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Sydowiols A–C: *Mycobacterium tuberculosis* protein tyrosine phosphatase inhibitors from an East China Sea marine-derived fungus, *Aspergillus sydowii*

Xinru Liu [a,†], Fuhang Song [a,†], Li Ma [a], Caixia Chen [a], Xue Xiao [b], Biao Ren [a], Xueting Liu [a], Huanqin Dai [a], Andrew M. Piggott [b], Yossef Av-Gay [c], Lixin Zhang [a,*], Robert J. Capon [b,*]

[a] Chinese Academy of Sciences, Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, PR China
[b] Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia
[c] Department of Medicine, Division of Infectious Diseases, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

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ABSTRACT

Chemical analysis of an East China Sea marine-derived fungus, *Aspergillus sydowii* (MF357) returned three new tris-pyrogallol ethers, sydowiols A–C (**1**–**3**), and two known bis-pyrogallol ethers, violaceols I (**4**) and II (**5**). Structures were assigned on the basis of detailed spectroscopic analysis and by consideration of symmetry. Sydowiols A (**1**) and C (**3**) were responsible for the inhibitory activity detected in the crude fungal extract against *Mycobacterium tuberculosis* protein tyrosine phosphatase A (PtpA).

Tuberculosis (TB) is a leading cause of death in the world today, exacerbated by the prevalence of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains.[1] TB causes nearly 3 million deaths annually, and is expected to result in >1 billion new infections by 2020.[2] Even drug-sensitive *Mycobacterium tuberculosis* (Mtb) infections currently require several months of treatment with a cocktail of vintage antibiotics, many of which expose patients to serious adverse side-effects. The need for new antitubercular drugs is urgent. Such medications need to be safe with minimal side effects, and effective over a shorter treatment window to encourage compliance. Most importantly, in order to break the cycle of resistance, next generation antitubercular drugs must be capable of treating all Mtb strains, including MDR-TB and XDR-TB, without selecting for new forms of resistance. To meet this challenge requires innovative new approaches to drug discovery.

Macrophages play a central role in host defense, and recognizing and destroying invading pathogens. However, Mtb overcomes these defenses by replicating within macrophage cells. To avoid destruction by macrophage phagocytosis, Mtb secretes the protein tyrosine phosphatase A (PtpA) into the cytosol of infected macrophages.[3] PtpA acts as an inhibitor of phosphorylated (activated) vacuolar sorting-associated protein 33B (VPS33B), inhibiting the key phagocytosis processes of phagosome–lysosome fusion and phagosome acidification.[4] Genetic deletion of PtpA significantly attenuates Mtb growth in human macrophages, suggesting that Mtb PtpA is an attractive target for the development of anti-tubercular drugs.[3] Although natural and synthetic small molecule Mtb PtpA inhibitors have been reported, including roseophilin,[5] phenyl difluoromethyl-phosphonic acid derivatives,[6] chalcones,[7] and cyclic peptides,[8] the threat to global human health posed by TB demands ongoing vigilance to discover new, safer, and more effective antibiotics.

To address this challenge, we embarked on an antitubercular microbial biodiscovery program,[9–11] which involved screening a library of marine-derived bacteria (4024) and fungi (533) for inhibitory activity against Mtb PtpA. This program detected promising inhibitory activity in an East China Sea sediment isolate of *Aspergillus sydowii* (MF357). Bioassay-guided fractionation of a scaled up cultivation yielded three new tris-pyrogallol ethers, sydowiols A–C (**1**–**3**), and two known[12] bis-pyrogallol ethers, and violaceols I (**4**) and II (**5**) (Fig. 1). Structures were assigned to **1**–**3** on the basis of detailed spectroscopic analysis and symmetry considerations

* Corresponding authors. Tel./fax: +86 10 6256 6511 (L.Z.); tel.: +61 7 3346 2979; fax: +61 7 3346 2090 (R.J.C.).
E-mail addresses: zhanglixin@im.ac.cn (L. Zhang), r.capon@uq.edu.au (R.J. Capon).
† These authors contributed equally to this work.

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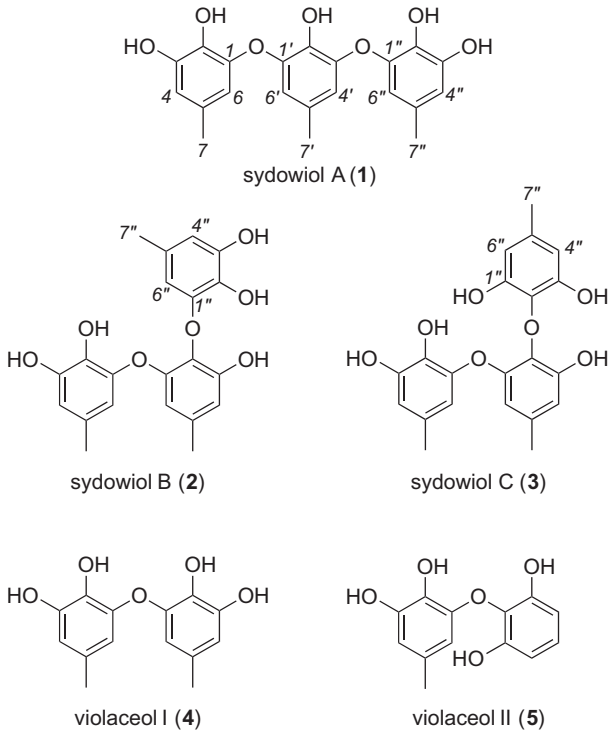

Figure 1. Structures of sydowiols A–C (**1**–**3**) and violaceols I (**4**) and II (**5**).

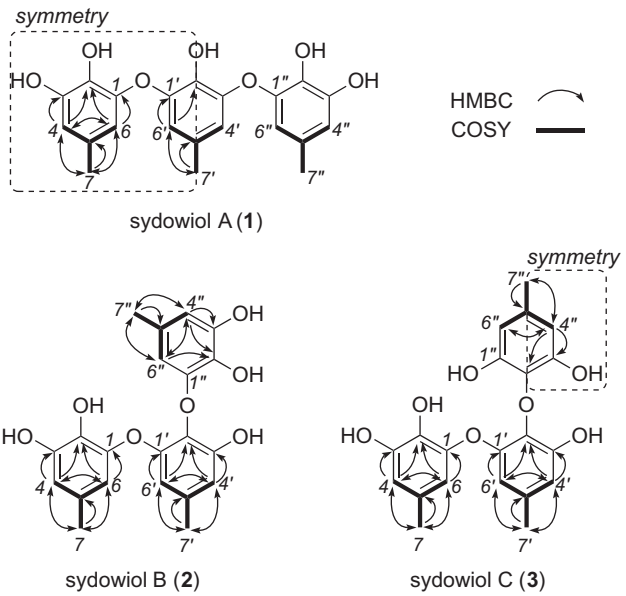

Figure 2. Key 2D NMR correlations for sydowiols A–C (**1**–**3**).

(see below), and to **4** and **5** on the basis of spectroscopic analysis and literature comparisons.[12]

HRESI(−)MS measurements on **1**–**3** returned identical molecular formulae of $C_{21}H_{20}O_7$. Detailed analysis of the 1D (Table 1) and 2D (Fig. 2) NMR data (methanol-d_4) confirmed the compounds as closely related isomers, each consisting of three ether-linked 5-methylpyrogallol moieties and differing only in the location of the ether linkages between the monomer units. Fortuitously, differing levels of symmetry ensure that each of the five possible assembly isomers (each with distinct ether linkage patterns) gives rise to a different number of magnetically unique aromatic protons (see Supplementary data, Fig. S16). As a result, careful analysis of the NMR data permitted identification of sydowiols **1**–**3**, as described below.

The ^{1}H NMR spectrum of **1** revealed three magnetically unique aromatic resonances—one pair of *m*-coupled aromatic protons (δ_H 6.43 and 6.20, d, J = 1.8 Hz) and one isolated aromatic proton (δ_H 6.40, s). Conversely, the ^{1}H NMR spectrum of **2** revealed six magnetically unique aromatic resonances, consisting of three pairs of *m*-coupled aromatic protons (δ_H 6.49 and 6.12, dd, J = 1.8, 0.6 Hz; δ_H 6.41 and 6.05, dd, J = 1.8, 0.6 Hz; δ_H 6.30 and 6.11, dd, J = 1.8, 0.6 Hz). Finally, the ^{1}H NMR spectrum of **3** revealed five magnetically unique aromatic resonances—two pairs of *m*-coupled aromatic protons (δ_H 6.42 and 5.97, dd, J = 1.8, 0.6 Hz; δ_H 6.33 and 6.31, d, J = 1.8 Hz) and one isolated proton (δ_H 6.25, d, J = 0.6 Hz). Significantly, as the two remaining possible assembly isomers give rise to two and four magnetically unique aromatic protons, respectively, the structures for **1**–**3** could be unambiguously assigned as indicated.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (methanol-d_4) for sydowiols A–C (**1**–**3**)

Position	Sydowiol A (**1**)		Sydowiol B (**2**)		Sydowiol C (**3**)	
	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C
1		146.3		148.5		148.4
2		135.1		133.3		132.4
3		147.6		147.1		147.9
4	6.43, d (1.8)	112.7	6.30, dd (1.8, 0.6)	111.6	6.33, d (1.8)	111.5
5		130.04		129.8		130.0
6	6.20, d (1.8)	111.8	6.11, dd (1.8, 0.6)	108.5	6.31, d (1.8)	108.5
7	2.15, s	21.01	2.09, s	21.3	2.10, s	21.1
1′		147.2		152.5		152.5
2′		137.8		132.6		130.8
3′		147.2		152.2		151.5
4′	6.40, s	115.1	6.49, dd (1.8, 0.6)	112.7	6.42, dd (1.8, 0.6)	111.6
5′		129.98		136.7		136.2
6′	6.40, s	115.1	6.12, dd (1.8, 0.6)	110.7	5.97, dd (1.8, 0.6)	107.4
7′	2.12, s	20.95	2.18, s	21.6	2.14, s	21.4
1″		146.3		145.3		151.7
2″		135.1		135.9		130.0
3″		147.6		147.7		151.7
4″	6.43, d (1.8)	112.7	6.41, dd (1.8. 0.6)	113.3	6.25, d (0.6)	109.5
5″		130.04		130.1		136.8
6″	6.20, d (1.8)	111.8	6.05, dd (1.8. 0.6)	113.3	6.25, d (0.6)	109.5
7″	2.15, s	21.01	2.10, s	21.1	2.20, s	21.6

Metabolites **1**–**5** were evaluated for inhibitory activity against Mtb PtpA and for antibiotic activity against Gram-positive and Gram-negative bacteria, and a fungus (Supplementary data, Table S1). The Mtb PtpA inhibitory activity detected in the crude *A. sydowii* (MF357) extract was attributed to sydowiols A (**1**) (IC_{50} 14 μg/mL) and C (**3**) (IC_{50} 24 μg/mL) (Supplementary data, Fig. S18), with the latter also exhibiting modest growth inhibitory activity against *Staphylococcus aureus* (MIC 12.5 μg/mL).

In conclusion, this investigation into the marine-derived fungus, *A. sydowii* MF357 has identified a new class of tris-pyrogallol ethers, sydowiols A (**1**) and C (**3**), as inhibitors of *M. tuberculosis* protein tyrosine phosphatase (PtpA) and potential antitubercular agents.

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Supplementary data

Supplementary data (general experimental procedures, taxonomic identification, full characterization of compounds, 1D and 2D NMR spectra and bioassay results) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.08.137. These data include MOL files and InChiKeys of the most important compounds described in this article.

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