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Research paper

Design, synthesis and biological evaluation of novel azaspiro analogs of linezolid as antibacterial and antitubercular agents

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ABSTRACT

The design, synthesis and antimicrobial evaluation of a novel series of azaspiro analogues of linezolid (**1**) have been described. Linezolid comprises of a morpholine ring which is known for its metabolism-related liabilities. Therefore, the key modification made in the linezolid structure was the replacement of morpholine moiety with its bioisostere, 2-oxa-6-azaspiro[3.3]heptane. Furthermore, the replacement of *N*-acetyl terminal of **1** with various aromatic or aliphatic functionalities was carried out. The title compounds were evaluated against a panel of Gram-positive and Gram-negative bacteria and *Mycobacterium tuberculosis*. Subsequent structure-activity relationship (SAR) studies identified several compounds with mixed antibacterial and antitubercular profiles. Compound **22** (IC_{50} 0.72, 0.51, 0.88, 0.49 μg/mL for *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, respectively) exhibited similar antibacterial profile as **1**. The *N*-acetyl derivative **18** was similar to **1** in antitubercular profile. Thus, the present study successfully demonstrated the use of azaspiro substructure in the medicinal chemistry of antibacterial and antitubercular agents.

1. Introduction

The bacterial resistance to established antimicrobial drugs has become a critical global problem. Infections caused by multidrug-resistant Gram-positive cocci, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PNSSP), etc., have emerged as major public health concern [1]. The number of effective antibiotics has reduced drastically, because microorganisms are able to create new mechanisms of resistance and rapidly spread the genes encoding them via mobile genetic elements, mostly plasmids and integrons [2]. Similarly, tuberculosis (TB) also remains a major global health challenge and has been declared as public health emergency by the World Health Organization (WHO) [3]. TB affects one-third of the world population. About 9 million new cases and 1.1 million TB-related deaths were reported in 2013 [3]. Multidrug-resistant strains of *Mycobacterium tuberculosis* (Mtb), the causative agent of TB (MDRTB) and extremely drug-resistant TB (XDRTB) have emerged [4,5]. There is an extremely urgent need of new antitubercular drugs which can tackle the TB menace.

As a prerequisite, newer antitubercular drugs should be active against the drug-resistant forms of Mtb. This implies that they must act preferably on molecular targets different than those of the current drugs. Importantly, such drugs need to be more active against persistent Mtb, which may lead to further reduction in the treatment duration. The treatment of TB is complicated by the tendency of its etiological agent, predominantly Mtb, to adopt a nonreplicating persistent state [6–8].

Linezolid (**1**, Zyvox®, Fig. 1), a newer generation synthetic

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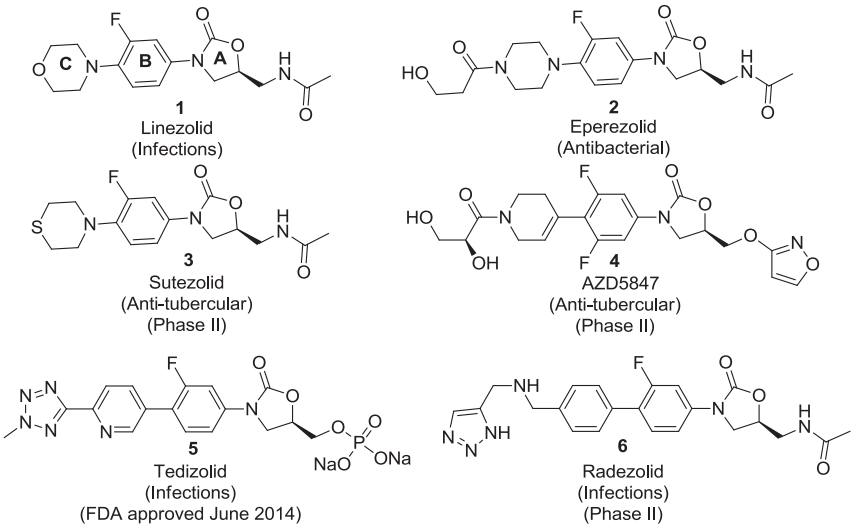

Fig. 1. Structures of approved and developmental oxazolidinone anti-bacterial.

antibiotic from oxazolidinone class, was developed by a team at Pharmacia and Upjohn Company (now part of Pfizer) [9]. It was approved in 2000 for the treatment of serious infections caused by Gram-positive bacteria resistant to several other antibiotics such as MRSA, VRE (responsible for soft tissue infections and PNSSN) [10]. Currently, **1** and related compounds are under clinical investigation for the treatment of TB (Fig. 1) [11]. Recently, cytoxazone-linezolid hybrids were shown to induce apoptosis and senescence in DU145 prostate cancer cell line [12].

The mechanism of action of **1** involves interaction with 50S A-site pocket at the peptidyl transferase center (PTC) of the bacterial ribosome, which overlaps with the aminoacyl moiety of an A-site bound tRNA, thereby inhibiting overall protein synthesis [12,13]. However, in some cases, linezolid-resistant *Staphylococcus aureus* and *Enterococci* were found among the hospital isolates [14,15]. Toxic effects of linezolid on prolonged use include reversible myelosuppression leading to anaemia, leucopenia and thrombocytopaenia. In addition to this, **1** has been shown to inhibit monoamine oxidase (MAO) enzymes, potentially leading to drug-drug interactions with adrenergic and serotonergic agents [2]. Overall, newer and potent antibiotics are expected to possess: i) extended spectrum of antibacterial activity covering fastidious Gram-negative organisms; ii) activity against linezolid-resistant strains; iii) improved safety profile leading to circumvention or at least minimization of myelosuppression and iv) activity against drug-resistant forms of Mtb.

In the present investigation, bioisosteric modifications of **1** were tried in order to develop potential new treatment against drug-resistant forms of bacteria, including Mtb, with reduced toxicity profiles. Here, replacement of the morpholine ring in **1** with 2-oxa-6-azaspiro[3.3]heptane was attempted. Morpholine is one of the common privileged structures found in several drugs such as linezolid, timolol, gefitinib, moracizine, nimorazole, emorfazone, etc. It is often used to raise aqueous solubility of drugs.

However, morpholine ring is regularly a target of the oxidative metabolism by non-cytochrome P450 (CYP) enzyme systems. This is evident from the oxidative metabolites reported for at least eight marketed drugs containing morpholine [16–18]. The 2-oxa-6-azaspiro[3.3]heptanes, a bioisostere of morpholine (Fig. 2) was selected for combating the oxidative metabolism issues associated with morpholine. The azaspiro analogue of morpholine, 2-oxa-6-azaspiro[3.3]heptanes, is better due to its improved chemical

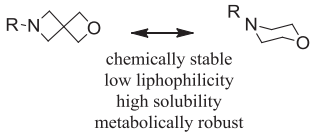

Fig. 2. 2-Oxa-6-azaspiro[3.3]heptanes, a bioisostere of morpholine.

stability, lower lipophilicity, higher solubility and metabolic robustness over morpholine [19]. The present study documents a systematic structure-activity relationship (SAR) investigation of the azaspiro analogue of **1** (compound **18**, Tables 1 and 2) leading to potent antibacterial and antitubercular agents.

2. Results and discussion

2.1. Chemistry

The azaspiro intermediates **9** and **10** were synthesized by reported method [20] in good yield. Condensation of **10** with 3,4-difluoronitrobenzene (**11**) under refluxing conditions in presence of DIPEA in ACN led to compound **12** which was subjected to catalytic hydrogenation to yield substituted aniline **13**. It was further reacted with commercially available **14** to afford secondary alcohol **15**. Use of DIPEA in DMF at 120 °C resulted in moderate yield compared to other bases and polar solvents (data not shown). Further, **15** was cyclized to oxazolidinone **16** using CDI, followed by deprotection of the pthalimide-protected 5-methylamino group using hydrazine hydrate to get alkylamine compound **17**.

The systematic exploration of the SAR at the 5-methylamino group of **17** (amide, sulfonamide, urea and thiourea derivatives) yielded a library of title compounds (see Supporting Information section) on reaction with substituted acid chlorides, sulphonyl chlorides, isocyanates and thioisocyanates, respectively, at 0–5 °C for 2 h. The crude products were purified using flash chromatography (2–5% MeOH in DCM) to afford title compounds **18**–**42** (Scheme 1). Compound **45** was synthesized by condensing commercially available intermediates **43** and **44** at 100 °C in presence of 2 M Na_2CO_3, $PdCl_2$ (dppf)-CH_2Cl_2 adduct in 1,4-dioxane (Scheme 2).

Further, we have synthesized novel azaspiro compound **52** which was central spiro ring analogue of **1** (Scheme 3). Compound