3. Anti-tubercular activity of natural products: Recent developments

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Abstract. An increasing incidence of deaths due to tuberculosis and the known drawbacks of the current existing drugs including the emergence of multi drug-resistant strains have led to a renewed interest in the discovery of new anti-tubercular agents. The recent researches focused on natural products have shown a useful way to obtain a potentially rich source of drug candidates. This review covers the most active naturally occurring compounds with antitubercular properties at minimal inhibitory concentrations (MICs) of 5 mg/mL or less. The literature from January 2001 to 2009 is reviewed. The compounds are presented in order of chemical type, namely alkynes, heterocyclic compounds, phenols and quinones, peptides, alkaloids, terpenoids and steroids.

1. Introduction

Tuberculosis is a chronic infectious disease, one of the major enemies of the humanity from times immemorial. Today it still remains one of the most serious medical and social problems. It is responsible for 3 million deaths per year and around 8 million cases of first-recorded disease. The advances in the chemotherapy of tuberculosis in the mid-20th century have recently given way to anxiety over the evolution of drug resistance based on the genetically
fixed mutations of *M. tuberculosis*. Moreover, nearly all drugs used for the treatment of tuberculosis and possessing different mechanisms of activity are able to cause adverse side effects on the human organism. Therefore, it is extremely important to search for new, low-toxic substances superior to the available drugs in their activity and efficiency. This primarily concerns the agents possessing activity against *M. tuberculosis* strains with multidrug resistance.

Modern tuberculosis is generally associated with *M. tuberculosis* and *M. bovis*, mycobacteria that are pathogenic to the human organism. Because of slow growth and pathogenicity of *M. tuberculosis* H₃₇Rv, many research groups used fast-growing and/or nonpathogenic mycobacteria including *M. tuberculosis* H₃₇Ra, *M. smegmatis*, *M. aurum*, and others as organisms to be tested. The antimycobacterial activity was also investigated on *M. avium* and *M. intracellulare*, which cause bird tuberculosis and are associated with human diseases in advanced countries (AIDS patients and immunocompromised individuals), to find compounds with a wide range of activity.

A special group of research works includes investigations on *M. tuberculosis* clinical isolates and strains possessing multidrug resistance. Multidrug-resistant tuberculosis (MDRTB) is strictly defined as *M. tuberculosis* strains showing resistance simultaneously against isoniazid and rifampicin [1, 2]. Tuberculosis with a different drug resistance (DDRTB) involves *M. tuberculosis* strains displaying mono- or polyresistance not including associated resistance against isoniazid and rifampicin [3]. The *M. tuberculosis* strains may be sensitive (inhibited by first series drugs such as isoniazid) or resistant (not inhibited by isoniazid). Since researchers use different analytical procedures and/or organisms under test, care should be taken in comparing the biological activities obtained by different authors.

The review covers publications from 2001 to first half 2009; the selected structures have minimum inhibiting concentrations (MIC) of 5 μg/mL or less. Due to this limitation, the most effective compounds were analyzed within one review. For better insight into the "structure-property" relationship, we occasionally gave structures with higher MIC values. The review includes the introduction section, two chapters on synthetic and natural compounds with an antimycobacterial activity, and the conclusions section. To reveal possible "structure-activity" relationships, we grouped the data according to chemical structures.

2. Alkynes and heterocyclic compounds

The metabolite of several strains of the endophytic fungus of the genus *Phomopsis*, 3-nitropropionic acid (1), actively inhibited growth of
*M. tuberculosis* H$_{37}$Ra (MIC 0.4 μg/mL). Although the high neurotoxicity of this compound was a hindrance to its use as a pharmaceutical, it could be used as a model for the synthesis of new inhibitors of isocitratelyase, an enzyme essential to the catabolism of fatty acids and virulence of *M. tuberculosis* [4]. Linoleic acid (2) that inhibits growth of *M. phlei* (MIC 2 μg/mL) is extracted from the plant *Humulus lupulus*. An example of polyacetylene compounds is 3S,8R stereoisomer (3) isolated from *Anethum graveolens* and having MIC 2-4 μg/mL when tested on a group of fast growing mycobacteria (*M. fortuitum* ATCC 6841, *M. smegmatis* ATCC 14468, *M. phlei* ATCC 11758, *M. aurum* Pasteur Institute 104482, and *M. abscessus* ATCC 19977; for ethambutol, MIC 0.5-4 μg/mL) [5]. However, cytotoxicity of this class of polyacetylene compounds can lower the interest in their biological activity [6]. Compounds (4a) and (4b), the synthetic analogs of the natural antibiotic thiolactomycin, inhibit growth of *M. tuberculosis* with MIC 1-16 μg/mL, including drug-resistant strains [7].

Investigation of the components of the plant *Cinnamomum kotoense* led to the isolation of a number of compounds, of which lincomolide B (5) with MIC 2.8 μg/mL had the highest antituberculosis activity [8]. Micromolide (6), which is a γ-lactone derivative of oleic acid, was isolated from the stem bark of *Micromelum hirsutum* and has MIC 1.5 μg/mL against *M. tuberculosis* (H$_{37}$Rv).

Further evaluation of activity on J774 mice cells infected with a more virulent strain of *M. tuberculosis* Erdman gave MIC 5.6 μg/mL [9]. 2-Substituted furans (7a,b) and (8a,b) isolated from the roots of *Polyalthia evecta* possess activity against *M. tuberculosis* (MIC 3.1 and 6.25 μg/mL, respectively) [10]. The synthesized natural compound pamamycin-607 (9) inhibits growth of *M. bovis* BCG, *M. smegmatis* and *M. tuberculosis* (MIC 0.5-4.7 μg/mL). It does not show cross resistance to isoniazid and rifampicin [11].
3. Phenols and quinones

Phenylpropanoids (10) and (11), metabolites of *Pimpinella* sp., inhibit growth of a number of mycobacteria, including *M. intracellulare*, *M. smegmatis*, *M. aurum*, and *M. phlei* (MIC 1.25-10 μg/mL) [12]. The tricyclic diphenol ether engelhardion (12) is very active against *M. tuberculosis* H37Rv (MIC 0.2 μg/mL) [13]. (-)-4-Hydroxy-1-tetralone (13, MIC 4.0 μg/mL) & 3-methoxycarboxy-1,5-dihydroxyantraquinone (15, MIC 3.125 μg/mL) were isolated as an antituberculosis component of *Engelhardia roxburghiana* [13]. As is known, the level of the intra- and extracellular inhibition of *M. tuberculosis* by 7-methyljuglon (14) (MIC 0.5 μg/mL) extracted from the plant *E. natalenis*, is comparable to that of streptomycin and ethambutol (MIC 1 and 2 μg/mL, respectively). Its derivatives, namely, 5-hydroxy-, 5-alkoxy-, and 5-acetoxy-8-substituted naphthoquinones, are less active (MIC 2.5- >20 μg/mL) and possess low antituberculosis selectivity, probably because of their nonspecific activity with various disulfide reductases found in mammal cells. Optimization of the specificity of these compounds for mycothiol disulfide reductase, which is one of the several biological targets for the antituberculosis activity of naphthoquinones of this structure, is required [14].
Marine metabolites pseudopyronines A and B (16a,b, MIC 0.78-3.125 μg/mL) inhibit the growth \( M. \text{tuberculosis} \) \( H_{37} \text{Rv} \) [15]. Pyrone (17, MIC 4 μg/mL) is a component of \( \text{Piper sanctum} \) that is active against \( M. \text{tuberculosis} \) \( H_{37} \text{Rv} \) [16]. Ferulenol (18a) isolated from the Sardinian giant fennel \( \text{Ferula communis} \) is effective against \( M. \text{smegmatis} \) (MIC 0.5 μg/mL), as well as \( M. \text{fortuitum} \), \( M. \text{phlei} \) and \( M. \text{aurum} \) (MIC 2 μg/mL). The analogs of this compound, (18b-d), were isolated from the same plant; compound (18b) with a benzyloxy group retained its activity against \( M. \text{smegmatis} \) and \( M. \text{phlei} \), and, to a lesser extent, against \( M. \text{fortuitum} \) and \( M. \text{aurum} \), while the activity of (18c) and (18d) with the hydroxy and acetoxy groups is considerably lower [17]. Ostruthin (19), the metabolite of \( \text{Peucedanum ostruthin Koch} \), inhibit the growth \( M. \text{aurum} \) (MIC 3.4 μg/mL) [18].
Compounds (20a-h), isolated from the lichen fungus *Microsphaeropsis* sp., show different activities against *M. tuberculosis* H₃₇Ra (MIC 25, 3.12, 3.12–6.25, 6.25, 12.5, 25, 1.56-3.12, 50 μg/mL, respectively), but are also characterized by cytotoxicity [19]. The dibenzofuran derivative, usnic acid (21), which is a secondary metabolite of lichen, inhibits growth of *M. tuberculosis* (MIC 2.5–5 μg/mL) [20]. One of the xanthone dimers isolated from the endophytic fungus of the genus *Phomopsis*, phomoxanthone A (22a), is very active against *M. tuberculosis* H₃₇Ra (0.5 μg/mL), while its deacetylated derivative (22b) is inactive. Phomoxanthone B (22c) is less active (MIC 6.25 μg/mL). Both active compounds are cytotoxic [21].

The anthraquinone celastramycin B (23), isolated from the unknown species *Streptomyces*, is active against *M. Vaccae* (MIC 3.1 μg/mL) [22]. The anti-HIV agent (+)-calanolide A (24) was tested for the antituberculosis activity; a combination of the anti-HIV and antituberculosis activities in one agent is very attractive in view of the concurrence of these diseases. This compound, isolated from the tropical tree *Calophyllum lanigerum*, also has an antituberculosis activity against *M. tuberculosis* (MIC 3.13 μg/mL) and a number of drug resistant strains (MIC 8–16 μg/mL) [23].
4. Peptides

Four cyclic peptides, namely, enniatins H (25a), I (25b), B (25c), and B4 (25d), which are the components of the pathogenic fungus *Verticillium hemipterigenum*, inhibit growth of *M. tuberculosis* H₃₇Ra (MIC 3.12–6.25 μg/mL) [24]. Syringomycin E (26), isolated from *Pseudomonas syringae* pv. *Syringae*, is active against *M. smegmatis* (MIC 1.5 μg/mL) [25].

The metabolite of *Nocardia* sp. (ATCC 202099), namely, the thiazole peptide nocathiacin (27) shows activity against *M. tuberculosis* ATCC 35828, *M. avium* A26778, and *M. avium* A26640 (MIC ≤ 0.008, 0.06, and 0.25 μg/mL, respectively). Unfortunately, compounds from this class typically show poor pharmacokinetics and solubility (the latter problem can be solved by synthesizing analogs with higher solubility in water) [26].

5. Alkaloids

Two compounds, namely, the known antibiotic pyrrolnitrin (28) and banegasine (29), isolated from the zoobacterium *Aristabacter necator*, act synergetically against *M. smegmatis* (MIC (29) >0.5 μg/mL, (28) 0.3 μg/mL, (28) + (29) 0.075 μg/mL) [27]. Their analog celastramycin A (30), which is a dichloropyrrole metabolite of the *Streptomyces* strain, has a broad spectrum of antimycobacterial activity (MIC 0.05-3.1 μg/mL against *M. smegmatis*, *M. aurum*, *M. vaccae*, and *M. fortuitum*) [22]. The bis-1-oxaquinolizidine alkaloid (−)–araguspongine C (32), isolated from the sea sponge *Xestospongia exigua*, inhibits growth of *M. tuberculosis* H₃₇Rv (MIC 1.9 μg/mL) [28].

In the series of quinolone alkaloids (33a-d), isolated from the fruits of *Evodia rutaecarpa*, compounds with unsaturated aliphatic chains at 2-position exhibited better antimycobacterial activity as compared with saturated chain compounds [18]. Agelasine E (33a) and agelasine D (33b) were previously isolated from the sea sponge *Agelas nakamura*. While agelasine E is inactive, its methoxy analogs (33c-g), having different terpenoid side chains,
(25a) $R^1 = R^2 = R^3 = R^5 = \text{i-Pr}; R^4 = \text{s-Bu}$;
(25b) $R^1 = R^2 = R^3 = R^6 = \text{i-Pr}; R^4 = R^5 = \text{s-Bu}$;
(25c) $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = \text{i-Pr}$;
(25d) $R^1 = \text{i-Bu}; R^2 = R^3 = R^4 = R^5 = R^6 = \text{i-Pr}$

(26): $R = \text{NHCOCH}_2\text{CH(OH)(CH}_2\text{)}_8\text{CH}_3$

(27)

(28) $\text{NH}_2$
(29) $\text{COOH}$
(30) $\text{Cl}$

(31a)
(31b)
(31c)
(31d)

(32)
demonstrate high activity against *M. tuberculosis* H$_{37}$Rv (MIC 3.13, 1.56, and 3.13 μg/mL respectively). Possibly, the presence of an alkoxy group at the terminal nitrogen atom is a very important factor for the antimycobacterial activity of these compounds. However, there is only slight difference between the activities of agelasine D (33b) and its alkoxy derivatives (33f) and (33g) [29]. It is interesting that the simpler analog of the compounds, 9-methyladenine (33h), has MIC of 6.25 μg/mL [30].

The tetracyclic alkaloid cryptolepine (34a), isolated from *Cryptolepis sanguinolenta*, is active against a number of fast-growing mycobacteria, including *M. aurum* (MIC 2 μg/mL), *M. phlei* (MIC 4 μg/mL), and *M. fortuitum* (MIC 16 μg/mL) [31]. Metabolite of *Allium neapolitanium* (34b)
displayed enhanced activity against the *M. smegmatis* (mc²2700), when compared with that for (34c) (MIC 2-8 μg/mL). Furthermore, the activity of (34b) was greater against *M. smegmatis* (mc² 2700) than *M. smegmatis* (ATCC 14468) (MIC 16 μg/mL for (34c) and 8 μg/mL for (34b)) [32].

The metabolites of the Thailand pathogenic fungus *Hirsutella nivea* BCC 2594 hirsutellones A-D (35a-d) inhibit growth of *M. tuberculosis* H₃₇Ra (MIC 0.78, 3.125, 0.78, 0.78 μg/mL, respectively). Compound (35d) exhibits moderate *in vitro* cytotoxicity, while other compounds are less cytotoxic [33]. Hirsutellone F (35e), which is a new dimer alkaloid isolated, together with known hirsutellones A, B, and C, from the seeds of the fungus *Trichoderma* sp. BCC 7579 shows a weaker antituberculosis activity against *M. tuberculosis* H₃₇Ra (MIC 3.12 μg/mL) than hirsutellones A, B, and C [34].

The known alkaloid ecteinascidin 770 (36a) and the new one, ecteinascidin 786 (36b), both isolated from *Ecteinascidia thurstoni*, inhibit growth of *M. tuberculosis* H₃₇Ra (MIC 0.1 and 1.6 μg/mL, respectively) [35]. Manzamine alkaloids isolated from sea sponges are promising from the viewpoint of their antituberculosis activity. Manzamines A (37a), E (37c), and F (37d) and their hydroxyl derivatives 6-hydroxymanzamine E (37e) and
(+)-8-hydroxymanzamine A (37b) show activity against *M. tuberculosis* H$_{37}$Rv (MIC 1.5, 3.8, 2.6, 0.4, and 0.9 μg/mL, respectively) [36]. Manadomanzamines A (38a) and B (38b) inhibit growth of *M. tuberculosis* H$_{37}$Rv (MIC 1.9 and 1.5 μg/mL, respectively) [37].
6. Terpenes

Compound (39), isolated from *Indigofera longeracemosa*, is active against *M. tuberculosis* (MIC 0.38 μg/mL) [39]. Diterpenes (40) and (41) from *Calceolaria pinnifolia* [40], the structurally related lecheronol A (42), isolated from *Sapium haematospermum* (MIC 4 μg/mL) [40], and metabolite of *Melica volkensii* 6-hydroxycyclactone (43) [18] have the same value of MIC against *M. tuberculosis* H37Rv. Ugandensial (44, from *Warbugia ugandensis*) inhibit growth of *M. aurum* and *M. phlei* at this value of MIC [18].

![Chemical structures](image)

The diterpenes diaportheines A (45a) and B (45b) were isolated from the fungus *Diaporthe* sp. Compound (45b) has antituberculosis activity against *M. tuberculosis* H37Ra (MIC 3.1 μg/mL) and cytotoxicity, while compound (45a) is much less active and cytotoxic (MIC 200 μg/mL) [41]. These data indicate that the presence of a carbonyl group is important for the antituberculosis activity. A metabolite of the African tree *Combretum imberbe*, traditionally used in folk medicine is imberbic acid (46), which shows activity against *M. fortuitum* (MIC 1.56 μg/mL) [42].
The chemical modifications of the parent structure of ursolic acid (at the C-3 position to cinnamate-based esters) resulted in an 4-fold increase in antimycobacterial activity ((47a), MIC 3.13 μg/mL for *M. tuberculosis* H37Ra, for ursolic acid MIC 12.5 μg/mL) [43].
Triterpene \((48)\), isolated from *Elateriospermum tapos*, is active against *M. tuberculosis* H\(_{37}\)Ra (MIC 3.13 μg/mL, for isoniazide and kanamicin sulfate MIC 0.05 & 1.25 μg/mL, respectively) [44]. Aegicerin \((49a)\) and protoprimulagenin A \((49b)\) were isolated from *Aegiceras* sp., *Embelia Schimperi*, and the Peruvian plant *Clavija procera*. Aegicerin \((49a)\) was tested on 37 different strains of tuberculosis (MIC 1.6-3.1 μg/mL against one strain of H\(_{37}\)Rv, 21 sensitive clinical strains, two clinical isolates resistant to isoniazid, and 13 MDR clinical strains). The inactivity of protoprimulagenin
A (49b) (MIC 200 μg/mL) demonstrates that as in the case of (45a) and (45b), the presence of a carbonyl group is critical for the antituberculosis activity. For the first time, an oleane type triterpene shows uniformly high activity against a wide range of both sensitive and resistant strains. Regrettably, for many MDR strains, its excellent antituberculosis activity (for comparison, MIC is 4-32 μg/mL for isoniazid and 2-16 μg/mL for rifampicin) has not yet been effected [45].

7. Steroids

Saringosterol, isolated from brown seaweeds Sargassum ringgoldianum and Lessonia nigrescens in the form of a 1:1 mixture of the 24R isomer (50a) and 24S isomer (50b), inhibits growth of M. tuberculosis H₃₇Rv (MIC 0.25 μg/mL) and has low cytotoxicity. In pure form these isomers possess different levels of activity (MIC is 0.125 μg/mL for the 24R isomer and 1 μg/mL for the 24S isomer) [46].

Lipids that inhibit growth of M. tuberculosis H₃₇Rv were isolated from an extract from Morinda citrifolia (Rubiaceae), traditionally used in folk medicine in the Philippines for the treatment of tuberculosis and respiratory diseases. The highest activity was found for a mixture of (51) and (52) (MIC <2.0 μg/mL for the 2:1 mixture) and endoperoxide (53) (MIC 2.5 μg/mL) [47]. Sterines (54a-d), isolated from an extract from the Argentinian plant Ruprechtia triflora, are active against M. tuberculosis (MIC 2-4 μg/mL) [39]. Synthetic analogues (55a,b) of 5(6→7) abeo-sterol from the Caribbean Sea sponge Svenzea zeai inhibit growth of M. tuberculosis H₃₇Rv ATCC 27294 (MIC 3.8 & 3.9 μg/mL, respectively) but possess moderate cytotoxicity [48].

8. Conclusions

More than 50 % of the medicines introduced in world medical practice are connected with natural compounds to some extent. It can be as native metabolites and synthetically the modified derivatives. Enthusiastic examples of discovery of natural compounds with remarkable pharmacological properties such as antitumoral metabolite taxol or antimalarial metabolite artemisinin and also tens other compounds of a plant and animal origin with various high biological activity allow to hope for prompt discovery of highly effective low-toxic natural compound which will be leader in struggle against a tuberculosis.
References