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Design and synthesis of novel quinoxaline derivatives as potential candidates for treatment of multidrug-resistant and latent tuberculosis

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ABSTRACT

Twenty-four quinoxaline derivatives were evaluated for their antimycobacterial activity using BacTiter-Glo microbial cell viability assay. Five compounds showed MIC values <3.1 μM and IC_{50} values <1.5 μM in primary screening and therefore, they were moved on for further evaluation. Compounds **21** and **18** stand out, showing MIC values of 1.6 μM and IC_{50} values of 0.5 and 1.0 μM, respectively. Both compounds were the most potent against three evaluated drug-resistant strains. Moreover, they exhibited intracellular activity in infected macrophages, considering log-reduction and cellular viability. In addition, compounds **16** and **21** were potent against non-replicating *Mycobacterium tuberculosis* and compound **21** was bactericidal. Therefore, quinoxaline derivatives could be considered for making further advances in the future development of antimycobacterial agents.

Tuberculosis (TB) is a chronic infection caused by *Mycobacterium tuberculosis* (*M. Tb.*), which is the second cause of death from a single infectious agent. Due to its high infectivity it is estimated that a third of the world population is a reservoir of *Mycobacterium*.

In 2000, within the Millennium Development Goals (MDG6), the United Nations proposed to halt the spread and reverse the incidence of TB by 2015. Some of these objectives have already been achieved. The TB mortality rate has decreased 45% since 1990 and more people have access to effective treatments and to early and sensitive diagnostic methods. However, epidemiological data are still alarming.[1] According to World Health Organization (WHO) data, 9 million people were infected by tuberculosis bacillus in 2013, including 1.1 million cases among people living with HIV. In 2013, 1.5 million people died due to tuberculosis infection, with this being the main cause of death among people affected by HIV. In addition, the number of infected people with multidrug-resistant TB (MDR-TB) is increasing annually. The ability of *M. Tb.* to develop resistance to drugs and the difficulties faced in the treatment of the TB-disease has facilitated the development of resistant strains. MDR-TB has been spreading rapidly in the last decades, especially in recent years; the number of individuals infected with MDR-TB tripled between 2009 and 2013, and reached 136,000 worldwide.[2]

These data indicate the urgent need to continue working in the development of new compounds against new targets and with new mechanisms of action for treating MDR-TB. It is also important to shorten the long periods of treatment because they are a common reason for treatment discontinuation on the part of the patient, and stopping the treatment too early is related with the appearance of resistant strains.[3] With this aim, our group has been working for several years on the synthesis and biological evaluation of new structures derived from quinoxalines. Quinoxaline derivatives show very interesting biological properties. Several studies have been published which justify the interest in these derivatives as antibacterial agents. As a result of our anti-tuberculosis research project, several papers have been published in which both synthesis and biological activity assessments have been described for a large number of quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives with a variety of substituents. Some of them have shown growth inhibition values of 99% and 100%.[4–9]

This indicates that these types of structures are very interesting for developing new anti-TB molecules. Furthermore, it has been reported that oxidation of the nitrogen in the quinoxaline ring

Abbreviations: TB, tuberculosis; *M. Tb.*, *Mycobacterium tuberculosis*; BTG, BacTiter-Glo microbial cell viability assay; MDG, Millennium Development Goals; BFX, benzofuroxan; NRP, non-replicating persistence; MBC, Minimal bactericidal concentration; LORA, Low Oxygen Recovery Assay; SDR, single drug-resistant; FBS, bovine serum; NA, not available; RIF, rifampin; INH, isoniazid; OFX, ofloxacin.

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provides a significant increase in the antibacterial biological activity.[10] It has also been reported that the quinoxaline 1,4-di-*N*-oxide derivatives suffer a bioreduction process under hypoxic conditions.[11] This behavior could be interesting because in the caseous core of the tuberculous granulomas there is a low concentration of oxygen where non-replicating persistence (NRP) forms of *M. Tb.* bacilli can survive.[12] These forms are thought to be the reason behind the need for long treatments and the development of tolerance to the treatment.[13]

Chalcones are precursors of flavonoids and isoflavonoids, which consist in two aromatic rings linked by a 3 carbons chain presenting an α,β-unsaturated ketone system. Chalcones exhibit a wide range of biological activities such as anticancer, anti-leishmaniasis, anti-inflammatory, anti-oxidant and anti-tuberculosis. With regard to the anti-mycobacterial activity of chalcones, there are numerous publications that justify their use in the search for new anti-TB compounds.[14,15]

Fluoroquinolones are an important group of broad spectrum antibiotics and they are present in many molecules with high anti-tuberculosis activity such as gatifloxacin and moxifloxacin, which are under development in clinical trials.[16]

With the aim of developing new antitubercular drug candidates, we have synthesized and evaluated 24 quinoxaline derivatives. The design was based on the molecular hybridization of the quinoxaline 1,4-di-*N*-oxide with chalcone and fluoroquinolones scaffolds.[17]

We report the design and synthesis of the quinoxaline 1,4-di-*N*-oxide derivatives **1**–**24**. The biological evaluation of the compounds included a *M. Tb.* $H_{37}Rv$ dose–response assay (primary screening) in which the IC_{50}, IC_{90} and MIC against *M. Tb.* were determined. The most active compounds were moved on to a more advanced testing stage for antimycobacterial activity. These assays included the evaluation against single drug-resistant (SDR) strains of *M. Tb.*, Minimal bactericidal concentration (MBC), Low Oxygen Recovery Assay (LORA) and intracellular drug activity. All results are reported and structure–activity relationships (SARs) are discussed.

The quinoxaline 1,4-di-*N*-oxide derivatives were synthesized through the synthetic process illustrated in Scheme 1.

The starting benzofuroxans, were commercially available or were obtained by previously described methods.[18] The synthesis of quinoxaline 1,4-di-*N*-oxide intermediates were carried out by a variation of the Beirut reaction following the procedure described in the literature.[19–21]

The 10 novel chalcone analogs (**1–10**) were prepared by Claisen–Schmidt condensation.[22] Benzaldehyde, 5-nitro-2-furaldehyde and 5-nitro-2-thiophenecarboxylaldehyde were used as the starting aldehyde. The corresponding quinoxaline 1,4-di-*N*-oxide previously synthesized was used as the ketone. The new derivatives **1**–**10** were unsubstituted or substituted in R^1 or R^2 positions by methyl moiety as electron-releasing groups and by chlorine moiety as electron-withdrawing groups.

The methodologies for the synthesis of compounds **11**–**15**, **17**–**20** and **22**–**24** were previously described by our group.[19] Compounds **16** and **21** were obtained by nucleophilic aromatic substitution of chlorine or fluorine linked to R^1/R^2 substituent on the quinoxaline ring. Morpholine and 1-(4-fluorophenyl)piperazine were used as the nucleophilic amine. Compound **16** was obtained by reflux using *N*,*N*-DMF and compound **21** was obtained using DBU as base.

The identification of novel interesting compounds begins with a primary screening. This initial dose response assay evaluates the ability of compounds to inhibit the *M. Tb.* replication. The IC_{90}, IC_{50} and MIC of the 24 quinoxaline 1,4-di-*N*-oxide derivatives were determined against *M. Tb.* $H_{37}Rv$ (ATCC 27294) using BacTiter-Glo (BTG) microbial cell viability assay.

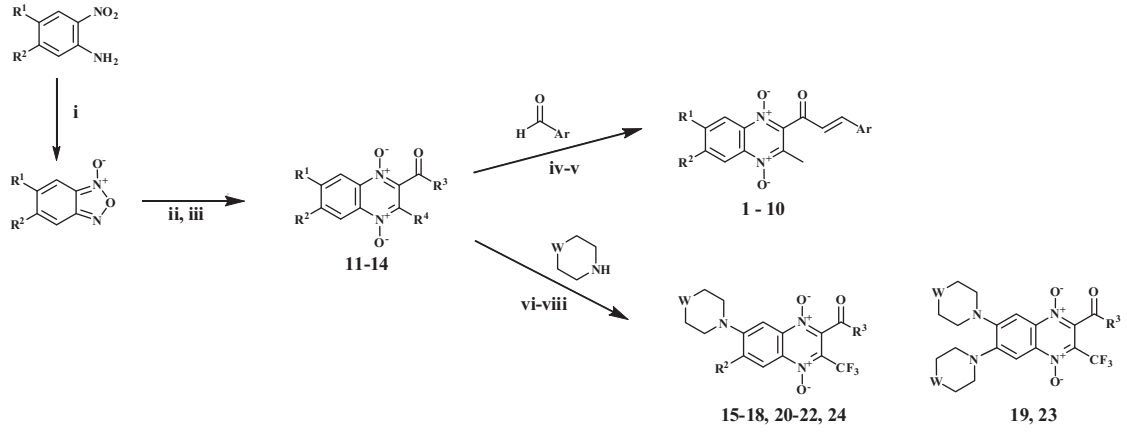
The structure and anti-tuberculosis activity data of the 24 compounds are shown in Table 1. Nine out of twenty-four evaluated compounds exhibited MIC values $\leqslant$6.2 μM and IC_{50} values <3.7 μM. Compounds **3**, **16**, **18**, **21** and **24** were the most active, showing MIC values among 1.6–3.1 μM and IC_{50} values among 0.5–1.5 μM. These MIC and IC_{50} values were lower than those of the second-line reference drugs cycloserine and pyrimethamine, and also by the first-line drug ethambutol. It is worth noting that 4 out of the 5 most active compounds (**16**, **18**, **21** and **24**) are fluoroquinolone analogs while compound **3** belongs to chalcone analogs.

In comparison with fluoroquinolones, the presence of an electron-withdrawing substituent (fluorine or chlorine) in only one of the R^1 and R^2 positions in the quinoxaline ring is important for biological activity. This behavior is observed comparing the biological results obtained for compounds **3** versus **1** and **2**, **6** versus **5**, **9** versus **7**, **8** and **10**, **13** versus **14**, **18** versus **19** and **24** versus **23**.

With respect to R^4 substitutions, 8 out of 9 most active compounds present a trifluoromethyl group. The substitution of hydrogen by its bioisostere fluorine is a widely used strategy in the search for new compounds. The presence of fluorine atoms can change and radically modulate the physicochemical properties of organic compounds modifying its biological behavior.[23] However, it is not possible to carry out a SAR study due to the limited structural variability in this position.

With regard to R^3 position, it seems to be less important for the anti-mycobacterial activity.

With regard to *N*-oxide groups of the quinoxaline ring, these groups seem to be essential for the activity as we can see



Scheme 1. General synthesis of quinoxaline 1,4-di-*N*-oxide derivatives. Reagents and conditions: (i) *N*,*N*-DMF, NaOCl, low temperature; (ii) ethanolamine, $CaCl_2$; (iii) microwave assisted synthesis; (iv) NaOH, MeOH, ice bath; (v) NaOH, MeOH, freezing bath; (vi) *N*,*N*-DMF, reflux; (vii) CH_3CN, Et_3N, rt; (viii) CH_3CN, DBU, 50 °C.