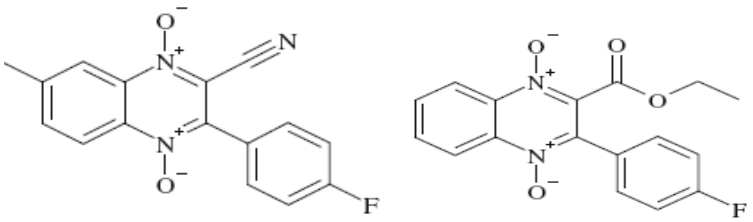



Ortega *et al.*, 2001 **III** MIC<0,2 µg/mL; SI>125 **1-56**




**57-70** **IV** MIC=0, 10 µg/mL; SI=470 Jaso *et al.*, 2005

**Figure 3**. Design of new 3-phenylquinoxaline 1,4-di-N-oxide derivatives, 1–70, as anti-TB drugs from structural modifications from the previous in vitro lead compounds, III and IV.




**12** MIC<0.2 µg/mL; SI>500 **64** MIC=0.39 µg/mL; SI=22.82

**Figure 4**. Lead compounds of the new series of quinoxaline 1,4-di-N-oxide derivatives, **12** and **64**.

In general, solubility problems detected in previous derivatives [33,34] have been resolved. More specifically, quinoxaline-2-carboxylates appear to be more soluble than quinoxaline-2-carbonitrile, as demonstrated by analogues 6 and 60. All of the compounds, with the exception of **55**, were active in preliminary assays, with more than 90% of growth inhibition at 6.25µg/mL. Table 1 shows the results obtained from the determination of the MIC values against H37Rv strain of Mtb, the $IC_{50}$ in VERO cells, and the selectivity index (SI) calculated as $IC_{50}$/MIC. As shown in **Table 1**, 60 derivatives showed an MIC value equal to or less than 1.56µg/mL. This value depends on several factors, such as compound structure, novelty, toxicity and potential mechanism of action; in general, MIC 61 µg/mL in a novel compound class may be considered an excellent lead compound. Moreover, thirty-four of these sixty compounds showed an MIC value less than 0.2µg/mL, a value on the order of MIC of rifampicin (RIF). Comparing the analogues, carbonitrile derivatives (**1,3,4,8,9,17,25** and 37) with carboxylate derivatives (**57, 58,**

**59, 63, 64, 65, 67** and **69**, respectively), quinoxaline-2-carbonitriles were more active against *Mtb* H37Rv strain but that they also displayed lower $IC_{50}$ values in VERO cells than ethyl quinoxaline-2-carboxylates. Within carboxylate's series, the best derivative found was ethyl 3-(40-fluoro) phenylquinoxaline-2-carboxylate 1,4-di-N-oxide, 64, with MIC=0.39 µg/mL and SI=22.82 (**Fig. 4**). Electron-donating group or an electron-withdrawing group leads to compounds with the same MIC value (e.g. compounds **67** and **70**; **Table 1**). However, cytotoxicity appears to be more important, with fluorinated derivatives (**9–16** and **64**) being the least cytotoxic and the most selective compounds. A similar behaviour is found regarding R6 and R7 positions. At these positions, the substituents do not influence the anti-TB activity but they do influence the cytotoxicity. The least cytotoxic compounds are those with a fluorine atom at $R_7$ position (**2, 10, 18, 26** and **50**) or those substituted by a methyl group in both positions (**8, 16, 24, 32** and **56**). Considering that a SI value >10 is required for a compound to be selected for further testing, the results indicate that very interesting anti-TB prototype agents were identified. Twenty-two of the 49 evaluated compounds in VERO cells have demonstrated selectivity indexes greater than the established cut-off; this means 45% of the derivatives showed a good in vitro activity/toxicity ratio. Outstanding compounds are 10,11,14,18 and 50 (all of them carbonitrile derivatives bearing, at least, a fluorine atom), with MIC <0.2 µg/mL and SI >100, and the new lead compound, 12 (**Fig. 4**), with MIC <0.2 µg/mL and SI >500. These derivatives are quite promising due to their low MIC value and, moreover, due to their great selectivity which improves in vitro results of best candidates (**Figs. 2** and **3**) [36,37].

The proposed modifications on previous lead-compounds give rise to a new series of 3-phenylquinoxaline-2-carbonitrile 1,4-di-N-oxide derivatives with greatly improved activities and selectivities and also to a new series of ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-N-oxide derivatives with maintained biological properties. The potency, low cytotoxicity and selectivity of these compounds make them valid lead-compounds for synthesizing new analogues that possess better activity, particularly compound 7-methyl-3-(4'-fluoro)phenylquinoxaline-2-carbonitrile 1,4-di-N-oxide (**12**, MIC <0.2µg/mL and SI >500), as it improves the in vitro results of best candidates. A primary screen was conducted at 6.25µg/mL against *Mtb* H37Rv in BACTEC 12B medium, using the MABA. Compounds effecting <90% inhibition in the primary screen (MIC >6.25µg/mL) were not further evaluated. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested against *Mtb* H37Rv at lower concentrations in order to determine the actual MIC in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampicin (RIF) was used as the reference compound (RIF MIC = 0.015–0.125 µg/mL). Concurrent with the determination of MIC's, compounds were tested for cytotoxicity ($IC_{50}$) in VERO cells at concentrations less than or equal to 62.5µg/mL or 10 times the MIC for *Mtb* H37Rv. The

Selectivity Index (SI=$IC_{50}$/ MIC) was also determined; it was considered significant when SI >10 (RIF $IC_{50}$ >100 µg/mL, SI >800).

**Table 1**. Anti-TB activity against *M. tuberculosis* (H37Rv strain), cytotoxicity in VERO cells and selectivity for new quinoxaline 1,4-di-N-oxide derivatives, **1–70**a.

| ID | $R_2$ | W | $R_6$ | $R_7$ | MIC[b] (lg/mL) | $IC_{50}$c (µg/mL) | SI[d] |
|---|---|---|---|---|---|---|---|
| **1** | CN | H | H | H | 0.20 | 1.9 | 9.5 |
| **2** | CN | H | H | F | <0.2 | 17.60 | >88.03 |
| **3** | CN | H | H | Cl | 0.39 | 0.36 | 0.92 |
| **4** | CN | H | H | $CH_3$ | 0.39 | 3.6 | 9.2 |
| **5** | CN | H | H | $CF_3$ | <0.2 | 3.78 | >18.89 |
| **6** | CN | H | H | $OCH_3$ | 3.13 | Insoluble | |
| **7** | CN | H | Cl | Cl | 0.20 | Insoluble | |
| **8** | CN | H | $CH_3$ | $CH_3$ | 0.39 | 3.9 10 | 10 |
| **9** | CN | F | H | H | <0.2 | 3.83 | >19.16 |
| **10** | CN | F | H | F | <0.2 | 24.93 | >124.67 |
| **11** | CN | F | H | Cl | <0.2 | 51.73 | >258.67 |
| **12** | CN | F | H | $CH_3$ | <0.2 | >100 | >500 |
| **13** | CN | F | H | $CF_3$ | <0.2 | 0.99 | >4.94 |
| **14** | CN | F | H | $OCH_3$ | <0.2 | 95.11 | >475.55 |
| **15** | CN | F | Cl | Cl | <0.2 | 7.41 | >37.06 |
| **16** | CN | F | $CH_3$ | $CH_3$ | 1.69 | >100 | >59.07 |
| **17** | CN | Cl | H | H | 0.39 | 0.36 | 0.92 |
| **18** | CN | Cl | H | F | <0.2 | 47.94 | >239.69 |
| **19** | CN | Cl | H | Cl | 0.39 | Insoluble | |
| **20** | CN | Cl | H | $CH_3$ | 0.39 | 0.45 | 1.15 |
| **21** | CN | Cl | H | $CF_3$ | <0.2 | 1.34 | >6.68 |
| **22** | CN | Cl | H | $OCH_3$ | 0.78 | 1.00 | 1.30 |
| **23** | CN | Cl | Cl | Cl | 0.20 | Insoluble | |
| **24** | CN | Cl | $CH_3$ | $CH_3$ | 0.78 | >10 | >12.8 |
| **25** | CN | $CH_3$ | H | H | 0.39 | 0.68 | 1.70 |
| **26** | CN | $CH_3$ | H | F | <0.2 | 14.64 | >73.19 |
| **27** | CN | $CH_3$ | H | Cl | 6.25 | 0.63 | 0.10 |
| **28** | CN | $CH_3$ | H | $CH_3$ | 0.78 | Insoluble | |
| **29** | CN | $CH_3$ | H | $CF_3$ | <0.2 | 0.28 | >1.42 |
| **30** | CN | $CH_3$ | H | $OCH_3$ | 1.56 | 3.80 | 2.40 |
| **31** | CN | $CH_3$ | Cl | Cl | 3.13 | 0.72 | 0.23 |
| **32** | CN | $CH_3$ | $CH_3$ | $CH_3$ | 1.56 | >62.5 | >40.0 |
| **33** | CN | $OCH_3$ | H | H | <0.2 | NDe | ND |
| **34** | CN | $OCH_3$ | H | F | <0.2 | 13.03 | >65.15 |
| **35** | CN | $OCH_3$ | H | Cl | <0.2 | ND | ND |
| **36** | CN | $OCH_3$ | H | $CH_3$ | <0.2 | ND | ND |
| **37** | CN | $OCH_3$ | H | $CF_3$ | <0.2 | 0.90 | >4.53 |
| **38** | CN | $OCH_3$ | H | $OCH_3$ | <0.2 | ND | ND |
| **39** | CN | $OCH_3$ | Cl | Cl | <0.2 | ND | ND |
| **40** | CN | $OCH_3$ | $CH_3$ | $CH_3$ | <0.2 | ND | ND |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **41** | CN | $OCF_3$ | H | H | <0.2 | <0.2 | ND |
| **42** | CN | $OCF_3$ | H | F | <0.2 | ND | ND |
| **43** | CN | $OCF_3$ | H | Cl | <0.2 | ND | ND |
| **44** | CN | $OCF_3$ | H | $CH_3$ | <0.2 | ND | ND |
| **45** | CN | $OCF_3$ | H | $CF_3$ | <0.2 | ND | ND |
| **46** | CN | $OCF_3$ | H | $OCH_3$ | <0.2 | ND | ND |
| **47** | CN | $OCF_3$ | Cl | Cl | <0.2 | ND | ND |
| **48** | CN | $OCF_3$ | $CH_3$ | $CH_3$ | 0.42 | ND | ND |
| **49** | CN | $COOCH_3$ | H | H | 0.78 | 4.80 | 6.20 |
| **50** | CN | $COOCH_3$ | H | F | <0.2 | 57.49 | >258.67 |
| **51** | CN | $COOCH_3$ | H | Cl | 3.13 | 20.87 | 6.70 |
| **52** | CN | $COOCH_3$ | H | $CH_3$ | 0.39 | 3.20 | 8.20 |
| **53** | CN | $COOCH_3$ | H | $CF_3$ | <0.2 | 1.93 | >9.64 |
| **54** | CN | $COOCH_3$ | H | $OCH_3$ | 3.13 | 3.90 | 1.20 |
| **55** | CN | $COOCH_3$ | Cl | Cl | >6.25 | ND | ND |
| **56** | CN | $COOCH_3$ | $CH_3$ | $CH_3$ | 6.25 | 9.66 | 1.50 |
| **57** | $COOC_2H_5$ | H | H | H | 1.56 | 24.76 | 15.87 |
| **58** | $COOC_2H_5$ | H | H | Cl | 1.56 | 3.33 | 2.13 |
| **59** | $COOC_2H_5$ | H | H | $CH_3$ | 1.56 | 18.61 | 11.93 |
| **60** | $COOC_2H_5$ | H | H | $OCH_3$ | 6.25 | 17.78 | 2.84 |
| **61** | $COOC_2H_5$ | H | F | F | 1.56 | 0.38 | 0.24 |
| **62** | $COOC_2H_5$ | H | Cl | Cl | <0.2 | ND | ND |
| **63** | $COOC_2H_5$ | H | $CH_3$ | $CH_3$ | 6.25 | >10 | >1.6 |
| **64** | $COOC_2H_5$ | F | H | H | 0.39 | 8.90 | 22.82 |
| **65** | $COOC_2H_5$ | Cl | H | H | 0.39 | 4.80 | 12.31 |
| **66** | $COOC_2H_5$ | Br | H | H | 0.39 | 6.12 | 15.69 |
| **67** | $COOC_2H_5$ | $CH_3$ | H | H | 0.78 | 11.29 | 14.47 |
| **68** | $COOC_2H_5$ | $CF_3$ | H | H | 1.56 | 2.24 | 1.44 |
| **69** | $COOC_2H_5$ | $OCH_3$ | H | H | 1.56 | 7.65 | 4.90 |
| **70** | $COOC_2H_5$ | $NO_2$ | H | H | 0.78 | 2.26 | 2.9 |
| RIFf | | | | | 0.125 | >100 | >800 |

a For a general structure and definition of W, $R_2$, $R_6$ and $R_7$; b MIC against H37Rv strain of *M. tuberculosis* (µg/mL); cMeasurement of cytotoxicity in VERO cells: 50% inhibitory concentrations (µg/mL); dSelectivity index (in vitro): $IC_{50}$ in VERO cells/MIC against *M. tuberculosis*; e Not determined; f Rifampin.

Quinoxaline-1,4-di-N-oxide derivatives even improve the biological results shown by their reduced analogues and are endowed with antiviral, anticancer, antibacterial and antiprotozoal activities [16,20, 40-41]. There are many publications regarding 1,4-di-N-oxide derivatives, and more specifically alkyl and arylcarboxamide derivatives, in which their antibacterial and antimicrobial activities have been reported or their capability to act as antitumoral agents [42] has been clearly demonstrated, thereby reflecting the growing interest in these structures over the past forty years. As a result of the anti-TB research project, our group has published several papers reporting a wide range of quinoxaline-1,4-di-N-oxide derivatives (**Fig. 5**) including a

great variety of substituents in positions 2,3,6 and 7. With regard to position 2, carbonitrile derivatives appeared to be quite toxic [28,29, 33,34]. Moreover, ketone, carboxylate and carboxamide quinoxaline-1,4-dioxydes derivatives were actually patented in the 70s for their antibacterial activity. These studies have facilitated a wide SAR analysis which lead us to design a group of thirty-six 3-methylquinoxaline-2-carboxamide 1,4-di-N-oxide derivatives that were prepared and tested against *Mtb* and to justify the design of the compounds presented in this paper [43]. Continuing with the anti-TB project and in an attempt to establish the structural requirements necessary for the development of anti-TB drugs, nine series of quinoxaline-2-carboxamide 1,4-di-N-oxide derivatives were proposed (**Fig. 6**).

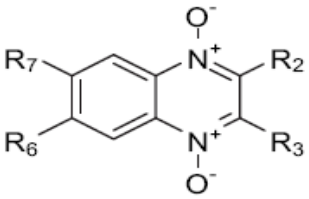



**Figure 5**. General structure and numeration of quinoxaline-1,4-di-N-oxide.

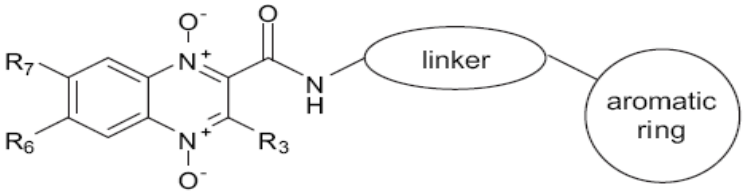



**Figure 6**. Design of the new series of quinoxaline-2-carboxamide 1,4-di-N-oxide.

Several structural modifications were designed and can be summarized as follows: a) variation in the length of the aliphatic linker between the carboxamide group and the aromatic ring; b) modification of the substituent in position 3 by a phenyl (**Figure 7-9**) and a methyl moiety (**Figure 10-15**); c) substitution of a variety of aromatic rings (**Figure 10-15**). Structure and biological values of synthesized quinoxaline-1,4-di-N-oxide derivatives are reported in **Table 2**. Compounds are assayed against *Mtb* H37Rv in order to determine the $IC_{90}$. Compounds showing values of ≤10 mg/mL are considered as active and move on to the secondary screening. Cytotoxicity is assayed in VERO cells and the $CC_{50}$ is determined from the dose response curve. Next, the $IC_{90}$ and $CC_{50}$ values are formed into a ratio termed Selectivity Index (SI). Compounds showing a SI ≥ 10 are considered active for anti-TB activity. As can be observed in Table 1, thirteen of the forty-three evaluated compounds passed the cut off established by the TAACF at the primary screening level and moved on to the secondary screening level. Compounds 2b and 4d were identified as the most interesting with a SI higher than 10. Some structure activity relationships were established. Looking at the values of compounds **7b, 7g, 8b, 10b, 10c, 10d, 10g, 12b, 12g, 13b** and **15b**, it can be said that the insertion of a halogen moiety, increases the anti-TB activity. Taking into account the biological values reported in **Table 2**, it can be

concluded that the insertion of a electron-withdrawing moiety, especially that of chlorine atom, is an essential requirement for the anti-TB activity, as previously established by our group [43]. With the aim of corroborating previous preliminary structureeactivity relationship observed by our group and identifying the most suitable length for the aliphatic chain between the carboxamide group and the aromatic ring, three series of compounds (**Figure 6, 7** and **9**) were prepared. Comparing the biological values shown by these compounds, it can be said that the preferred length for the aliphatic chain is one methylene group. In previous investigations, three series of 1,4-di-N-oxide-quinoxaline-2-carboxylic acid aryl amide derivatives were synthesized, containing a methyl moiety in position 3. The SAR of these types of compounds, a phenyl group was substituted in position 3 of the quinoxaline ring (**Figure 6, 7** and **9**) and reported in this paper. This modification led to a reduction of the anti-TB activity as can be observed by comparing the biological values of compounds from Figure **6, 7** and **9** with their analogues containing a methyl group in position 3, described in previous reports [27]. Taking into account the biological values of the structures which present a phenyl group substituted in position 3 and the compounds with a methyl group in this position, [43]. The methyl moiety in position 3 and modify the substitution on the aromatic ring. Different substituents were introduced on para position of the phenyl ring considering chloro, bromo or trifluoromethyl moiety as electron-withdrawing groups and methyl as electron-releasing group (**Figure 10, 11, 12, 13**). In this fragment of the structure other substituents as byphenyl or a benzodioxol have been considered (**Figure 14, 15**). Taking into account the biological values showed by these derivatives, it can be said that the insertion of a substituent on para position of the phenyl group did not improve the anti-TB activity suggesting that the most suitable aromatic ring is the unsubstituted phenyl.

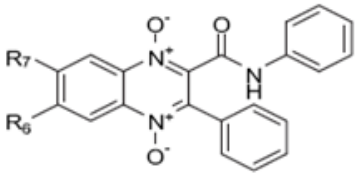


**Figure 7**

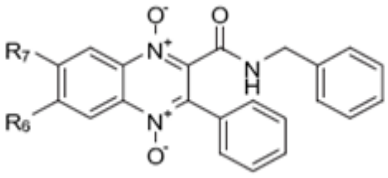


**Figure 8**

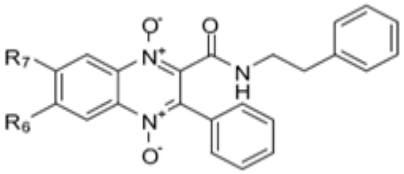


**Figure 9**

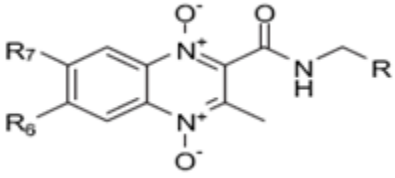


**Figure 10-15**

General structure of compounds quinolone compounds

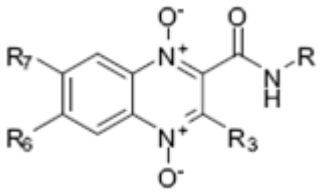
| General structure | Compounds | $R_6$ | $R_7$ |
|---|---|---|---|
|  | **A** | H | H |
| | **B** | H | Cl |
| | **C** | H | F |
| | **D** | H | $CF_3$ |
| | **E** | H | $CH_3$ |
| | **F** | H | $OCH_3$ |
| | **G** | Cl | Cl |

**Table 2**. Anti-tubercular activity of compounds (Series **6-15**).

| Compound | R3 | R | Anti-TB activity | Cytotoxicity | |
|---|---|---|---|---|---|
| | | | $IC_{90}$a H37Rv | $CC_{50}$b VERO | SIc $CC_{50}$/MIC |
| **7a** | $C_6H_5$ | $C_6H_5$ | 26.99 | N.T.d | N.T. |
| **7b** | | | 6.71 | 8.97 | 1.34 |
| **7c** | | | 17.93 | N.T. | N.T. |
| **7d** | | | 19.10 | N.T. | N.T. |
| **7e** | | | 24.65 | N.T. | N.T. |
| **7f** | | | 26.84 | N.T. | N.T. |
| **7g** | | | 6.63 | 5.541 | 0.84 |
| **8a** | $C_6H_5$ | $CH_2$-$C_6H_5$ | 6.71 | >40 | >5.96 |
| **8b** | | | 3.39 | >40 | >11.79 |
| **8c** | | | 3.86 | 17.86 | 4.62 |
| **8d** | | | >100. | N.T. | N.T. |
| **8e** | | | 13.91 | N.T. | N.T. |
| **8f** | | | 14.58 | N.T. | N.T. |
| **8g** | | | 25.45 | N.T. | N.T. |
| **9a** | $C_6H_5$ | $CH_2$-$CH_2$-$C_6H_5$ | 18.61 | N.T. | N.T. |
| **9b** | | | 15.42 | N.T. | N.T. |
| **10a** | $CH_3$ | $CH_2$-$C_6H_5$-4-$CF_3$ | 16.81 | N.T. | N.T. |
| **10b** | | | 6.13 | >40 | >6.52 |
| **10c** | | | 4.48 | >40 | >8.94 |
| **10d** | | | 3.38 | >40 | >11.82 |
| **10e** | | | >100 | N.T. | N.T. |
| **10f** | | | >100 | N.T. | N.T. |
| **10g** | | | 6.58 | >40 | >6.08 |

| | | | | | |
|---|---|---|---|---|---|
| **11a** | $CH_3$ | $CH_2$-$C_6H_5$-4-Cl | 11.04 | N.T. | N.T. |
| **11b** | | | 29.68 | N.T. | N.T. |
| **11e** | | | 14.56 | N.T. | N.T. |
| **11g** | | | 51.86 | N.T. | N.T. |
| **12a** | $CH_3$ | $CH_2$-$C_6H_5$-4-Br | 15.61 | N.T. | N.T. |
| **12b** | | | 5.33 | >40 | >7.50 |
| **12e** | | | 78.22 | N.T. | N.T. |
| **12g** | | | 6.92 | >40 | >5.78 |
| **13a** | $CH_3$ | $CH_2$-$C_6H_5$-4-$CH_3$ | 6.76 | >40 | >5.92 |
| **13b** | | | 32.04 | N.T. | N.T. |
| **13e** | | | 99.91 | N.T. | N.T. |
| **13g** | | | >100 | N.T. | N.T. |
| **14a** | $CH_3$ | $CH_2$-CH-$(C_6H_5)_2$ | 15.99 | N.T. | N.T. |
| **14b** | | | 60.43 | N.T. | N.T. |
| **14e** | | | 16.79 | N.T. | N.T. |
| **14g** | | | 66.54 | N.T. | N.T. |
| **15a** | $CH_3$ | $CH_2$-benzo[d][1,3]dioxol | 22.75 | N.T. | N.T. |
| **15b** | | | 6.99 | >40 | >5.72 |
| **15e** | | | 13.22 | N.T. | N.T. |
| **15g** | | | 34.92 | N.T. | N.T. |
| **RIFe** | | | 0.015-0.125 | >100 | >800 |

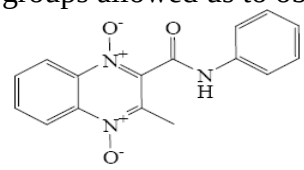
a$IC_{90}$ against *Mtb* H37Rv; bCytotoxicity in VERO cells; cSelectivity index; dNot tested; eRifampin.

**2.1 Primary screening** (Dose Response):

The initial screening is conducted against *Mtb* H37Rv in BACTEC 12B medium using the MABA [30]. Compounds are tested in ten 2-fold dilutions, typically from 100 mg/mL to 0.19 mg/mL. Any $IC_{90}$ value of ≤10 mg/mL is considered "Active" for anti-TB activity. Cytotoxicity is determined from the doseeresponse curve as the $CC_{50}$. Then the $CC_{50}$ is divided by the $IC_{90}$ for calculating a Selectivity Index (SI) value. SI values of ≥10 are considered for further testing. Forty-three new 1,4-di-N-oxide-quinoxaline-2-carboxylic acid aryl amide derivatives were synthesized using a variation of the Beirut reaction. All of the compounds were evaluated against *MTb* H37Rv stain; thirteen were active in the primary screening, showing an $IC_{90}$ ≤10 mg/mL, and were then moved on to the secondary screening level. Two of the compounds were active at this level, showing a SI ≥10. Taking into account the biological values obtained, it can be said that the lead general structure for developing new anti-TB agents should consider the 1,4-di-N-oxide-quinoxaline ring with a carboxamide functionalized on position 2 and a methyl moiety on

position 3. The most suitable substituent on positions 6 or/and 7 should be an electron-withdrawing group and a methyl moiety on position 3. With regard to the linker and the aromatic ring attached to it, one methylene group and an unsubstituted phenyl ring are considered to be the most appropriate substituents.

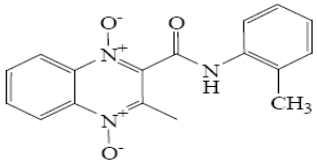
Many of quinoxaline derivatives possess excellent anti-TB activity, and the range of possible substituents affords an opportunity to tailor both the pharmacokinetic and activity profiles [32]. As a part of anti-TB research project, we have synthesized different series of quinoxaline-1,4-di-*N*-oxide derivatives, with a great variety of substituents in positions 2,3,6 and 7. Regarding the 2 position, where the main group is linked, our team has principally worked with carbonitrile, amide, ketone and ester derivatives. The groups studied in position 3 are, among others, methyl, amine, trifluoromethyl, piperazinyl and phenyl groups. Finally, the substitutions of the hydrogens of positions $R_6$ and/or $R_7$ by fluorine, chlorine, methyl, trifluoromethyl and methoxy groups allowed us to obtain a great variety of compounds.



16a

MIC=3.13 µg/mL; SI>20

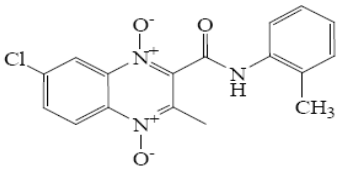
$EC_{90}$/MIC=0.89



16b2

MIC=6.25 µg/mL; SI>10

$EC_{90}$/MIC=0.42

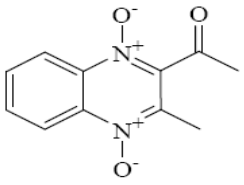


16c3

MIC=6.25 µg/mL; SI>10

$EC_{90}$/MIC=0.42

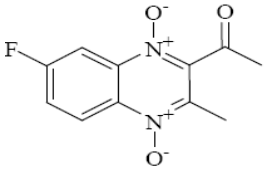
**Figure 16.** 3-methyl-*N*-phenylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives.



**17a4**

MIC=3.13 µg/mL; SI>20

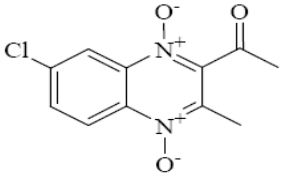
$EC_{90}$/MIC=0.87



**17b5**

MIC=3.13 µg/mL; SI>20

$EC_{90}$/MIC=0.79



**17c6**

MIC=0.78 µg/mL; SI>20

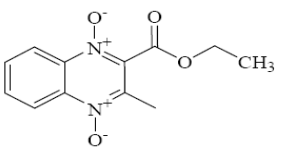
$EC_{90}$/MIC=3.13

**Figure 17.** 2-acetylquinoxaline 1,4-di-*N*-oxide derivatives.

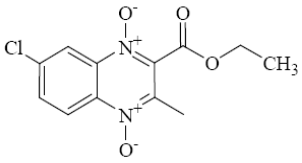
The quinoxaline derivatives as anti-TB agents date back to the end of the 1990s. Herein, 2-quinoxalinecarbonitrile derivatives obtained from the first screening [44]. During these past few years, studied the influence of the 1,4-di-*N*-oxide groups in quinoxaline ring on the anti-TB activity, confirming their importance to increase the activity [24-26]. In the early 2000s, positive results from several new quinoxaline 1,4-di-*N*-oxide derivatives were synthesized and tested against *Mtb*. The 2-quinoxalinecarbonitrile derivatives appeared to be quite toxic [33,34]; thus,

the replacement of the carbonitrile group with a carboxamide, acetyl, benzoyl or carboxylate groups [28,29] was proposed. A group of thirty-one 3-methylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives was prepared and tested [27]. Eight showed a MIC value lower than 6.25µg/mL although only 3 presented enough selectivity and good results in macrophage assay in order to merit continuation of their study. We should point out the interesting anti-TB activities shown by certain 3-methyl-*N*-phenylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives, particularly the compounds with a chlorine atom in 7 position (**16c**) and corresponding nonsubstituted derivatives (**16a** and **16b**) which showed the best results (**Figure 16**). With regard to the benzene moiety, greater effectiveness resulted for the *ortho*-methyl substituents (**16b** and **16c**). Among twenty-seven 2-acetylquinoxaline 1,4-di-*N*-oxide and seven 2- benzoylquinoxaline 1,4-di-*N*-oxide derivatives, eighteen demonstrated MIC values equal or better than the first cut-off established by the TAACF. Six of these derivatives also showed good selectivity and maintained activity in the macrophage assay, among which stand out compounds **17a-17c** (**Figure 17**), exhibiting $EC_{90}$/MIC values between 0.79 and 3.13. Twenty-two of the twenty-nine 2-quinoxaline carboxylate 1,4-di-*N*-oxide derivatives evaluated possessed improved MIC values [29].

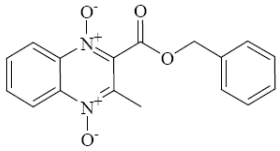
In addition, fifteen were selective as assessed by the cytotoxicity assay and were active in the macrophage assay. The results from the *in vitro* assays show that of the series tested ethyl and benzyl 3-methylquinoxaline-2-carboxylate 1,4-di-*N*-oxide derivatives with the chlorine group in position 7 of the benzene moiety (**18c** and **18d**) and the unsubstituted derivatives (**18a** and **18b**) had the best anti-TB activity, showing $EC_{90}$/MIC values between 0.01 and 2.30 (**Figure 18**). These new quinoxaline 1,4-di-*N*-oxide derivatives emerged as new lead candidates for the treatment against TB because of their potency, selectivity and low cytotoxicity. Meanwhile, reduced quinoxaline derivatives as anti-TB agents [45-47]. A series of 3-methyl-2-alkylsulfanyl quinoxalines, but none were active against *Mtb*. The prepared 9 quinoxaline-2-carboxylates, but only two achieved the MIC values of 6.25 µg/mL; Guillon´s group focused on pyrrolo[1,2-*a*]quinoxaline-carboxylic acid hydrazide derivatives and, again, only two compounds reached the first cut-off (MIC<6.25 µg/mL). A series of 3-methyl quinoxaline 1,4-di-*N*-oxide derivatives with a phenylthio, phenylsulfonyl or phenylsulfinyl linked in $R_2$ position of quinoxaline subunit. They completed this work studying 6,7-difluoro quinoxaline derivatives, analogues to the previous compounds, in which the phenylthio group was substituted for benzylamino or phenylamino (**Figure 19**). The series of 3-methyl-2-phenyl thioquinoxaline 1,4-di-*N*-oxide derivatives presented the best MIC data, ranging between 0.39 and 0.78 µg/mL, whereas the oxidation of sulphur bridge to yield phenylsulfinyl and phenyl sulfonyl derivatives or its replacement with benzylamino or phenylamino group slightly reduces its activity [16,17].



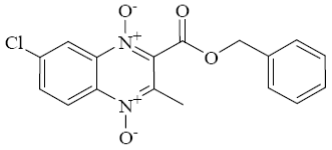
**18a**
MIC=1.56 µg/mL; SI>30.13; $EC_{90}$/MIC=0.56



**18b**
MIC=0.20 µg/mL; SI>50; $C_{90}$/MIC=2.30



**18c9**
MIC=0.10 µg/mL; SI>470; $EC_{90}$/MIC=1.50



**18d10**
MIC=0.10 µg/mL; SI>76.9; $EC_{90}$/MIC=0.01

**Figure 18.** Ethyl and benzyl 3-methylquinoxaline-2-carboxylate 1,4-di-*N*-oxide derivatives.

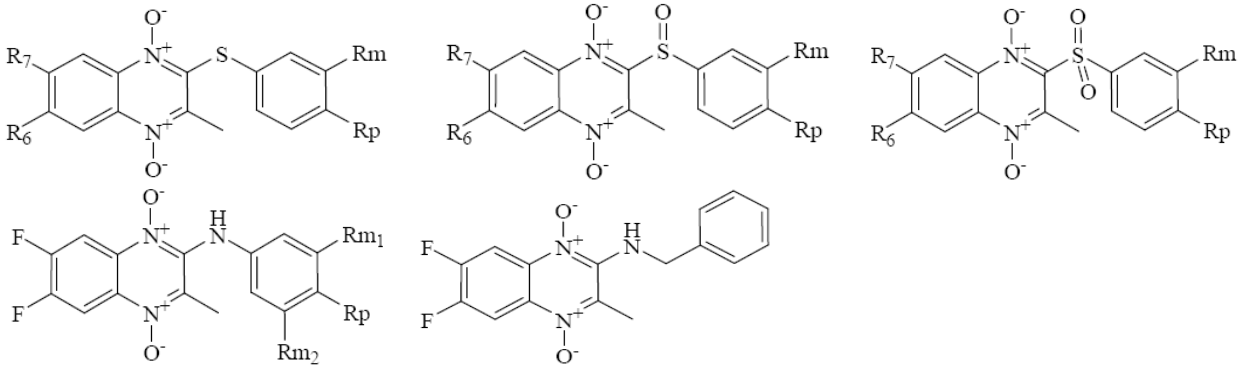
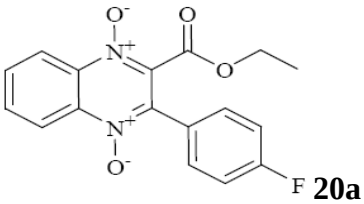
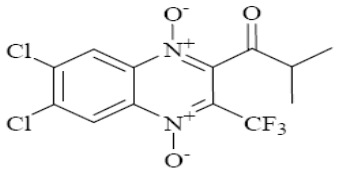


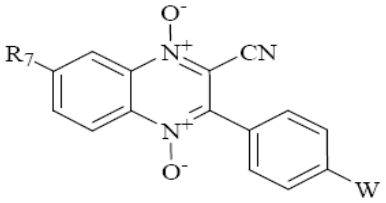
**Figure 19.** General structure of quinoxaline 1,4-di-*N*-oxide derivatives.



**20a**
MIC=0.39 µg/mL; SI=22.82



**20b**
MIC=0.2 µg/mL; SI<50



**20**c $R_7$=Cl; W=F (MIC<0.2 µg/mL; SI>258)
**20d** $R_7$=$CH_3$; W=F (MIC<0.2 µg/mL; SI>500)
**20e** $R_7$=$OCH_3$; W=F (MIC<0.2 µg/mL; SI>475)
**20f** $R_7$=F; W=F (MIC<0.2 µg/mL; SI>124)
**20g** $R_7$=F; W=Cl (MIC<0.2 µg/mL; SI>239)
**20h** $R_7$=F; W=$COOCH_3$(MIC<0.2 µg/mL; SI>258)

**Figure 20**. *In vitro* lead-compounds of new series of quinoxaline 1,4-di-*N*-oxides.

These studies have facilitated a wide-ranging SAR analysis.

## 3. Recent developments

These *in vitro* results indicate that 1,4-di-*N*-oxide quinoxalines hold promise for the treatment of TB. Therefore, an extended evaluation of the *in vitro* and *in vivo* **anti-TB** activity of most interesting quinoxaline 1,4-di-*N*-oxide derivatives was performed.

### *3.1. Amide derivatives*

MIC values for the most potent amide derivatives from our previous studies (**16a-c**) (Figure **16**) were determined against different single-drug resistant strains of *Mtb*. In general, the three derivatives showed good MIC values against resistant strains. The MBCs against strain H37Rv of compounds **16a** and **16b** were determined. Derivative **16a** could be considered bactericidal but, on the other hand, compound **16b**, which showed higher MBC/MIC ratios for H37Rv, indicated bacteriostatic rather than bactericidal activity [36].

### *3.2. Ketone derivatives*

The ketone derivatives **4-6** (**Figure 5**) were subjected to the following set of tests: determination of MIC against different SDR strains of *Mtb*, MBC and *in vivo* efficacy testing, including the determination of oral bioavailability and the MTD. Almost all of these derivatives displayed good inhibitory activity against resistant strains. The most moderate activity was observed against the CIP-resistant strain. The susceptibilities of RIF, TAC-, EMB- and PAS resistant strains were comparable to that of H37Rv, with ratios of MICs against resistant and non-resistant strains about 1. This indicates that there is no cross-resistance with the current anti-TB drugs supporting the premise that 1,4-di-*N*-oxide quinoxaline derivatives have a novel mode of action unrelated to the currently used **anti-TB** drugs. The promising developments of new effective compounds were against the growing number of drug resistant strains. Compounds **17a** and **17c** could be considered to be bactericidal due to the low MBC/MIC ratios obtained. In addition, specific derivatives were further evaluated in a series of *in vivo* assays and compound **6** was active in reducing CFU counts in both the lung and spleen of infected mice following oral administration (**Figure 17**). This *in vivo* efficacy is comparable to clinically used TB drugs, although a relatively high dose of compounds was required to obtain equivalent reductions in lung CFU [36]. Compound **17c** was active *in vivo* via oral administration when dosed at 100 or 300 mg/kg and *in vivo* cidal activity was indicated at 300mg/kg dosing. This compound is most likely bioreduced to an active metabolite and is active on PA-824 resistant *M. bovis*, thereby indicating that the pathway of bioreduction/activation was different from PA-824, a bioreducible nitroimidazole in clinical trials [48]. In addition compound **17c** was tested against non-replicating bacteria (NRP) adapted to low oxygen and showed very good activity, indicating that activation occurred in both growing and non replicating bacteria leading to cell death. The activity of compound **17c** is unique in that it is

1) bactericidal;
2) active on single-drug resistant strains,
3) and more importantly active on poly-drug resistant clinical isolates, MDR-TB, including strains with resistance to additional TB drugs and quinolones;
4) orally bioavailable and strongly active *in vivo*, in a murine model of TB infection;
5) active against PA-824 resistant strains of *M. bovis* with defined resistance determinants; and
6) active on non-replicating persistent mycobacteria.
This later activity may prove important for attaining cures in a shorter amount of time, and this is being further evaluated *in vitro* and *in vivo* [36].

### *3.3. Ester derivatives*

Following the protocol for the ketone derivatives, several ester compounds were subjected to the same *in vitro* and *in vivo* assays. The MIC against different single-drug resistant strains of *Mtb* and *in vivo* efficacy testing of the most potent compounds from previous studies were evaluated. Similar to the results for the ketone derivatives there is little to no cross-resistance with the current anti-TB drugs as all compounds showed ratios of MICs against resistant and non-resistant strains of about 1 [49,50]. Compound **18b** is most likely bioreduced to an active metabolite and is active on PA-824 resistant *M. bovis*, thereby indicating that the pathway of bioreduction/activation was different from PA-824, a bioreducible nitroimidazole in clinical trials [48]. Quinoxaline *N*-oxides are likely activated via bioreduction in bacteria. Compound **18b** is most likely bioreduced to an active metabolite and is active on PA-824 resistant *M. bovis*, thereby indicating that the pathway of bioreduction/activation was different from PA-824via of the nitroimidazole PA-824 was used to compare if compound **18b** was activated through the same pathway. Again, the quinoxaline derivative **18b** was active on all PA-824 resistant *M. bovis* strains tested showing lack of cross-resistance and supporting a different pathway of drug activation. The *in vivo* activity of ethyl 7-chloroquinoxaline derivative (**18b**) was evaluated at oral doses of 25, 100 and 300 mg/kg. This compound was active in the lung and spleen at 100 and 300 mg/kg with a reduction of the CFU of 2.03 and 5.58 (lung) and 2.72 and 5.51 (spleen), respectively [36]. As with compound **17c,** compound **18b** appeared cidal *in vivo* when dosed at 300mg/kg. Several structural modifications were introduced onto the lead compounds of the series of 3-methylquinoxaline-2-carboxylate 1,4-di-*N*-oxide derivatives. Homologation of the substituents linked at C-3 of quinoxaline scaffold was obtained by the replacement of a methyl with a phenyl group, keeping the ethyl carboxylate group linked to C-2 of the quinoxaline subunit. Variations of the electronic profile of the group W linked to the *para* position of the phenyl moiety were carried out [51]. All of the modifications were conducted in order to establish the contributions of electronic and steric parameters for the optimization of the lead compounds. Certain parameters of potency, cytotoxicity and selectivity (levels 1 and 2) were

established for the fourteen synthesized derivatives. The unsubstituted derivatives in positions $R_6/R_7$ and the 7-methyl derivative demonstrated selectivity indexes greater than the established cut-off (SI>10) and were selected for more additional studies. One compound with unique activity is ethyl 3-(4'-fluoro) phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide, **2a** (**Figure 20**) [49].

### *3.4. Other derivatives*

Other quinoxaline 1,4-di-*N*-oxide derivatives [40] in order to combat other diseases such as malaria [52,53] and cancer which were also assayed as anti-TB candidates. Twenty seven 2-alkylcarbonyl- and thirty 2-arylcarbonyl-3-trifluoromethyl quinoxaline 1,4-di-*N*-oxide derivatives, designed as anticancer [54,55] and antiplasmodial agents [56], were tested against *Mtb*. Among these compounds, only eight of them did not pass the first screening but almost all of them were not selective enough (SI<10) to move forward. The four derivatives with SI>10 were all alkylcarbonyl derivatives (the least potent against cancer cell lines), outstanding 6,7-dichloro-2-isobutyryl-3-trifluoro methyl quinoxline 1,4-di-*N*-oxide, **20b**. Synthesized seventy two 3-arylquinoxaline-2-carbonitrile 1,4-di-*N*-oxide derivatives, designed as antiplasmodial agents [36]. The activity against *Mtb* of all of them were established, showing that sixty-nine derivatives attained the first cut-off (MIC <6.25 μg/mL). Moreover, forty-five of these sixty-nine compounds showed an MIC value equal to or less than 0.2μg/mL, a value on the order of MIC of rifampicin (RIF). Cytotoxicity and selectivity of thirty-seven analogs were determined revealing that the fluorinated derivatives (**20c-20h**) were the least cytotoxic and the most selective compounds. One compound of particular interest is 7-methyl-3-(4'-fluoro) phenylquinoxaline-2-carbonitrile 1,4-di-*N*-oxide, **20d**, with MIC<0.2 μg/mL and SI>500 [48].

Infections caused by *Mtb* and *M. avium* complex are the most commonly reported opportunistic bacterial infections in people with HIV disease [57]. *M. avium* is a human pathogen that causes infection in immunocompetent as well as immune-compromised patients [58]. The TAACF has tested some of our selected compounds for activity against *M. avium* but only one was active. This compound, **17c**, the ethyl 7-chloro-3-methylquinoxaline-2-carboxylate 1,4-di-*N*-oxide demonstrated MIC 8 μg/mL for 4 of the 5 strains tested. The reason for lack of, or reduced activity on *M. avium* could be due to differences in potential activating enzymes and or uptake of compounds into the bacteria.Recently some select analogs were found active against a panel of single-drug-resistant strains and in the TAACF macrophage model. Two derivatives, compounds **17c** and **18b**, proved efficacious *in vivo* in a murine model of low dose aerosol infection. Moreover, theses two compounds also showed activity against non-replicating bacteria. If the bactericidal activity and activity on NRP bacteria *in vitro* translate to *in vivo* conditions, quinoxaline 1,4-di-*N*-oxides may lead to shortened therapy, because the presence of NRP

bacteria is believed to be a major factor responsible for the prolonged nature of anti-TB therapy. In conclusion, quinoxaline 1,4-di-*N*-oxides represent a new class of orally active anti-TB drugs.

## 4. Discussion

Tuberculosis (TB) is a respiratory transmitted disease affecting more than any other infectious disease worldwide. However, the impact of TB on morbidity and mortality has varied widely during the centuries. Since the beginning of the clinical treatment of TB with streptomycin it was observed that the bacillus was capable of rapidly developing drug resistance [59]. By the drug combination regimens were established to prevent drug resistance [60]. Currently, MDR-TB presents a high incidence, with rates varying widely between different countries and regions [61]. The continuing emergence of MDR-TB will inevitably make it more difficult in the future to control TB [62]. The increasing spread of TB is the coexistence of HIV with TB. HIV infected patients who progress to AIDS is at high risk for infection with TB and other mycobacteria (such as *M. avium*). While *M. avium* does not usually cause disseminated disease in healthy individuals, in immunocompromised patients, such as AIDS, disseminated disease is common [63]. So the pandemic of AIDS and the evidence of its association with TB is now of serious concern [64,65]. The development of resistance by *Mtb* to commonly used anti-TB drugs, the increasing incidences of disease in immuocompromised patients, and longer durations of therapy that are required as a result of resistance development, highlights the need for new drugs to extend the range of effective TB treatment options [66].

## 5. Conclusion

Over the past years quinoxaline 1,4-di-*N*-oxide derivatives were found to possess activity against *Mtb*. Continued evaluation of *in vitro* anti-TB activity and cytotoxicity of our quinoxaline 1,4-di-*N*-oxide series, allowed identification of novel anti-TB candidates based on their potency, selectivity and low cytotoxicity, making them valid new lead for synthesizing additional analogs that might improve the anti-TB activity. Quinoxaline derivatives show very interesting biological properties (antibacterial, antiviral, anticancer, antifungal, antihelmintic, insecticidal) and evaluation of their medicinal chemistry is still in progress.

## References

[1] Global Tuberculosis Control WHO REPORT 2009, http://www.who.int/tb/publications/global_report/2009/pdf/full_report.pdf (accessed 08.03.10).
[2] WHO Fact sheet No. 104. 2007.
[3] WHO Weekly epidemiological record No. 15. 2003, 78, 121.
[4] M. Asif, A. Singh, Lakshmayya, A. Husain, A.A. Siddiqui. *Lett. Drug Design & Discov*., 10 (2013) 651-660.

[5] M. Asif, A.Singh, Lakshmayya. *Chronicle of Young Scientist*., 4(1) (2013) 1-8.
[6] M. Asif, A.A. Siddiqui, A. Husain, *Med Chem Res.*, 22 (2013), 1029-1042.
[7] M. Asif, *World J. Org. Chem*., 1 (2013) 14-19
[8] Y.L. Janin, *Bioorg. Med. Chem.,* 15 (2007) 2479.
[9] NIAID MDR/XDR TB Research Agenda June 6, 2007, http://www3.niaid.nih. gov/topics/tuberculosis/ (accessed 08.03.10).
[10] Tuberculosis Fact Sheet, No. 104. 2007, http://www.who.int/tb/publications/ global_report/2007/pdf/full.pdf.
[11] A. Carta, P. Corona, M. Loriga, *Curr. Med. Chem.,* 12 (2012) 2259.
[12] L. Novacek, L. Polasek, *Biol. Chem. Zivocisne*, 18 (1982) 17.
[13] H.U. Gali-Muhtasib, M.J. Haddadin, D.N. Rahhal, I.H. Younes, *Oncol. Rep.*, 8 (2001) 679.
[14] G. Aguirre, H. Cerecetto, R. Di Maio, M. Gonzalez, M.E.M. Alfaro, A. Jaso, B. Zarranz, M.A. Ortega, I. Aldana, A. Monge-Vega, *Bioorg. Med. Chem. Lett.*, 14 (2004) 3835-3839.
[15] C. Urquiola, M. Vieites, G. Aguirre, A. Marin, B. Solano, G. Arrambide, P. Noblia, M.L. Lavaggi, M.H. Torre, M. Gonzalez, A. Monge, D. Gambino, H. Cerecetto, *Bioorg. Med. Chem.*, 14 (2006) 5503-5509.
[16] A. Carta, M. Loriga, G. Paglietti, A. Mattana, P.L. Fiori, P. Mollicotti, L. Sechi, S. Zanetti, *Eur. J. Med. Chem.*, 39 (2004) 195-203.
[17] A. Carta, G. Paglietti, M.E. Rahbar Nikookar, P. Sanna, L. Sechi, S. Zanetti, *Eur. J. Med. Chem.,* 37 (2002) 355.
[18] A. Azqueta, G. Pachon, M. Cascante, E.E. Creppy, A.L. de Cerain, *Mutagenesis*. 20 (2005) 165.
[19] G. Chowdhury, D. Kotandeniya, J.S. Daniels, C.L. Barnes, K.S. Gates, *Chem. Res. Toxicol.*, 17 (2004) 1399.
[20] B. Ganley, G. Chowdhury, J. Bhansali, J.S. Daniels, K.S. Gates, *Bioorg. Med. Chem.* 9 (2001) 2395-2401.
[21] D.J. Brown, E.C. Taylor, P. Wipf, *Quinoxalines. Supplement II*, Wiley & Sons: New York, 2004.
[22] A. Azqueta, L. Arbillaga, G. Pachon, M. Cascante, E.E. Creppy, A. Lopez de Cerain, *Chem.-Biol. Interact.*, 168 (2007) 95.
[23] R.C. Goldman, K.V. Plumley, B.E. Laughon, *Infect. Disord. Drug Targets.*, 7 (2007) 73.
[24] M.E. Montoya, Y. Sainz, M.A. Ortega, A.L. De Cerain, A. Monge, *Farmaco*., 53 (1998) 570.
[25] Y. Sainz, M.E. Montoya, F.J. Martinez-Crespo, M.A. Ortega, A.L. de Cerain, A. Monge, *Arzneim.-Forsch*., 49 (1999) 55.
[26] M.A. Ortega, Y. Sainz, M.E. Montoya, A.L. De Cerain, A. Monge, *Pharmazie*, 54 (1999) 24.

[27] B. Zarranz, A. Jaso, I. Aldana, A. Monge, *Bioorg. Med. Chem.*, 11 (2003) 2149-2156.
[28] A. Jaso, B. Zarranz, I. Aldana, A. Monge, *Eur. J. Med. Chem.*, 38 (2003) 791-800.
[29] A. Jaso, B. Zarranz, I. Aldana, A. Monge, *J. Med. Chem.*, 48 (2005) 2019-2025.
[30] K. Andries, P. Verhasselt, J. Guillemont, H.W.H. Gohlmann, J.M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, V. Jarlier, *Science*, 307 (2005) 223.
[31] J.C. Sacchettini, E.J. Rubin, J.S. Freundlich, *Nature Rev Microbiol.*, 6 (2008) 41.
[32] R.C. Goldman, B.E. Laughon, R.C. Reynolds, J.A. Secrist, J.A. Maddry, M.A. Guie, A.C. Poffenberger, C.A. Kwong, S. Ananthan, S. *Infect. Disord. Drug Targets.*, 7 (2007) 92.
[33] M.A. Ortega, M.E. Montoya, A. Jaso, B. Zarranz, I. Tirapu, I. Aldana, A. Monge, *Pharmazie*, 56 (2001) 205-207.
[34] M.A. Ortega, Y. Sainz, M.E. Montoya, A. Jaso, B. Zarranz, I. Aldana, A. Monge, *Arzneim.-Forsch.*, 52 (2002) 113-119.
[35] E. Vicente, R. Villar, A. Burguete, B. Solano, S. Perez-Silanes, I. Aldana, J.A. Maddry, A.J. Lenaerts, S.G. Franzblau, S.H. Cho, A. Monge, R.C. Goldman, *Antimicrob. Agents Chemother.*, 52 (2008) 3321.
[36] R. Villar, E. Vicente, B. Solano, S. Perez-Silanes, I. Aldana, J.A. Maddry, A.J. Lenaerts, S.G. Franzblau, S.H. Cho, A. Monge, R.C. Goldman, *J. Antimicrob. Chemother.*, 62 (2008) 547.
[37] E. Vicente, LM. Lima, E. Bongard, S. Charnaud, R. Villar, B. Solano, A. Burguete, S. Pérez-Silanes, I. Aldana, L. Vivas, A. Monge, *Eur. J. Med. Chem.*, 43 (2008) 1903.
[38] M.J. Haddadin, C.H. Issidorides, *Heterocycles.*, 35 (1993) 1503.
[39] M.A. Ortega, M.J. Morancho, F.J. Martinez-Crespo, Y. Sainz, M.E. Montoya, A.L. de Cerain, A. Monge, *Eur. J. Med. Chem.*, 35 (2000) 21.
[40] E. Vicente, R. Villar, B. Solano, A. Burguete, S. Ancizu, S. Pérez-Silanes, I. Aldana, A. Monge, A. An., *R. Acad. Nac. Farm.*, 73 (2007) 927-945.
[41] C. Urquiola, M. Vieites, G. Aguirre, A. Marin, B. Solano, G. Arrambide, P. Noblia, M.L. Lavaggi, M.H. Torre, M. Gonzalez, A. Monge, D.H. Gambino, H. Cerecetto, *Bioorg. Med. Chem.*, 14 (2006) 5503.
[42] K.M. Amin, M.F. Ismail, E. Noaman, D.H. Soliman, Y.A. Ammar, *Bioorg. Med. Chem.*, 14 (2006) 6917-6923.
[43] S. Ancizu, E. Moreno, B. Solano, R. Villar, A. Burguete, E. Torres, S. Pérez-Silanes, I. Aldana, A. Monge, *Bioorg. Med. Chem.*, 18 (2010) 2713-2719.
[44] P. Sanna, A. Carta, M. Loriga, S. Zanetti, L. Sechi, *Farmaco*, 54 (1999) 169.
[45] J. Kunes, M. Spulak, K. Waisser, M. Slosarek, J. Janota, *Pharmazie*, 55 (2000) 858.
[46] L.E. Seitz, W.J. Suling, R.C. Reynolds, *J. Med. Chem.*, 45 (2002) 5604.
[47] J. Guillon, R.C. Reynolds, J.M. Leger, M.A. Guie, S. Massip, P. Dallemagne, C. Jarry, *J. Enzym. Inhib. Med. Chem.*, 19 (2004) 489.

[48] U.H. Manjunatha, H. Boshoff, C.S. Dowd, L. Zhang, T.J. Albert, J.E. Norton, L. Daniels, T. Dickl, SS. Pang, C.E. Barry, *Proc. Natl. Acad. Sci. U. S. A.*, 103 (2006) 431.
[49] E. Vicente, S. Pérez-Silanes, L.M. Lima, S. Ancizu, A. Burguete, B. Solano, R. Villar, I. Aldana, A. Monge, *Bioorg & Med. Chem.,* 17 (2009) 385–389.
[50] E. Vicente, S. Charnaud, E. Bongard, R. Villar, A. Burguete, B. Solano, S. Ancizu, S. Pérez-Silanes, I. Aldana, L. Vivas, A. Monge, *Molecules.,* 13 (2008) 69.
[51] L. M. Lima, B. Zarranz, A. Marín, B. Solano, E. Vicente, S. Pérez-Silanes, I. Aldana, A. Monge, *J. Heterocycl. Chem.,* 42(2005) 1381.
[52] B. Zarranz, A. Jaso, I. Aldana, A. Monge, S. Maurel, E. Deharo, V. Jullian, M. Sauvain, *Arzneim.-Forsch.,* 55 (2005) 754.
[53] B. Zarranz, A. Jaso, L.M. Lima, I. Aldana, A. Monge, S. Maurel, M. Sauvain, *Braz. J. Pharm. Sci.,* 46 (2006) 357.
[54] B. Solano, V. Junnotula, A. Marín, R. Villar, A. Burguete, E. Vicente, S. Pérez-Silanes, I. Aldana, A. Monge, S. Dutta, U. Sarkar, K.S. Gates, *J. Med. Chem.,* 50 (2007), 5485.
[55] B. Zarranz, A. Jaso, I. Aldana, A. Monge, *Bioorg. Med. Chem.,* 12 (2004) 3711.
[56] A. Marín, L.M. Lima, B. Solano, E. Vicente, S. Pérez-Silanes, S. Maurel, M. Sauvain, I. Aldana, A. Monge, E. Deharo, *Exp. Parasitol.,* 118 (2008), 25.
[57] C.A. Benson, *Curr. Opin. Infect. Dis.,* 7 (1994) 95.
[58] L. Danelishvilli, L.E. Bermudez, *Curr. Pharm. Des.,* 9 (2003) 61.
[59] D.A. Mitchison, *Am. J. Respir. Crit. Care Med.,* 171 (2005) 699.
[60] C. Saltini, *Respir. Med.,* 100 (2006) 2085.
[61] G. Fatkenheuer, H. Taelman, P. Lepage, A. Schwenk, R. Wenzel, *Diagn. Microbiol. Infect. Dis.,* 34 (1999) 139.
[62] L. Ballell, R. Field, A.K. Duncan, R.J. Young. *Antimicrob. Agents Chemother.,* 49 (2005) 2153.
[63] Inderlied, C.B.; Kemper, C A.; Bermudez, L.E.M. *Clin. Microbiol. Rev.,* 1993, *6*, 266.
[64] L.A. Collins, S.G. Franzblau, *Antimicrob. Agents Chemother.,* 41 (1997) 1004.
[65] A. Nayyar, R. Jain, *Curr. Med. Chem.,* 12 (2005), 1873.
[66] E. Moreno, S. Ancizu, S. Pérez-Silanes, E. Torres, I. Aldana, A. Monge, *Eur. J. Med. Chem.,* 45 (2010) 4418-4426.