European Journal of Medicinal Chemistry 64 (2013) 239–251

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Anti-tubercular agents. Part 7: A new class of diarylpyrrole–oxazolidinone conjugates as antimycobacterial agents

Ahmed Kamal [a,*], P. Swapna [a], Rajesh V.C.R.N.C. Shetti [a], Anver Basha Shaik [a], M.P. Narasimha Rao [a], Farheen Sultana [a], Inshad Ali Khan [b], Sandeep Sharma [b], Nitin Pal Kalia [b], Sunil Kumar [b], Bagul Chandrakant [c]

[a] Medicinal Chemistry and Pharmacology, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India
[b] Clinical Microbiology Division, CSIR-Indian Institute of Integrative Medicine, Jammu 180001, India
[c] Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Hyderabad 500037, India

ARTICLE INFO

Article history:
Received 24 September 2012
Received in revised form
10 March 2013
Accepted 13 March 2013
Available online 22 March 2013

Keywords:
Anti-tubercular agents
Mycobacterium tuberculosis
MDR- and XDR-TB
Antibacterial
Oxazolidinones

ABSTRACT

In an effort to discover new anti-tubercular agents, a series of new diarylpyrrole–oxazolidinone conjugates have been designed and synthesized. The anti-tubercular activity of these new conjugates (**4a**–**n** and **5a**–**d**) against *Mycobacterium tuberculosis* $H_{37}Rv$ and drug resistance strains such as *M. tuberculosis* Rif^R and *M. tuberculosis* XDR are discussed, wherein compound **4i** has been found to be the most potent amongst the series. MTT assay was performed on the active conjugates of the series (**4b**–**f**, **4i** and **5c**) against mouse macrophage (J-774) cells to evaluate cytotoxic effects and selective index values. In addition, these conjugates (**4a**–**n** and **5a**–**d**) are also tested against a panel of Gram-positive and Gram-negative bacterial strains. The docking studies have been carried out to provide some insight into the mechanism of action for this class of compounds.

1. Introduction

Despite the availability of efficacious treatment and diagnosis for tuberculosis (TB), caused by the bacillus *Mycobacterium tuberculosis* (MTB), TB still remains a major health problem. In 1993, the World Health Organization (WHO) declared TB as a global emergency [1]; at that time when an estimated 7–8 million cases and 1.3–1.8 million deaths occurred each year. In 2010, there were an estimated 8.8 million cases of TB, 1.1 million deaths among HIV-negative cases of TB and an additional 0.35 million deaths among people who were HIV-positive [2]. Inspite of improved sanitation, better lifestyle and the widespread use of the bovine Calmette–Guerin vaccine, MTB still persists as a leading bacterial infection problem [3,4]. The efficacy of currently available drugs used in standard tuberculosis treatment regimen is seriously limited by several factors: including HIV-1 infection, long treatment regimens and multiple drug treatment regimens [5,6]. Moreover, the increasing emergences of drug resistant TB, especially multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) are particularly alarming [7,8]. There has been an increase in the number of TB patients diagnosed with MDR-TB in the last five years.

For the first time in 40 years, there is a coordinated portfolio of promising new drugs on the horizon. There are 10 new or repurposed TB drugs in trials, which have the potential to shorten the treatment of drug-susceptible TB and to improve the treatment of multidrug-resistant TB. Some notable examples that are under different stages of clinical trials include gatifloxacin (**1a**), moxifloxacin (**1b**), levofloxacin (**1c**) and linezolid (**2a**) as shown in Fig. 1 [1,9]. Linezolid is the first and only oxazolidinone which is effective for the treatment of Gram-positive bacteria including first-line drug resistance infections in humans and recently has been suggested as an alternative treatment for patients infected with *M. tuberculosis* isolates [10–12]. The oxazolidinones, represent a new class of synthetic antibacterial agents that emerged in the last four decades having potent activity against multidrug-resistant Gram-positive bacteria. Oxazolidinones target the bacterial protein synthesis prior to the chain initiation step by binding to the 23S rRNA of 50S ribosomal subunit and interfering with the initiator fMET-rRNA binding to the P-site of the ribosomal peptidyltransferase center [13,14]. Many efforts have been made on developing new

* Corresponding author. Tel.: +91 40 27193157; fax: +91 40 27193189.
E-mail address: ahmedkamal@iict.res.in (A. Kamal).

0223-5234/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.ejmech.2013.03.027

oxazolidinones toward extending the spectrum of activity and reducing the toxic effects. Recent studies have shown that the conversion of the acetamide group of linezolid to the triazolyl, example PH-027 (**2b**) as shown in Fig. 1 could restore its antibacterial activities [15–18]. On the other hand, diarylpyrroles were first reported by Biava and coworkers as antimycobacterials and amongst them BM212 (**3**) is the most potent derivative which showed promising activity toward several strains of MTB [19,20].

In an effort to global fight against tuberculosis, from our laboratory many series of compounds have been designed and synthesized that exhibit promising antimycobacterial activity and some are undergoing detailed investigations [21–23]. In the present investigation we have introduced a new class of diarylpyrrole–oxazolidinone conjugates as potential antimycobacterial agents. In this context the diarylpyrrole group has been attached to (*R*)-3-(3-fluoro-4-(piperazin-1-yl)phenyl)-5-((4-aryl/heteroaryl-1*H*-1,2,3-triazol-1-yl)methyl)oxazolidin-2-one at the distal piperazine N–H position in order to identify new molecules with improved antimycobacterial activity. Additionally, phenyl, *m*-tolyl, 2-pyridyl and 1*H*-methyl-5-imidazolyl groups have been introduced at the 4th position of the 1*H*-1,2,3-triazole ring. Attempts have also been made to correlate the structure to antimycobacterial activity of these compounds.

2. Chemistry

The synthetic pathways employed for the generation of different products in the series are described in Schemes 1 and 2. Various substituted diarylpyrroles have been prepared from the corresponding 1,4-diketones and anilines. Synthesis of diarylpyrroles is depicted in Scheme 1, wherein the reaction of a suitable benzaldehyde with methyl vinyl ketone, under Stetter conditions to yield 1,4-diketones (**8a**–**f**) [20], followed by modified Paal–Knorr condensation procedure using $Gd(OTf)_3$ as catalyst. In the Paal–Knorr condensation, the intermediates (**8a**–**f**) were treated with the appropriate amines in the presence of $Gd(OTf)_3$ for cyclization to yield the required diaryl pyrroles (**9a**–**h**) in good yields.

The key intermediate (*S*)-*N*-[[3-(3-fluoro-4-(piperzinyl)phenyl)-2-oxo-5-oxazolidinyl]methyl]-azide (**10**) has been synthesized as previously described [24,25]. The union of these two fragments (**9a**–**h**) and **10** was achieved by employing Mannich reaction between them to produce **11a**–**h** (Table 1). The azido compounds (**11a**–**h**), thus obtained were then converted to diarylpyrrole–triazolyloxazolidinone conjugates (**4a**–**n**) by click chemistry using aryl/heteroaryl acetylenes in the presence of copper sulfate and catalytic amount of sodium ascorbate in *t*-BuOH and water (3:1) as illustrated in Scheme 2 [26,27].

In addition, the diarylpyrrole–oxazolidinone conjugates (**5a**–**d**) were prepared as outlined in Scheme 2. The amino compounds **12a**–**d** were obtained from the reduction of azide group of **11a**, **11b**, **11f** and **11h** in the presence of ammonium formate and catalytic amount of zinc dust in methanol (Table 1). On acylation of the amino compounds (**12a**–**d**) in the presence of acetyl chloride in dry CH_2Cl_2 employing triethylamine as a base, the corresponding acetamides were obtained (**5a**–**d**).

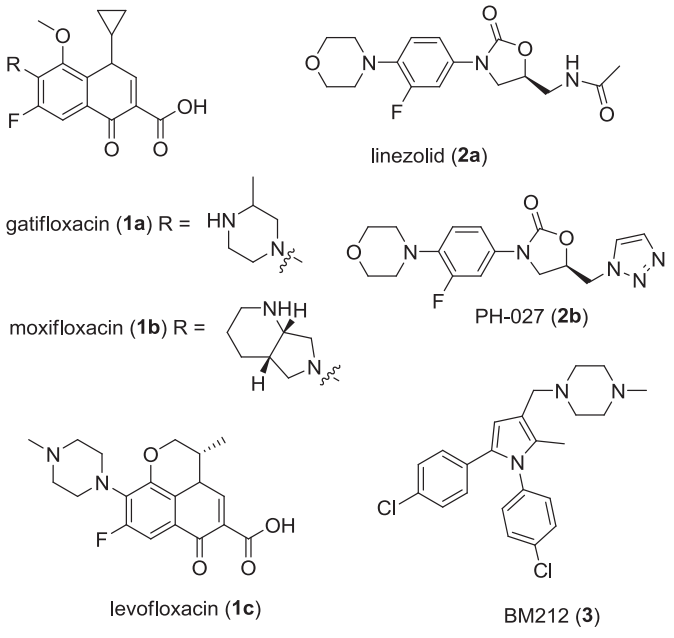

Fig. 1. Potent anti-tubercular agents and antibacterial agents.

3. Results and discussion

3.1. Antimycobacterial activity

These newly synthesized diarylpyrrole–oxazolidinones (**4a**–**n** and **5a**–**d**) were evaluated for their antimycobacterial activity against *M. tuberculosis* isolates using the microplate dilution assay at 1–16 μg/mL concentrations. The drugs in clinical use rifampicin and linezolid were used as reference compounds. The *in vitro* test results for this new class of compounds are outlined in Table 2 as minimum inhibitory concentration (MIC) and the activity ranges from 2 to >6.25 μg/mL. Moreover, these compounds have also been screened against *M. tuberculosis* Rif^R and *M. tuberculosis* XDR. At primary screening, the compounds **4a**–**d**, **4e**–**i** and **5a**–**c** showed significant inhibition against MTB. Amongst the evaluated conjugates, the conjugate (5*R*)-3-(3-fluoro-4-(4-((1-(4-fluorophenyl)-2-methyl-5-*o*-tolyl-1*H*-pyrrol-3-yl)methyl)piperazin-1-yl)phen-yl)-5-((4-(1-methyl-1*H*-imidazol-5-yl)-1*H*-1,2,3-triazol-1-yl)methyl) oxazolidin-2-one (**4i**) was found to be more active against MTB (2 μg/mL) compared to the other compounds in this series. Moreover, compound **4i** exhibited very good activity against the resistant strains such as, rifampicin resistant MTB (4 μg/mL) and XDR-TB (8 μg/mL).

In the structure activity relationship (SAR) studies, some interesting trends have been observed in these conjugates. In the substituted triazole series, the conjugates having pyridyl group (**4a**–**e**) and 1-methyl-1*H*-imidazol-5-yl group (**4f**–**i**) at 4-position of triazole ring have shown promising activity (2–16 μg/mL) compared to the compounds having phenyl/*m*-tolyl group at 4-position of triazole ring (**4j**–**n**). This observation indicates the importance of the presence of 2-pyridyl/1-methyl-1*H*-imidazol-5-yl group (**4f**–**i**) at 4-position of the triazole ring which is playing key role in exhibiting the activity against *Mycobacterium*. Compounds **5a**–**d** that possess a C5-acetamide group as in case of linezolid also showed moderate activity against *M. tuberculosis* (4–16 μg/mL).

3.2. Cytotoxicity assay

As described in experimental procedure, the maximum tolerated test (MTT) was performed to evaluate the *in vitro* cytotoxicity of the promising compounds (**4b**–**f**, **4i** and **5c**) against mouse macrophage (J-774) cell lines. These compounds were not cytotoxic as indicated by their IC_{50} values. The IC_{50} and selective index (SI) values of these compounds are shown in Table 3. The good selectivity index values for these compounds indicate its potential usefulness in the drug development for tuberculosis.