Bioorganic & Medicinal Chemistry Letters 26 (2016) 4146–4153

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, 3D-QSAR analysis and biological evaluation of quinoxaline 1,4-di-*N*-oxide derivatives as antituberculosis agents

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ARTICLE INFO

Article history:
Received 12 September 2015
Revised 11 January 2016
Accepted 22 January 2016
Available online 22 January 2016

Keywords:
Quinoxaline 1,4-di-*N*-oxide
Tuberculosis
Antimycobacterial activity
3D-QSAR
CoMFA
CoMSIA

ABSTRACT

A series of quinoxaline 1,4-di-*N*-oxide derivatives variously substituted at C-2 position were synthesized and evaluated for in vitro antimycobacterial activity. Seventeen compounds exhibited potential activity (MIC ⩽6.25 μg/mL) against *Mycobacterium tuberculosis* (H37Rv), in particular the compounds **3d** and **3j** having an MIC value of 0.39 μg/mL. None of the compounds exhibited cytotoxicity when using an MTT assay in VERO cells. To further investigate the structure–activity relationship, CoMFA (q^2 = 0.507, r^2 = 0.923) and CoMSIA (q^2 = 0.665, r^2 = 0.977) models were performed on the basis of antimycobacterial activity data. The 3D-QSAR study of these compounds can provide useful information for further rational design of novel quinoxaline 1,4-di-*N*-oxides for treatment of tuberculosis.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), remains a major global health problem and ranks as the second leading cause of death from an infectious disease worldwide. According to the latest report of World Health Organization (WHO), there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths.[1] The standard treatment for TB is generally comprised of several effective anti-TB drugs such as isoniazid, rifampicin, ethambutol, and pyrazinamide.[2,3] However, the emergence of new Mtb strains as like multi drug resistant (MDR) and extensively drug resistant (XDR) TB severely threatens tuberculosis control.[4,5] So it becomes necessary to discover newer anti-TB agents which can target a novel pathway so as to be effective against Mtb-resistant strains.[6–8]

Quinoxaline-1,4-di-*N*-oxide derivatives have attracted continuing interest over the years because of their varied biological activities, including antibacterial, antiviral, antifungal, antiprotozoal,[9–12] anticancer and hypoxia-selective properties.[13–18] Recently, a wide range of quinoxaline-1,4-di-*N*-oxide derivatives with variable substituents at different positions and their anti-TB activities have been reported and many of these compounds possess excellent anti-TB activity.[19–27] The studies have revealed that the antimycobacterial activity of the synthesized compounds significantly depends on the presence of the two *N*-oxide groups. The presence of electronegative group such as chlorine or fluorine atom as well as trifluoromethyl group at C-6 or C-7 position on the quinoxaline ring was favorable for higher antimycobacterial activity against *Mtb* H 37Rv.[11,21] In addition, quinoxaline-1,4-di-*N*-oxide derivatives with different C-2 substituents displayed distinct antimycobacterial activity suggested that C-2 position substituent play a crucial role in their antimycobacterial activities. For example, a series of quinoxaline-1,4-di-*N*-oxide derivatives bearing ester groups at C-2 position exhibited significant antimycobacterial activity (MIC 0.10–6.25 μg/ml),[22] while the analogs with amide groups at C-2 position exhibited weak activities.[24]

In this study, aim to optimization of the structure at C-2 position of the quinoxaline ring, a series of new quinoxaline 1,4-di-*N*-oxide derivatives were synthesized and subjected to preliminary screening for their anti-TB activity against *Mtb* H37Rv strain in order to discover potential anti-TB agents. Scheme 1 depicts the synthetic route of new quinoxaline-1,4-di-*N*-oxide derivatives. Compound **1** was synthesized by treating benzofuroxane with acetyl acetone as previously described.[21] Bromination of **1** with bromine in glacial acetic acid to yield the key intermediates compound **2**, 2-bromoacetyl-3-methylquinoxaline-1,4-di-*N*-oxide. Then compounds **2a**–**2j** were obtained by the nucleophilic substitution reaction of **2** and the corresponding amino derivatives. Similarly, various mercapto compounds were allowed to react with the

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http://dx.doi.org/10.1016/j.bmcl.2016.01.066
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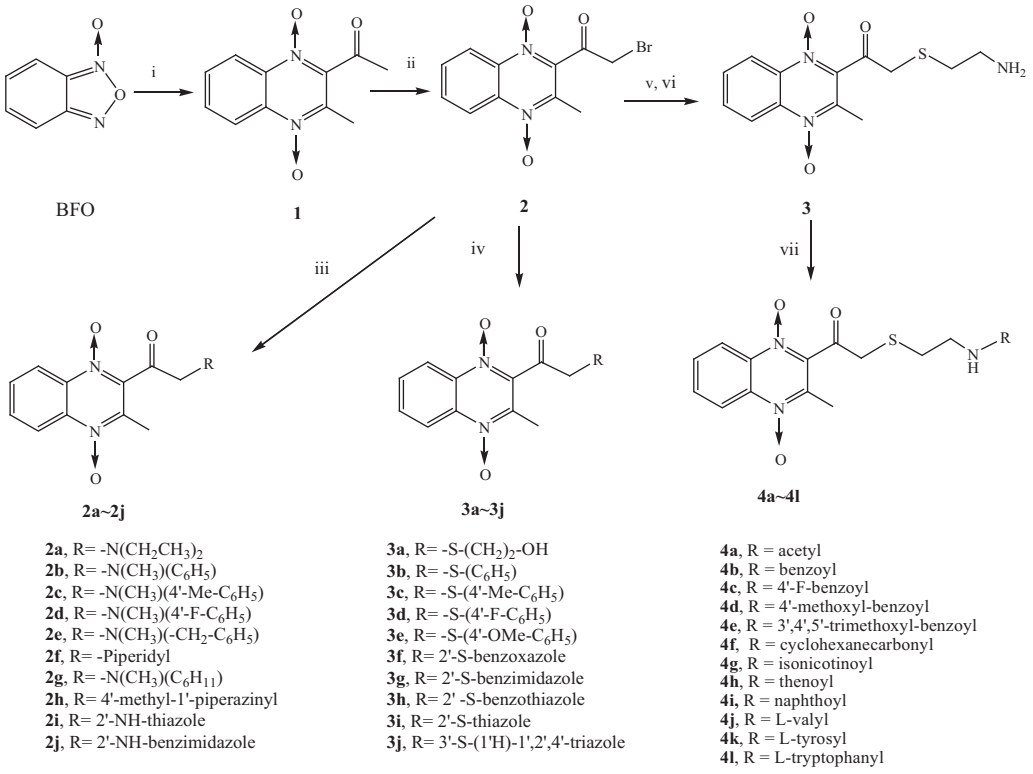

Scheme 1. Reagents and conditions: (i) acetylacetone, CH_2Cl_2, TEA, 25 °C, 12 h, 80%; (ii) Br_2, glacial acetic acid, 60 °C, 8 h, 62%; (iii) RNH-, EtOAc, TEA, 25 °C, 1 h, 65–82%; (iv) R-SH, DMF, 25 °C, 1 h, 82–92%; (v) *N*-(Boc)-2-aminoethanethiol, DMF, TEA, 25 °C, 1 h, 90%; (vi) HCl, EtOAc, 4 h, 84%; (vii) **4a–4i**: RCOCl, CH_2Cl_2, 0–25 °C, 1 h, 80–92%; **4j–4l**: (a) *N*-(Boc)-amino acid, DCC/NHS, DMAP, CH_2Cl_2, 25 °C, 12 h, 70%; (b) TFA, EtOAc, 4 h, 80%.

intermediate **2** in DMF to give compounds **3b–3j** with excellent yields ranged from 82% to 92%. For the synthesis of compounds **4a–4l**, the intermediate **2** was converted into **3** by reaction with *N*-(Boc)-2-aminoethanethiol and deprotection of Boc group with hydrochloric acid in ethyl acetate. After that, **4a–4i** were obtained by reacting **3** with various acyl chloride in dichloromethane. **4j–4l** were synthesized via two steps including coupling of **3** with different *N*-protected amino acids by using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) and then deprotection of Boc group with trifluoroacetic acid in ethyl acetate. All the compounds were chemically characterized by LC–MS, melting point, infrared (IR) and nuclear magnetic resonance (^{1}H NMR) spectra, as well as element analysis.

The newly synthesized quinoxaline-1,4-di-*N*-oxide derivatives were evaluated for their antimycobacterial activity against *Mtb* H37Rv strain using the Microplate Alamar Blue Assay (MABA) with isoniazid as the positive control. The theoretical lipophilicity (*C*log*P*) of the compounds were calculated by SYBYL-X 2.0 (Tripos, Inc.) and compared with their anti-TB activity as shown in Table 1. Compounds **2a–2j**, bearing amino substituents at C-2 position showed little antimycobacterial activity (MIC >6.25 μg/mL) against *Mtb* H37Rv strain except **2d**, which exhibited an MIC value of 6.25 μg/mL. Aliphatic amines attached at C-2 side chain were less active than aromatic amines substituents, such as **2a**, **2e**, **2g** and **2h**, exhibited no activity (MIC = 50 μg/mL). Compounds **3b–3j** displayed promising antimycobacterial activity (MIC ⩽6.25 μg/mL) against *Mtb* H37Rv, among then compounds **3d** and **3j** exhibited most potent anti-TB activity (MIC = 0.39 μg/mL). It seemed that thioether substituents of **3b–3h** are responsible for their higher lipophilicity and significantly enhance their antimycobacterial activities. When an amino or hydroxyethyl group was attached to sulfur, such as compounds **3** and **3a** (*C*log*P* = −1.84 and −1.90), the antimycobacterial activity was strongly reduced (MIC >50 μg/mL). It was worth noting that compounds **3i** and **3j** also exhibited good anti-TB potency, whereas they have a lower lipophilicity (*C*log*P* <0). This result indicated that the steric and electrostatic effect, as well as hydrogen bond of the side chain at C2 position may also play an important role in its antimycobacterial activity. Compounds **4a–4l** which also contain a thioether group at C-2 position but have a longer alkyl chain than **3a–3j** exhibited moderate antimycobacterial activity. Compound **4c**, containing 4-fluorobenzamide moiety, exhibited MIC value of 1.56 μg/mL. It seemed that 4-fluorophenyl substituents of **2d**, **3d** and **4c** were favorable for their antimycobacterial activity, which may due to the increased lipophilicity and enhanced permeability into cell membrane. When amino acid was introduced into the C-2 side chain, the antimycobacterial activity was impaired. For example, compounds **4j–4l**, containing L-valyl, L-tyrosyl and L-tryptophanyl moiety, exhibited MIC of 25, 25 and 6.25 μg/mL, respectively.

The compounds which exhibited promising antimycobacterial activity against *Mtb* H37Rv (MIC ⩽6.25 μg/mL) were tested for in vitro cytotoxicity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against VERO cell line. The IC_{50} and the selectivity index (SI = CC_{50}/MIC) was calculated and list in Table 1. The compounds showed less toxicity against VERO cells (SI >10).

In order to better understand the structure activity relationship, CoMFA and CoMSIA models were established based on the antimycobacterial activity data of the newly synthesized quinoxaline-1, 4-di-*N*-oxide derivatives. In this study, the MIC values were converted to *p*MIC values according to the following formula:

$$p\mathrm{MIC} = -\lg(\mathrm{MIC} \times 10^{-3})/(\text{molecular weight})$$