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Review

New tuberculosis drug leads from naturally occurring compounds

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SUMMARY

Tuberculosis (TB) continues to be a significant cause of mortality and morbidity worldwide. An estimated 2 billion individuals are infected with *Mycobacterium tuberculosis* and annually there are approximately 10 million new cases of clinical TB and 1.5 million deaths. Currently available drugs and vaccines have had no significant impact on TB control. In addition, the emergence of drug resistant TB is considered a public health crisis, with some strains now resistant to all available drugs. Unfortunately, the growing burden of antibiotic resistance is coupled with decreased effort in the development of new antibiotics. Natural sources are attractive starting points in the search for anti-tubercular drugs because they are extremely rich in chemical diversity and have privileged antimicrobial activity. This review will discuss recent advances in the development of TB drug leads from natural products, with a particular focus on anti-mycobacterial compounds in late-stage preclinical and clinical development.

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Emergent drug-resistant tuberculosis

The spread of drug resistant TB is a major threat to global TB control. These strains are now entrenched in most countries and are spreading at an alarming rate. Multi-drug resistant (MDR) TB isolates are resistant to isoniazid (INH) and rifampicin, the two frontline drugs for TB treatment, and have been detected in every country surveyed. In 2015 there were an estimated 480,000 new cases of MDR-TB, however only 50% of patients on MDR-TB treatment were successfully treated.[1] This means hundreds of thousands of people worldwide are going untreated

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and continuing to spread drug resistant forms of the disease. Extensively drug-resistant (XDR) TB strains, first detected in 2006, are resistant to front-line and second-line anti-tubercular antibiotics. XDR-TB is now present in over 100 countries and represents approximately 10% of MDR-TB cases.[1] Delayed diagnosis and inappropriate treatment leads to multiplication of resistance; this is best highlighted by the alarming emergence of totally drug resistant (TDR) TB, which is essentially untreatable using current drugs.[2] In addition, TB treatment is long; standard treatment for drug sensitive strains is 6 to 12 months, while patients with drug resistant TB must endure a longer course of treatment (24 months or longer) with harsh side effects, high cost and a low chance of cure. The combination of long treatment and side effects results in poor compliance, which is a major contributor to the development of resistance. Thus it is evident that current methods of treatment and control for TB are not sustainable in the face of highly drug resistant TB; there is an obvious and urgent need for the development of new TB drugs that are effective against drug resistant *M. tuberculosis* strains, as well as strategies to reduce duration of treatment regimens.

Natural products as new treatments for TB

The search for new anti-TB agents has been slow; the last major anti-TB drug to be licensed for human use was rifampicin in 1963. Since that time a handful of compounds have entered human trials, and encouragingly two compounds, bedaquiline and delamanid, have recently received fast-tracked approval for use against MDR-TB.[3] However both drugs are associated with side-effects and are only recommended for those without other treatment options. Considering the restrictions on bedaquiline use, and the fact that XDR and TDR strains cannot be adequately treated with currently available antibiotics, many more compounds must enter the TB drug development 'pipeline' in order to adequately combat the TB problem. New anti-TB compounds must overcome the issues with current treatments (Table 1). The ideal anti-TB drug must display high potency, particularly against drug-resistant strains, and possess an adequate safety profile. In addition drugs should be active against latent and replicating forms of *M. tuberculosis* and have limited drug/drug interactions, particularly with anti-retroviral agents.

In recent years the field of drug discovery has focused on target-based and genetics-driven approaches to identify new antibiotics. However, this strategy has not been overly successful, as inhibition of enzyme activity often does not correlate with killing of whole bacteria.[4] Large high-throughput (HTS) screening programs have also been employed with a view to rapidly elucidate 'hit' molecules. However, these studies have been typically performed using small molecule 'corporate' chemical libraries that are relatively limited in diversity. Furthermore, antibacterials that are successful in the clinic do not generally follow Lipinski's 'rule of five' for drug likeness, while most corporate compound collections are heavily biased towards such compounds.[4] A review of HTS campaigns by Novartis revealed that natural products were the most diverse compound class tested, with significantly higher hit rates compared to the compounds sourced from synthetic and combinatorial libraries.[5] Indeed, in recent years there has been renewed interest in the use of natural products, due to the wide range of pharmacophores and a high degree of stereochemistry, and therefore three-dimensionality that natural products possess.[6] Identification of bioactive molecules from natural sources involves a defined series of steps to characterize/synthesize the products of interest (Figure 1). In addition, natural products are often bioactive molecules that may display high degrees of bioavailability, thus increasing their capacity to access their site of action within target cells.

The remainder of this review focuses on recent advances in the identification of natural products as anti-mycobacterial agents and potential TB drug leads. We will focus predominately on natural products, their derivatives and 'nature-inspired' compounds that have entered lead optimization and pre-clinical development stages, as well as products that have entered clinical trials (Figure 2). We have kept the definition of natural products relatively broad in order to include the major TB drug candidates in development, with a focus on 5 major compound classes.

Phenazines

Phenazines are a diverse class of aromatic compounds produced both synthetically in the dye industry as well as biosynthetically by many species of the *Actinobacteria* phylum.[7] As biological molecules, phenazines are involved in redox reactions as well as competitive and symbiotic interactions.[8,9] The role of phenazines as inhibitory molecules translates to broad-spectrum antibiotic activity against bacteria and fungi. Antifungal phenazines were first isolated from *Pseudomonas fluorescens*[10] and since then, novel phenazines have been synthesised as potential antitumour drugs[11] as well as antibiotics.[12] **Riminophenazines** are currently under re-investigation as lead compounds for TB treatment. Historically derived from lichens, riminophenazines were developed decades ago as potential TB drugs.[13] Recent years have revived interest in this class of compounds due to the antitubercular activity of clofazimine. Several chemical series of novel riminophenazine derivatives have been synthesised and evaluated for lead development, aiming to improve activity and reduce lipophilicity.[14]

Clofazimine is a riminophenazine originally discovered in 1954 through structural modifications of diploicin, extracted from *Buellia canescens*.[15] While development of clofazimine for TB treatment was delayed by studies showing inactivity in guinea pig and monkey models,[16] it is currently used as a WHO group five drug for MDR-TB.[17] This is due to a reassessment of clofazimine as a TB drug, which discovered that when used in combination with gatifloxacin, ethambutol, pyrazinamide, prothionamide, kanamycin and high-dose isoniazid for 9 months, clofazimine was able to

Table 1
Desired properties of new anti-TB drugs.

Problem with existing therapy	Desired characteristics of new drugs
Lengthy treatment	Increased capacity to inhibit bacterial growth and shorten treatment time (e.g. <4 month).
High pill burden	Lower the number of pills and frequency of doses by using highly potent and bioavailable drugs. Also aim for intermittent treatment.
Expensive	Cheap to make and easily available to the developing world.
Side effects	Less toxic drugs. Intermittent treatment.
Interaction with other drug	Minimal drug-drug interaction with anti-virals, diabetes and non-TB drugs.
Drug resistant *M. tuberculosis* strains	Novel drugs with new mechanism of action.
Lack of efficacy against latent TB	Active against non-replicating bacteria and work effectively in hypoxic conditions. Drugs that can penetrate granulomas.