

# A Wish List of New Anti-Tuberculous Candidate Agents

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**T**uberculosis, or TB, is an infectious bacterial disease caused by rod-shaped and obligate aerobic bacteria, *Mycobacterium tuberculosis* (*M. tb*), which belongs to the order Actinomycetales, family Mycobacteriaceae. Usually, the disease attacks the lungs (as pulmonary TB) but can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin.<sup>1</sup> Although this disease has been discovered for more than a hundred years, it still has been claimed to be one of the global emerging bacterial infectious diseases by the World Health Organization (WHO) and needs to be taken seriously when there is coinfection with HIV. Currently, the potential effectiveness of standard therapy for a drug sensitive disease is a combination of 4 first-line drugs: isoniazid, rifampin, pyrazinamide and ethambutol or streptomycin for at least 6 months.<sup>2</sup> However, there are some limitations and problematic issues with the present therapy, largely from the lengthy treatment period and complicated nature of the first-line drugs, such as their complexity and associated adverse effects or toxicities. Furthermore, as a result of inadequate adherence to the treatment course and the widespread prevalence of HIV, multi-drug resistant (MDR) and extensively drug resistant (XDR) TB strains have been appearing, leading to cases that are even more difficult to manage and provide therapy. MDR-TB is defined as resistance to at least two first-line drugs: isoniazid and rifampin and often taking a further two years to treat with the second-line drugs (e.g. ethionamide, cycloserine, capreomycin, para-aminosalicyclic acid, and kanamycin/amikacin) that are less efficacious, more toxic and much more expensive than the first-line drugs.<sup>3-5</sup> XDR-TB is a subset of the MDR-TB strains that is resistant not only to the isoniazid and rifampin, but also to the second-line drugs (capreomycin, kanamycin and amikacin) and causes very high mortality.<sup>5</sup> In HIV-positive TB patients, the treatment is even more complicated because of the drug antagonism between some of the antiretroviral agents (ARVs) and key antituberculous drugs, especially rifampin.<sup>5</sup> According to the require-

ments, there is a clear need for more field-effective TB treatments since the early TB drug discovered which was the rifamycin (discovered in the late 1950s and the first used to treat TB in the early 1960s), to the new antituberculous drugs and new effective treatments against drug sensitive and resistant strains have been researched and developed in the past 10 years.<sup>3</sup> The primary goals of the activity are to shorten and simplify the treatment of the active TB, provide safer and more efficacious treatments for the drug-resistant TB, and simplify treatment of TB-HIV coinfection by eliminating troublesome drug-drug interactions. Hence, in this paper, all new antituberculous candidates for the TB treatment are listed and described with a high tendency to be a new antituberculous drug in the future and as a future hope for TB patients.

## Emerging antituberculous drugs: candidates in clinical development

To date, there are seven candidate antituberculous drugs under investigation and clinical development as summarized in Table 1 and described below.

### Gatifloxacin (GATI) and Moxifloxacin (MOXI):<sup>3-6</sup>

Both drugs belong to the family C8-methoxy fluoroquinolones. Their action against *M. tuberculosis* is specifically inhibition of the ATP-dependent enzyme topoisomerase II (DNA gyrase), resulting in the interference of the DNA replication, transcription and repair. *In vitro* potency against *M. tb* H37Rv: the minimum inhibition concentration (MIC) of GATI and MOXI are 0.25 and 0.5 µg/ml, respectively. The results of the phase 2 trials of both drugs suggested that each drug had been advanced into late-stage development to evaluate their ability as part of the first-line regimen to shorten treatment duration. Subsequently, both fluoroquinolones have been launched to the phase 3 trials.

### TMC-207:<sup>3-6</sup>

In the past, this novel compound was named as R207910. It is a diarylquinoline and was first discovered by whole-cell phenotypic screening. Its action is binding

**TABLE 1.** New antituberculous drug candidates in clinical development.

Drug	Drug class	Development stage	Mode of action (if known)	Source or organization
Gatifloxacin	Fluoroquinolone	Phase 3	Inhibit the activity of DNA gyrase (topoisomerase II)	Bristol-Myers Squibb (USA)
Moxifloxacin	Fluoroquinolone	Phase 3	Similar to Gatifloxacin	Bayer Healthcare Pharmaceuticals
TMC-207 (or R027910 in a past)	Diarylquinoline	Phase 2	Inhibit ATP synthase	Johnson & Johnson and Tibotec
PA-824	Nitroimidazo-oxazine	Phase 2	Prodrug, Inhibit mycobacterial mycolic acid and protein synthesis	Novartis and TB Alliance
OPC-67683	Nitroimidazo-oxazole	Phase 2	Prodrug, Inhibit mycobacterial mycolic acid and protein synthesis	Otsuka Pharmaceutical
SQ-109 (a novel 1,2-ethylenediamine)	Ethylenediamine	Phase 1	Inhibit cell wall synthesis	Sequella and the National Institute of Allergy and Infectious Disease of the U.S. National Institutes of Health
LL-3858	Pyrrole	Phase 1	Unknown	Lupin

Table modified from Ref.3-6.

to, and inhibiting the adenosine triphosphate (ATP) synthase subunit C. TMC-207 shows significant *in vitro* potency against both drug sensitive and multidrug resistant strains. Its MIC *in vitro* to *M. tb* H37Rv is in between 0.03-0.12 µg/ml. Presently, the compound is in phase 2 of clinical development, to evaluate it for safety and its ability to improve the efficacy of MDR-TB treatment when combined with an optimized regimen of the second-line drugs.

#### PA-824 and OPC-67683:<sup>3-6</sup>

Both drugs are in a novel class of nitroimidazole for TB treatment. Although the drug action mechanism is still unclear, it has been known that they are prodrugs which have a metabolic effect on mycobacterial mycolic acid and protein biosynthesis. The MICs *in vitro* of PA-824 and OPC-67683 to *M. tb* H37Rv are 0.13-0.3 and 0.012 µg/ml, respectively. Now both nitroimidazoles are in phase 2 of clinical development: PA-824 being evaluated for drug sensitive TB, and OPC-67683 is being tested in MDR-TB patients.

#### SQ-109:<sup>3-6</sup>

This is a novel 1, 2-ethylenediamine-based ethambutol analog. Its mode of action appears to involve inhibition of cell wall synthesis and it has a distinct mechanism compared with ethambutol. Its MIC *in vitro* to both drug sensitive and resistant *M. tb* is in a range from 0.16 to 0.64 µg/ml. Based on *in vitro* data, SQ-109

showed its synergism in its bactericidal activity with rifampin and isoniazid. To date, the drug has been evaluated in a phase 1 study.

#### LL-3858:<sup>3-6</sup>

This agent is a pyrrole derivative with unknown action and demonstrated *in vitro* synergy with rifampin. Its MIC *in vitro* against *M. tb* H37Rv has been reported to be 0.12 - 0.25 µg/ml. Based on a recent publication about this drug, it is now in phase 1 trial in India.

#### New antituberculous candidate agents discovered in Thailand

Regarding the urgent requirement of the new antituberculous agents for solving the TB problems with the concern of the safety issue, natural agents/compounds are the other option to investigate. With the natural biodiversity of sources in Thailand and a mission of the National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA by performance of an antituberculous screening assay unit, as a result of screening many synthetic and natural extract compounds, several agents showing good antituberculous activity have been discovered and presently in progress of investigation for further development. A few significant agents are herein described and mentioned as listed in Table 2.

#### 1'-acetoxychavicol acetate (ACA):<sup>8</sup>

This agent is in the majority of natural compounds

**TABLE 2.** New antituberculous candidate agents being discovered and patented by BIOTEC, NSTDA, Thailand.

Agents	Source	Its MIC (µg/ml)*
1'-acetoxychavicol acetate (ACA)	<i>Alpinia galanga</i>	0.1-0.5
Hirsutellones A, B and C	<i>Hirsutella nivea</i> and <i>Trichoderma sp.</i>	0.78
5-chloroquinolin-8-ol (cloxyquin)	Synthetic	0.125

Table modified from Ref. 8-10.

Note: \*The Minimal Inhibition Concentration (MIC) value was determined by performing Microplate Alamar Blue Assay (MABA) with *M. tb* H37Ra strain.<sup>7</sup>

extracted from *Alpinia galanga* or Khar in S-form. It demonstrated the significant growth inhibition to *M. tb* H37Ra, 30 in clinical isolates with MICs *in vitro* 0.1-0.5  $\mu\text{g}/\text{ml}$  and to *M. tb* H37Rv 0.6-1.6  $\mu\text{g}/\text{ml}$ . Instead, testing with the synthetic ACA compound, representing both L- and D-forms, its MICs *in vitro* to *M. tb* H37Ra and H37Rv are 0.4 and 2.7  $\mu\text{g}/\text{ml}$ , respectively. The toxic level of this natural extract ACA to the Vero cells (monkey kidney cells) was 2  $\mu\text{g}/\text{ml}$ . To other cell lines, the toxic level of the extracted ACA for L929 (mouse lung cells) and BHK21 (Hamster kidney cells) were 7-8.5  $\mu\text{g}/\text{ml}$ , while for HepG2 (Human liver cells) was 2-3.4  $\mu\text{g}/\text{ml}$ . Because of its significant antituberculous activity and lack of knowledge about its mode of action in *M. tuberculosis*, the gene responding profiles of *M. tb* to this compound are currently being explored with the hope to better understanding the activity of this compound for future development.

#### **Hirsutellones A, B and C:<sup>9</sup>**

Hirsutellones A, B and C are three new alkaloids produced in cultures of fungus *Hirsutella nivea* and *Trichoderma* sp. strains. They exhibited potent growth inhibitory activity against *M. tb* H37Ra with MIC *in vitro* value of 0.78  $\mu\text{g}/\text{ml}$ . In addition, with IC<sub>50</sub> data, these three alkaloids showed weak or no cytotoxicity to multiple human cancer cell lines especially Hirsutellone A which has an excellent selectivity index. At this moment, the productive and other tests done with these agents are in progress.

#### **Cloxyquin (5-chloroquinolin-8-ol):<sup>10</sup>**

Cloxyquin or 5-chloroquinoline-8-ol is a synthetic compound known to possess activity against bacteria, fungi and protozoa, but has never been documented about its antimycobacterial activity. After testing, its MICs against *M. tb* H37Ra, *M. tb* drug sensitive and drug resistant clinical isolates ranged from 0.125 to 0.25  $\mu\text{g}/\text{ml}$ . So far, there is no clear information regarding the safe use of this cloxyquin. However, the excellent *in vitro* activity against *M. tb* especially to MDR-TB deserves further investigation that is now in progress.

Despite these new candidate antituberculous drugs /

agents are well documented, discovered and under development, understanding which combination regimen or regimens will be most effective is also acquired. With the growing pipeline of new compounds, the opportunity for potential TB treatment should occur more rapidly. Nevertheless, improved therapies for this TB disease must not only be discovered and developed, but must also be made affordable, accessible and adoptable worldwide to help eliminate TB.

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