European Journal of Medicinal Chemistry 156 (2018) 580–586

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Design, synthesis and antimicrobial evaluation of propylene-tethered ciprofloxacin-isatin hybrids

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ARTICLE INFO

Article history:
Received 30 January 2018
Received in revised form
7 May 2018
Accepted 9 July 2018

Keywords:
Ciprofloxacin
Isatin
Hybrids
Antimicrobial
Antibacterial
Antimycobacterial
Structure-activity relationship

ABSTRACT

Twelve novel propylene-tethered ciprofloxacin-isatin hybrids **3a-f** and **4a-f** were designed, synthesized and characterized by MS, HRMS, ^{1}H NMR and ^{13}C NMR. All hybrids were evaluated for their *in vitro* antimicrobial activities against representative Gram-positive, Gram-negative and mycobacterial pathogens, cytotoxicity in VERO cell line as well as metabolic stability and *in vivo* pharmacokinetic (PK) properties. The preliminary results indicated that all mono-isatin-ciprofloxacin hybrids exhibited excellent antibacterial activities with MIC ranging from ≤0.03 to 0.5 μg/mL against most of the tested strains. In particular, ciprofloxacin-isatin hybrid **3d** was highly potent against all tested Gram-positive and Gram-negative strains including clinically important drug-resistant pathogens, which was comparable to or more potent than the parent ciprofloxacin and reference levofloxacin. Whereas, conjugate **3b** (MIC: 0.10 and 0.5 μg/mL) was 4- and 8-fold more active than ciprofloxacin (MIC: 0.78 μg/mL) and rifampicin (MIC: 0.39 μg/mL) against MTB H_{37}Rv, and 4->256 times more potent than the three references ciprofloxacin (MIC: 2.0 μg/mL), rifampicin (MIC: 32 μg/mL) and isoniazid (>128 μg/mL) against MDR-TB. Both hybrid **3b** and **3d** with low cytotoxicity (CC_{50}: 64 and 256 μg/mL) also showed acceptable metabolic stability and *in vivo* PK properties, could act as leads for further optimization.

1. Introduction

Bacterial infections, which are caused by Gram-positive, Gram-negative and mycobacterial pathogens, are responsible for the majority of hospital-acquired infections, and lead to extensive mortality and burden on global healthcare systems [1,2]. It's estimated that infectious diseases result in around 10 million deaths every year (over 15% of all deaths), and tuberculosis (TB) is the ninth leading cause of death throughout the world and the leading cause from a single infectious agent according to the latest World Health Organization (WHO) report [3,4]. The emergency and a wide spread of drug-resistant organisms such as methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE), vancomycin-resistant *S. aureus* (VRSA), extended-spectrum β-lactamase (ESBL)-producing *E. coli* and drug-resistant TB (DR-TB) has already increased up to alarming level in the recent decades and is associated with considerable mortality [3,4]. Thus, it's imperative to develop novel antimicrobial agents active against both drug-sensitive and drug-resistant bacterial infections.

Fluoroquinolones (**FQs**), are a family of synthetic broad spectrum antibiotics, and are the second widest used antibiotics in clinical practice for various bacterial infections including upper and lower respiratory infections, and their value and role in the treatment of bacterial infections continue to expand [1]. **FQs** predominately act by binding two type II bacterial topoisomerase enzymes, DNA gyrase (main target for Gram-negative bacteria) and topoisomerase IV (primary target for Gram-negative bacteria) [5,6], and DNA gyrase is deemed as the only type II topoisomerase present in MTB and is thus the only target for **FQs** action [7,8]. Besides their typical antibacterial activities, **FQs** also demonstrated various atypical biological properties such as antimalarial [9,10], antitumor [11] and anti-TB activities [12–15], play a pivotal role in new drug discovery.

However, as other antimicrobial agents, the resistance of pathogens to fluoroquinolones develops rapidly and spreads widely mainly attribute to the long-term, broad, inappropriate use and even abuse, makes **FQs** more and more ineffective. Therefore, enhancing the potency of fluoroquinolones has become

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https://doi.org/10.1016/j.ejmech.2018.07.025
0223-5234/

increasingly urgent.

Isatin is an endogenous compound identified in many organisms, endows with various biological properties such as antibacterial [1], anticancer [16], anti-HIV [17], antimalarial [10], and anti-TB activities [12] which may ascribed to its ability to exert non-covalent interactions such as electrostatic interactions and hydrogen bonds *etc*. Furthermore, many isatin related compounds such as semaxanib and nintedanib have been approved for clinical use. Thus, exploitation of isatin moiety in antimicrobial area will be especially fruitful.

Various FQs-isatin hybrids tethered with different linkers exhibited excellent *in vitro* and *in vivo* potency against diverse microorganisms [18–35]. The previous studies demonstrated that the linkers between FQs and isatin play a crucial role for the anti-TB activity [28–35], so the linkers are worth to be further optimized.

Ciprofloxacin (**CPFX**), as the second generation fluoroquinolone, endows with excellent antimicrobial activity, notable pharmacokinetic properties and few side effects, is used widely in clinical practice for the treatment of various bacterial infections [8]. Moreover, **CPFX** has been recommended as the second-line agents by the WHO for the treatment of TB mainly in cases involving resistance or intolerance to first-line anti-TB therapy [8]. Thus, ciprofloxacin derivatives have caused continuous interests.

Based on the above considerations, a novel set of propylene-tethered CPFX-isatin hybrids **3a-f** and **4a-f** with more flexible propylene as linker was designed, synthesized and examined for their *in vitro* antimicrobial activities against representative Gram-positive, Gram-negative and mycobacterial pathogens as well as cytotoxicity in VERO cell line. Our primary objective was to optimize the potency of these hybrids against clinically important pathogens. A preliminary structure-activity relationship (SAR) study is also explored to facilitate the further design. The design strategy is illustrated in Fig. 1.

2. Results and discussion

The synthetic pathway for propylene-tethered CPFX-isatin hybrids **3a-f** and **4a-f** is outlined in Scheme 1. Alkylation of C-5 substituted isatins **1a,b** was performed with 1,3-dibromopropane in presence of K_2CO_3 to provide *N*-(3-bromopropyl)isatins **2a,b**, which were then incorporated into **CPFX** core to afford the desired targets **3a,b** and bis-isatin-CPFX hybrids **4a,b**. Subsequently, condensation of hybrids **3a,b** or **4a,b** with methoxylamine or ethoxylamine hydrochloride in the presence of sodium bicarbonate provided other conjugates **3c-f** and **4c-f**.

All propylene-tethered isatin-CPFX hybrids **3a-f** and **4a-f** were assessed for their *in vitro* antibacterial activities against clinically important Gram-positive, Gram-negative pathogens as well as anti-mycobacterial activities against MTB $H_{37}Rv$ and MDR-TB strains [34]. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give 90% inhibition of bacterial growth and MICs of the compounds are reported in Table 1, **2** and **3**, respectively.

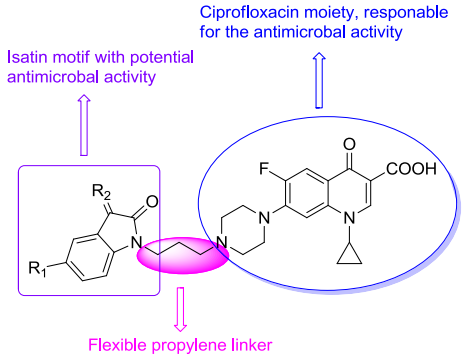

Fig. 1. Illustration of design strategy for propylene-tethered isatin-ciprofloxacin hybrids.

As it can be seen from Table 1, all mono-isatin-CPFX **3a-f** displayed promising activities against the tested Gram-positive strains with MIC ranging from 0.06 to 64 μg/mL. Among them, the most active hybrid **3d** was highly potent against all tested Gram-positive strains, especially against clinically important pathogens MSSE, MRSA, MSSA, MRSA and *Enterococcus faecalis* with MIC of 0.06, 0.06, 0.06 and 0.5 μg/mL, respectively, which was 2–64 folds more potent than the references **CPFX** (MIC: 0.125–64 μg/mL) and levofloxacin (MIC: 0.125–4 μg/mL).

From Table 2, it can be concluded that all mono-isatin-CPFX 3a-f displayed excellent potency against the tested Gram-negative strains with MIC in a range of ≤0.03–2 μg/mL. Interestingly, hybrid **3d** also demonstrated highest potent against all tested Gram-negative strains with MIC of ≤0.03–0.5 μg/mL, respectively, which was comparable to or slightly more active than the references **CPFX** (MIC: ≤0.03–2 μg/mL) and levofloxacin (MIC: ≤0.03–2 μg/mL).

The SAR revealed that the antibacterial activity of mono-isatin-CPFX **3a-f** were far more potent than the corresponding bis-isatin-CPFX **4a-f** (MIC: 4->128 μg/mL) against all tested Gram-positive and Gram-negative strains, suggesting carboxylic acid at C-3 position is essential for gyrase binding and bacterial membrane transport; introduction of imine at C-3 position of isatin motif could boost up the activity, and the relative contribution order was -NOMe > -NOEt > -O; Substituents at C-5 position of isatin moiety have great influence on the activity, and hybrids with electron-donating -Me were more potent than the corresponding unsubstituted analogs.

The antimycobacterial results showed that all hybrids exhibited considerable activity with MIC in a range of 0.1–64 μg/mL against MTB $H_{37}Rv$ and MDR-TB, and all mono-isatin-CPFX hybrids except **3f** were no inferior to the parent **CPFX**. The SAR indicated that mono-isatin-CPFX hybrids were more potent than the corresponding bis-isatin-CPFX hybrids which was in accordance with the previous antibacterial study; for mono-isatin-CPFX hybrids, introduction of imines at C-3 position and electron-donating -Me at C-5 position of isatin moiety reduced the activity against both of the tested strains generally. In particular, the most potent hybrid **3b** with MIC of 0.1 and 0.5 μg/mL was 4- and 8-fold more active than the parent **CPFX** (MIC: 0.78 μg/mL) and rifampicin (**RIF**, MIC: 0.39 μg/mL) against MTB $H_{37}Rv$, and 4->256 times more potent than the three references **CPFX** (MIC: 2.0 μg/mL), **RIF** (MIC: 32 μg/mL) and isoniazid (**INH**, >128 μg/mL) against MDR-TB.

The propylene-tethered isatin-CPFX hybrids **3a-f** and **4a-f** were subsequently examined for toxicity (CC_{50}) in a mammalian VERO cell line [36]. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product and the results are reported in Table 3. All hybrids (CC_{50}: 16–256 μg/mL) showed acceptable cytotoxicity, and fortunately, the cytotoxicity of the most potency **3b** (CC_{50}: 64 μg/mL) and **3d** (CC_{50}: 256 μg/mL) were comparable to that of **CPFX** (CC_{50}: 128 μg/mL).

The metabolic stability and *in vivo* pharmacokinetic (PK) properties of hybrid **3b** and **3d** were evaluated in mice after oral (po) administration 50 mg/kg, respectively [37]. As shown in Table 4, after oral dosing, both hybrids reached a maximum concentration in plasma within 1.5 h; their elimination half-life was favorable (4.1 and 3.3 h, respectively); and the area under curve (AUC) was 4988 and 2,865, respectively. In general, the metabolic stability and *in vivo* PK profiles were less favorable than the parent **CPFX**, need to