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Marine Natural Products as Antituberculosis Agents

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Abstract—In an attempt to characterize additional structural classes that could serve as lead antituberculosis agents, 48 structurally diverse marine-derived natural and semisynthetic compounds were examined for in vitro activity against *Mycobacterium tuberculosis*. Three new classes of compounds including C-19 hydroxy steroids, scalarin sesquiterpenoids and tetrabromo spirocyclohexadienylisoxazolines have been identified as having potential as leads for continued investigations as new antituberculosis agents. New additions to the established antituberculosis structural classes quinone-methide and peptide are also reported.

Tuberculosis (TB) is a vicious disease that has infected man since the earliest of times and currently infects about 15 million Americans of the two billion people carrying the disease worldwide.[1,2] Although a vaccine (BCG) and effective chemotherapy against TB were available 50 years ago, TB was declared a global emergency in 1993.[3] Over the past 10 years, the area of tuberculosis therapy has undergone a basic change in emphasis for drug therapy. The increase of tuberculosis coinciding with the AIDS epidemic has resulted in additional drug-resistant isolates of *Mycobacterium tuberculosis*.[4,5] HIV infection has increased the incidence of tuberculosis by causing immunosuppression, which enables latent infection to clinically progress.[6] The risk of developing tuberculosis among AIDS patients is over 100 times higher than among normal individuals.[7] Tuberculosis is a unique serious disease in that unlike other diseases associated with AIDS, it may be spread by airborne transmission to adults and children who are not at risk of AIDS.[7,8]

During 1992, worldwide tuberculosis mortality was two million, with the report of eight million additional new cases. In 1999, 90 million new cases and 30 million deaths from tuberculosis worldwide are expected despite the availability of powerful drugs with activity against the causative pathogen, *M. tuberculosis*.[9–11] Resistance to the current antituberculosis therapy is another threatening problem. Multi-drug-resistant strains of *M. tuberculosis*, resistant to as many as nine drugs, are 50–80% fatal even with intensive treatment. In the U.S., drug-resistant strains have been identified in 17 states since 1989.[12] Isoniazid resistance in the U.S. is present in 5.3% and secondary resistance in 19.4% of isolates while the figures for rifampin are 0.6 and 3.2%, respectively.[5,13] The resurgence of drug-resistant-tuberculosis has generated a renewal of interest in a strategic search for prototype leads. The oceans, with their unique and wide range of biodiversity, producing unusual metabolites, emerges as a good candidate for new antituberculosis agents. In this paper we report the antituberculosis activity for our small library of chemically diverse marine-derived metabolites.

Marine-Derived Antimycobacterium Compounds

To date, there are only two reports of in vitro anti-TB activity from marine origin. Massetolide A (**1**) and viscosin (**2**) are cyclic depsipeptides isolated from cultures of two *Pseudomonas* species isolated from a marine alga and tube worm, respectively.[14] When tested against *M. tuberculosis*, massetolide A and viscosin displayed MIC values of 5–10 and 10–20 μg/mL, respectively. When tested against *M. avium-intracellulare*, massetolide A (**1**) and viscosin (**2**) had MICs of 2.5–5 and 5–10 μg/mL, respectively.[14] Pseudopteroxazole (**3**) and *seco*-pseudopteroxazole (**4**) are new benzoxazole diterpene alkaloids isolated from the West Indian gorgonian *Pseudopterogorgia elisabethae*.[15] Both compounds induced 97 and 66%, respectively, growth inhibition for *M. tuberculosis* H37Rv at a concentration of 12.5 μg/mL without significant cytotoxicity.[15] These reports illustrate the significance of examining marine-derived secondary metabolites, as a possible unexplored resource for new antimycobacterial leads.

Keywords: antibiotics; natural products; marine metabolites; biologically active compounds.

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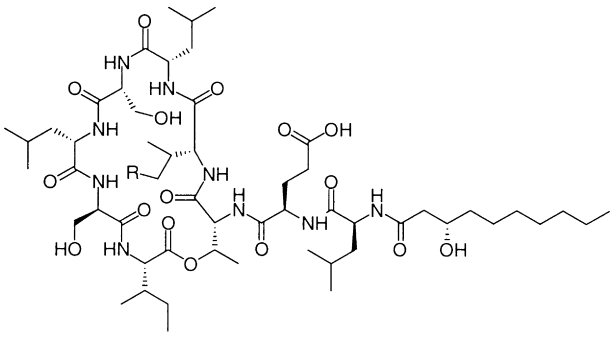
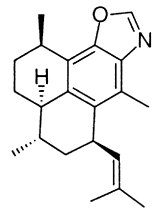
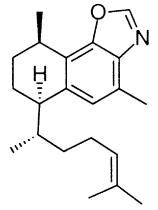


Massetolide A (**1**): R = CH_3 Viscosin (**2**): R = H



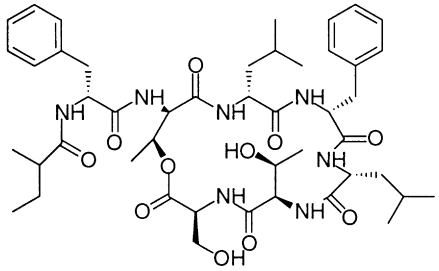
Pseudopteroxazole (**3**)



Seco-pseudopteroxazole (**4**)

Discussion

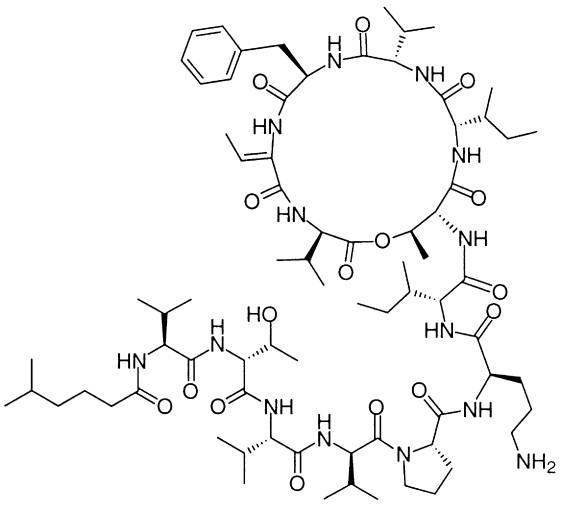
Forty-eight chemically diverse marine-derived secondary metabolites were tested against *M. tuberculosis* (H37Rv) (Table 1). Three classes of compounds show promising and previously unreported anti-TB activity in the range of 70–99% inhibition of this OI pathogen. Kahalalides A (**5**) and F (**6**) are two known polypeptides isolated from the sacoglossan mollusk *Elysia rufescens*.[16,17] Unlike kahalalide F (**6**), **5** did not show significant cytotoxicity against various tumor cell lines. Kahalalide A inhibited 83% of the growth of *M. tuberculosis* (H37Rv) at 12 μg/mL (Table 1). This is the third example of antimycobacterial tuberculosis activity for a marine-derived peptide, after massetolide A and viscosin. Further SAR studies of **5** may lead to an improved activity with minimal toxicity indicating that kahalalide A is a good candidate for future investigations.



Kahalalide A (**5**)

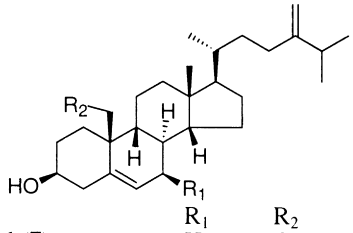
Table 1. In vitro inhibitory activity results against *M. tuberculosis* (H37Rv)

Compound	% Inhibition 12.5μg/mL	Structural class	Reference
Kahalalide A (**5**)	83	Polypeptide	17
Kahalalide F (**6**)	67	Polypeptide	16
Litosterol (**7**)	90	Steroid	18
Nephalsterol-B (**8**)	69	Steroid	19
Nephalsterol-C (**9**)	96	Steroid	19
Puupehenone (**10**)	99	Shikimate-sesquiterpene	20,21
15-Cyanopuupehenone (**11**)	90	Shikimate-sesquiterpene	20,21
20-*O*-Acetylpuupehenone(**12**)	6	Shikimate-sesquiterpene	22
Puupehedione (**13**)	0	Shikimate-sesquiterpene	20,21
15-Oxopuupehenol (**14**)	7	Shikimate-sesquiterpene	20,21
15α-Methylpuupehenol (**15**)	36	Shikimate-sesquiterpene	22
15α-Cyanopuupehenol (**16**)	96	Shikimate-sesquiterpene	22
15α,19,20-Tri-*O*-acetyl-puupehenol (**17**)	78	Shikimate-sesquiterpene	22
15α-Methyl-19,20-di-*O*-acetylpuupehenol (**18**)	7	Shikimate-sesquiterpene	22
15α-Cyano-19,20-di-*O*-acetylpuupehenol (**19**)	64	Shikimate-sesquiterpene	22
15α-Nitromethyl-19,20-di-*O*-acetylpuupehenol (**20**)	22	Shikimate-sesquiterpene	22
15α-Nitroethyl-19,20-di-*O*-acetylpuupehenol (**21**)	15	Shikimate-sesquiterpene	22
Heteronemin (**22**)	99	Sesterterpene	23
11-Hydroxyaerothionin (**23**)	70	Tetrabromo isoxazoline	24
12-*epi*-11-Oxo-12-hydroxyaerothionin (**24**)	60	Tetrabromo isoxazoline	25
11-Oxoaerothionin (**25**)	0	Tetrabromo isoxazoline	26,27



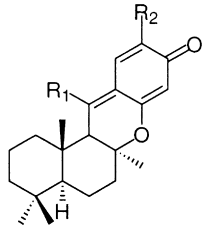
Kahalalide F (**6**)

Litosterol (**7**), nephalsterols B (**8**), and C (**9**) are known C19-hydroxysteroids reisolated by our group from a Red Sea *Nephthea* sp.[18,19] Compounds **7** and **9** inhibited 90 and 96% of the growth of *M. tuberculosis* (H37Rv) (Table 1), with MICs of 3.13 and 12.5 μg/mL, respectively. The poor solubility of litosterol and nephalsterols B in the aqueous tissue culture media obscured accurate IC_{50} and cytotoxicity determinations. Unlike **7** and **9**, nephalsterol B (**8**) inhibited only 69% of growth of *M. tuberculosis* (H37Rv), which indicates that C-7 hydroxylation reduces the activity. When this C-7 hydroxyl is blocked by an acetate, as in **9**, or completely absent, as in **7**, the activity is significantly improved. This is the first report of antimycobacterial activity of this class of compounds. Compounds **7** and **9** are also good candidates for further investigation since they show minimal toxicity.

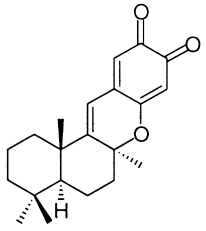


	R_1	R_2
Litosterol (**7**)	H	OH
Nephalsterol-B (**8**)	OH	OH
Nephalsterol-C (**9**)	OAc	OH

Puupehenone (**10**), 15-cyanopuupehenone (**11**), puupehedione (**13**), 15-oxopuupehenol (**14**), 15α-methylpuupehenol (**15**), 15α-cyanopuupehenol (**16**), compounds **12**, and **17–21** are natural sesquiterpene-shikimate derived metabolites or semisynthetic derivatives of puupehenone, which is isolated from sponges of the orders Verongida and Dictyoceratida.[20–22] Puupehenone (**10**), 15-cyanopuupehenone (**11**), and 15α-cyanopuupehenol (**16**) induced 99, 90, and 96% inhibition of *M. tuberculosis* (H37Rv) growth, respectively. Puupehenone shows an MIC of 12.5 μg/mL and an IC_{50} of 2.0 μg/mL. Clearly, the quinone-methide system in ring D of puupehenone is essential for activity as illustrated by both compounds **10** and **11**. Compounds with substitution or addition of cyano functionality at position C-15 retain activity and show reduced toxicity (Table 1). On the other hand the 15 oxo- or methyl derivatives were shown to be inactive (Table 1).

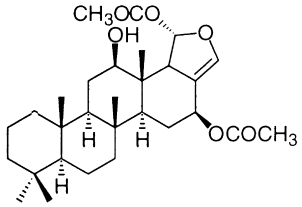


	R_1	R_2
Puupehenone (**10**)	H	OH
15-Cyanopuupehenone(**11**)	CN	OH
20-*O*-Acetylpuupehenone(**12**)	H	OAc



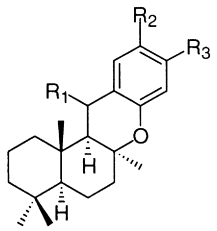
Puupehedione (**13**)

Heteronemin (**22**) is a scalarin-type sesterterpene previously isolated from the sponge *Heteronema erecta* and recently isolated by our group from a Red Sea sponge.[23] Heteronemin displayed a 99% inhibition of *M. tuberculosis* (H37Rv) with an MIC 6.25 μg/mL and IC_{50} 1.3 μg/mL. This is the first report of anti-TB activity for the scalarin-type sesterterpene class of compounds. The high cytotoxicity of these compounds prohibited further testing; however, microbial and/or chemical modifications of these compound may produce less toxic and more active derivatives.



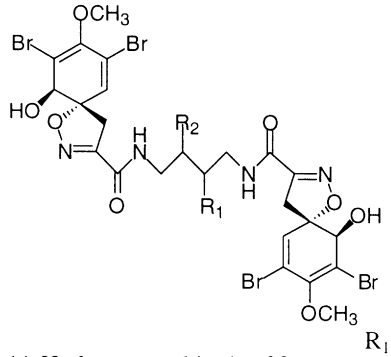
Heteronemin (**22**)

Aerothionins are a group of brominated spirocyclohexadienylisoxazolines isolated from Verongid sponges.[24–27] Both 11-hydroxyaerothionin (**23**) and 11-oxo-12 *epi*-hydroxyaerothionin (**24**) induced 70 and 60% inhibition, respectively, of *M. tuberculosis* growth while 11-oxoaerothionin (**25**) induced no inhibition at all (Table 1). This suggests that hydroxylation at positions 11 or 12 is essential for the activity of these compounds. Despite the moderate activity, of **23** and **24**, their low cytotoxicity and common occurrence in Verongid sponges suggest that they could be possible leads



	R_1	R_2	R_3
15-Oxopuupehenol (**14**)	=O	OH	OH
15α-Methylpuupehenol (**15**)	α-Me	OH	OH
15α-cyanopuupehenol (**16**)	α-CN	OH	OH
15α,19,20-Tri-*O*-acetylpuupehenol (**17**)	OAc	OAc	OAc
15α-Methyl-19,20-di-*O*-acetylpuupehenol (**18**)	α-Me	OAc	OAc
15α-Cyano-19,20-di-*O*-acetylpuupehenol (**19**)	α-CN	OAc	OAc
15α-Nitromethyl-19,20-di-*O*-acetylpuupehenol (**20**)	α-CH_2NO_2	OAc	OAc
15α-(1-Nitroethyl)-19,20-di-*O*-acetylpuupehenol (**21**)	α-$CH(CH_3)NO_2$	OAc	OAc

if additional chemical or microbial hydroxylation is accomplished. This report and the recent report of antimycobacterial activity of pseudopteroxazole suggests that further evaluation of other related isoxazoline and oxazole derivatives could also afford improved activity.



	R_1	R_2
11-Hydroxyaerothionin (**23**)	H	OH
11-Oxo-12-*epi*-hydroxy-aerothionin (**24**)	OH	=O
11-Oxoaerothionin (**25**)	H	=O

Conclusion

The marine environment clearly holds an enormous potential for providing new leads for the development of antituberculous agents. The identification of new structural classes active against *M. tuberculosis* will provide undescribed mechanisms of action and better treatments for resistant strains. Much of the disease burden lies in regions possessing coastal areas rich in marine life. As a result the eventual establishment of mariculture facilities for the production of bioactive materials provides a viable alternative to total synthesis. The reasonably high yields of many sophisticated marine natural products (sponge metabolites in particular) provide an opportunity for the utilization of endemic resources to combat this devastating disease. Cultivation of marine sponges for metabolite production is continuing to make solid progress as an alternative for production of complex marine natural products that are not amenable to cost-effective, large scale synthesis.[28] In this paper we report three new antimycobacterial structural classes; C-19 hydroxy steroids, scalarin sesquiterpenoids, and tetrabromo spirocyclohexadienyl-isoxazolines as well as two classes related to previous reports; kahalalides (peptides), and puupehenones (quinone-methide).

Experimental

Marine-derived compounds

The 48 compounds (>90% purity) tested represent our small marine-derived compounds library. The structure determination of each compound was based on the detailed analysis of 1-D, 2-D NMR and mass spectra and authenticated by comparison of their data with literature values.[29,30]

Test strains

Ten strains of *M. tuberculosis* were used. H37Rv and nine drug-susceptible clinical isolates from *M. tuberculosis* were subcultivated in 7H9 broth. When turbidity of the broth cultures equaled that of a no. 1 McFarland standard, 1.0 mL aliquots of each culture were made and stored at −70°C.[31]

In vitro preliminary screening

All compounds were dissolved, at a concentration of 12.5 μg/mL against *M. tuberculosis* H37Rv, in BACTEC 12B medium using the BACTEC 460 radiometric system.[31] Table 1 shows the preliminary in vitro antituberculosis results of the tested compounds.

MIC determination

Two sets of vials with 7H12 broth and a pH of 6.8 and 6.0, respectively were used. Compounds which showed 99% inhibition in the preliminary screening were dissolved in distilled water and filter sterilized. Double dilutions were made to obtain solutions of 1.280–2.5 μg/mL. The same

experimental procedure was adopted as previously reported.[32]

Acknowledgements

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