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# Design, synthesis, and biological evaluation of dihydroartemisinin–fluoroquinolone conjugates as a novel type of potential antitubercular agents

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ABSTRACT

Tuberculosis remains a global public health problem in recent years. To develop novel type of potential antitubercular agents, twelve novel dihydroartemisinin–fluoroquinolone (DHA–FQ) conjugates (three types of molecules) were gradually designed and conveniently synthesized. All the newly synthesized conjugates were well characterized and evaluated against different *Mycobacterium tuberculosis* strains in vitro. The screening results showed that five DHA–FQ conjugates were active toward *M. tuberculosis* H37Rv, and compound **3a** exhibited the strongest inhibitory activity (MIC = 0.0625 μg/mL), which was comparable to the positive control Moxifloxacin and even stronger than Ofloxacin. Conjugates **2a** and **3a** also displayed comparable activities against various clinically isolated sensitive and resistant *M. tuberculosis* strains (MIC = 0.125–16 μg/mL) to Moxifloxacin. All target compounds possessed selective anti-*M. tuberculosis* ability. Preliminary structure–activity relationship demonstrated that short linker between DHA and FQ was favorable for strong antitubercular activity. This study provides a new clue for the development of novel antitubercular lead molecules.



Tuberculosis (TB), originated mainly from various strains of *Mycobacterium tuberculosis* (*M. tuberculosis*), is a highly infectious and chronic disease with high infection rate since ancient times.[1,2] There are approximately one third of population infected with TB bacillus and 1.7 million people die from TB annually, and in the year of 2010, new TB patient is estimated at 8 million or more.[3] TB has been therefore identified by the WHO as one of the three priority diseases for drug research and development.[4] Although the treatment of TB has been investigated over 100 years, and lots of effective drugs have been developed, TB remains a global public health concern. Clinical antitubercular (anti-TB) drugs such as Rifampicin, Isoniazid, Pyrazinamide and Ethambutol[5] are satisfactorily efficacious in the management of ordinary TB, but accompanied by poor compliance, long treatment period, certain side effects and other shortcomings.[6] Multidrug-resistant tuberculosis (MDR-TB)[7] and extensively drug-resistant tuberculosis (XDR-TB) make this problem become more complex.[8,9] The most challenging aspect is the combination of TB and human immunodeficiency virus (HIV), which gives infected patients more risk to death.[10] Therefore there is an urgent need to discover and develop new anti-TB agents with stronger efficacy, lower toxicity, shorter treatment duration and ideally compatible with anti-HIV drugs.[11]

During the last two decades, many naturally occurring compounds and their derivatives have been reported to exhibit significant inhibitory activity against *M. tuberculosis*, some of which have been selected as the prototype molecules for the development of the new anti-TB drug.[12] Pyrrole derivative LL3858, nitroimidazole derivatives PA824 and OPC67683, diamine derivative SQ109 have been found in different stages of clinical trials.[13] A new candidate, diaryl quinoline derivative TMC207, was approved as bedaquiline (Sirturo™, Fig. 1) by United States FDA in 2012 for clinical treatment of multi-drug resistant pulmonary tuberculosis,[14] representing the very first new TB drug for over 40 years. These prompt us to develop novel structural anti-TB agents.

Fluoroquinolones (FQs), with the advantages of high efficacy, low toxicity, good selectivity and no cross resistance to antibiotics,[15] have become a major clinical drugs used for the treatment

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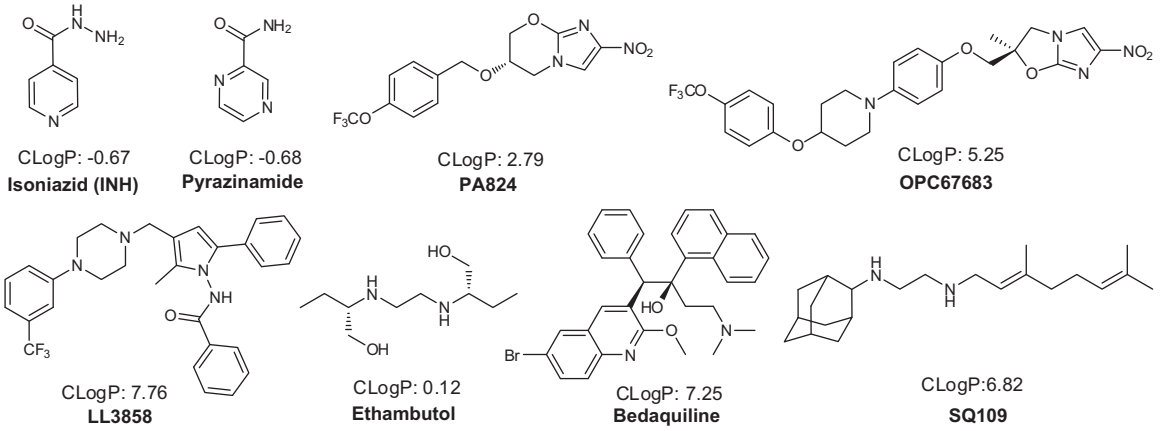



**Figure 1.** Representative anti-TB first-line drugs and candidates under clinical trials.

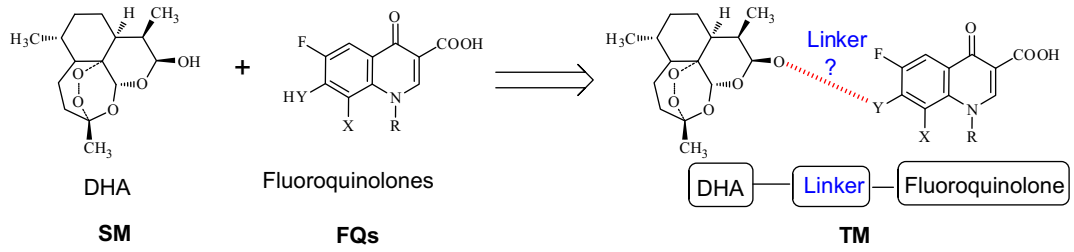
of community and nosocomial infections of the respiratory, gastrointestinal and urinary tracts, skin and soft-tissue infections, chronic osteomyelitis, and sexually transmitted diseases.[16–19] Several FQ drugs even show excellent oral bioavailability, strong macrophage infiltration capacity and potent killing activity on *M. tuberculosis*.[20] Importantly, the WHO also recommended the use of Levofloxacin (LF) or Moxifloxacin (MF) for the treatment of XDR-TB.[21] Therefore, the design and synthesis of new class of FQ molecules, or modification of the existing FQ drugs, with effective anti-TB activity in vitro and in vivo, is a critical direction for the development of new types of anti-TB agents.

Artemisinin, isolated from *Artemisia annua*,[22] is a new class of antimalarial drugs with unique characteristics, especially effective to drug-resistant *Plasmodium falciparum* strains.[23] Dihydroartemisinin (DHA), as the most important derivative of artemisinin, not only possesses better solubility, bioavailability and biological activity than artemisinin, but also has a hemiacetal hydroxyl group, which provides a key reactive site for further modification without destroying its parent structure. Starting from DHA, a lot of DHA derivatives were successfully designed and synthesized, which exhibited either excellent antimalarial potency or other potential activities including anticancer,[24] anti-allergic,[25] antibacterial enhancement[26] as well as anti-helminth,[27] anti-cytomegalovirus,[28] anti-viral[29] and anti-HIV[30] effects, etc. Nevertheless, there is little report on the anti-TB activity of DHA derivatives. It was not until 2011 when Miller et al reported that certain conjugates of DHA with Mycobactin have potent activity against TB,[31] which reminds us that we may find new anti-TB molecules just via structural modification of DHA. Then the most burdensome task is to identify ideal molecule(s) or substituent(s) as coupling unit of DHA. Taking into consideration of the distinctive anti-TB potency of some FQs, it may be a wise choice to incorporate FQ into DHA to design new anti-TB molecules. As an attempt, we intended to design and synthesize some DHA–FQ conjugates (Scheme 1) using the principle of hybridization and to evaluate their anti-TB activity for the purpose to discover new type of anti-TB agents.

Drug modification through succinic anhydride has had a good track record of success. One of the famous examples is sodium artesunate, the salt of the succinic acid half-ester derivative of DHA, which has been listed for the treatment of acute and severe malaria.[32] The free carboxyl in artesunate provides a convenient reactive site for further modification, which prompts us to design some conjugates of DHA with succinyl linker. In the initial attempt to obtain certain conjugates of DHA and FQs, we selected Ciprofloxacin (CPF), Norfloxacin (NF), Sarafloxacin (SF) and Clinafloxacin (CF) to couple with artesunate (**IM1**), which led to novel target molecules **1** (Scheme 2). **IM1** can be quickly generated without impurities under mild condition, and with simple post-processing procedure resulting in significant yield (over 90%). Through the coupling of **IM1** with FQ under the catalytic activation of DCC and HOBt, 4 target molecules compound **1**, mostly white crystals, were obtained smoothly by the classic amide coupling method, with a single-step yield between 75% and 90%.

The inhibitory activities against *M. tuberculosis* H37Rv (ATCC27294) of compound **1** were conducted by Microdilution Alamar Blue Assay in 96-well culture plates with the termination time between 7 and 14 days.[33] MF and Ofloxacin (OF) were used as positive controls. The maximum concentration of **1** was set at 2 μg/mL. The concentration of the reference strains was at 1 μg/mL and 0.5 μg/mL, respectively. The screening results are shown in Table 1.

Table 1 showed that compound **1a** (MIC = 1 μg/mL) and **1b** (MIC = 2 μg/mL) exhibited good anti-TB activities as expected. However, the DHA–FQ conjugates gave lower activity in comparison with the positive controls. This is probably attributed to the ester bond in compound **1**, which results in molecular instability. Thus, changing the linker to increase the molecular stability may be one of the ways to improve the anti-TB activity. The hemiacetal




**Scheme 1.** Design of target molecules.