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# Asperterpenoid A, a New Sesterterpenoid as an Inhibitor of *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase B from the Culture of *Aspergillus* sp. 16-5c

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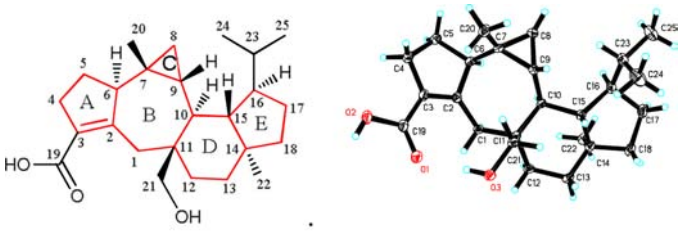
## ABSTRACT



Asperterpenoid A (1)

**Asperterpenoid A (1), a novel sesterterpenoid with a new carbon skeleton, has been isolated from a mangrove endophytic fungus *Aspergillus* sp. 16-5c. Its structure was characterized by extensive spectroscopic methods, and the absolute configuration was determined by single crystal X-ray diffraction analysis. Asperterpenoid A (1) exhibited strong inhibitory activity against *Mycobacterium tuberculosis* protein tyrosine phosphatase B (*m*PTPB) with an $IC_{50}$ value of 2.2 $\mu$M.**

Tuberculosis (TB) is a major infectious disease caused by *Mycobacterium tuberculosis*. For 2011 the World Health Organization reported that about 1.4 million people died from TB and 8.7 million new cases were recorded. A major concern with TB is its predilection for development drug resistance; drug-resistant TB (DR-TB) is a global public health concern dictating the urgent need to discover and develop new chemotherapeutic approaches to TB treatment.[1] The *M. tuberculosis* protein tyrosine phosphatase (*m*PTPB) is secreted by the microbe and manipulates host signal transduction pathways that facilitate host infection.[2] Recently, there has been increased interest in finding new inhibitors of *m*PTPB, which is considered to be a promising target for pulmonary tuberculosis cure.[3]

Natural products are biologically compatible with many living systems and often possess unique and useful biological activities, in large part, because their scaffolds are the result of evolutionarily significant selective pressures. Among these, sesterterpenoids ($C_{25}$), the smallest group of terpenoids, have been isolated from various natural sources, such as terrestrial fungi, lichens, higher plants, insects, sponges, and various marine organisms,[4] and

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exhibit a broad range of biological activities, such as cytotoxicity, phosphatase inhibition, and antimicrobial effects.[5] However, sesterterpenoids from marine-derived fungi are rare; only 17 sesterterpenoids have been reported to date.[6] In past the decade, we have been dedicated to investigating novel bioactive compounds from mangrove endophytic fungi collected from the South China Sea.[7] Recently, one novel 5/7/(3)6/5 pentacyclic sesterterpenoid named asperterpenoid A (**1**) has been isolated from a mangrove fungus which was identified as *Aspergillus* sp. Herein we report the isolation and structural elucidation of asperterpenoid A (**1**) and its ability to inhibit the *M. tuberculosis* protein tyrosine phosphatase B (*m*PTPB).

The fungus *Aspergillus* sp. 16-5c was fermented on autoclaved rice solid-substrate medium supplemented with 3% sea salt for 28 d at room temperature. The mycelia and rice medium were extracted with MeOH. The MeOH layer was dried in vacuo to yield 6.8 g of organic extract, which was separated by column chromatography (CC) over silica gel by elution with a gradient of $CHCl_3$/MeOH from 1:0 to 1:45 to afford five fractions (Fr.1−Fr.5). Fr.3 (120 mg) was applied to Sephadex LH-20 CC and eluted with $CHCl_3$/MeOH (1:1) to render 0.8 mg of **1**[8] which was isolated as colorless crystals (MeOH). Its molecular formula, $C_{25}H_{38}O_3$, was established by HR-EI-MS, indicating seven degrees of unsaturation. The IR spectrum suggested the presence of an OH group (3403 $cm^{-1}$) and C=O group (1677 $cm^{-1}$). The $^1H$ and $^{13}C$ NMR spectra, with the aid of HSQC spectra, revealed the presence of two singlet and two doublet methyls, nine methylenes, including an oxygen-bearing methylene ($\delta_H$ 3.61, $\delta_C$ 61.4, C-21), six methines, and six quaternary carbons, including a carbonyl carbon ($\delta_C$ 169.8, C-19) and two olefinic carbons ($\delta_C$ 161.0 and 127.1, C-2 and C-3) (Table 1). Furthermore, the presence of α,β-unsaturated carbonyl groups was also proposed by the UV band at 236 nm (log $\varepsilon \approx 4.1$)[4d] and the related carbon chemical shifts. The carbonyl and olefinic carbons accounted for two degrees of unsaturation, indicating that compound **1** possessed five rings. The basic carbon skeleton was established by comprehensive analysis of 2D NMR spectroscopic data, in particular with $^1H-^1H$ COSY and HMBC correlations. The $^1H-^1H$ COSY spectrum revealed $^1H-^1H$ spin systems of H-4/H-5/H-6, H-8/H-9/H-10/H-15/H-16(H-23(H-25)/H-24)/H-17/H-18, and H-12/H-13, allowing for assignment of the fragments $-CH_2-CH_2-CH-$, $-CH_2-CH-CH-CH-CH(CH(CH_3)-CH_3)-CH_2-CH_2-$, and $-CH_2-CH_2-$ (Figure 1, red, blue, and black, respectively). In the HMBC spectrum, correlations from the singlet methyl signal at $\delta_H$ 0.77 ($H_3$-22) to C-14 ($\delta_C$ 42.8), C-15 ($\delta_C$ 51.2), and C-18 ($\delta_C$ 35.5) established the structure of ring E. This methyl signal also showed an HMBC correlation to C-13 ($\delta_C$ 39.8), as well as HMBC correlations from the protons of the oxygen-bearing methylene ($\delta_H$ 3.61, $CH_2$-21) to C-11 ($\delta_C$ 44.8), C-12 ($\delta_C$ 22.9). The HMBC correlation from H-10 ($\delta_H$ 1.36) to C-11 defined the six-membered ring system (ring D). Another singlet methyl signal at $\delta_H$ 0.92 ($H_3$-20) showed HMBC correlations to C-7 ($\delta_C$ 21.6), C-8 ($\delta_C$ 26.1), and C-9 ($\delta_C$ 28.9), enabling assignment of the three-membered ring (ring C). Furthermore, the protons of $CH_3$-20 showed a correlation with C-6 ($\delta_C$ 57.1) and the protons of $CH_2$-21 showed a correlation with C-1 ($\delta_C$ 43.1). The signals of the methylene ($\delta_H$ 3.61, H-1a) displayed HMBC correlations with C-6, C-10 ($\delta_C$ 47.9), and the $sp^2$ quaternary C-2.

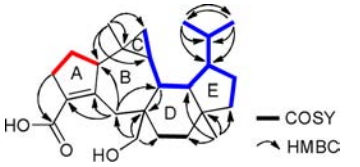


**Figure 1.** COSY and key HMBC correlations in **1**.

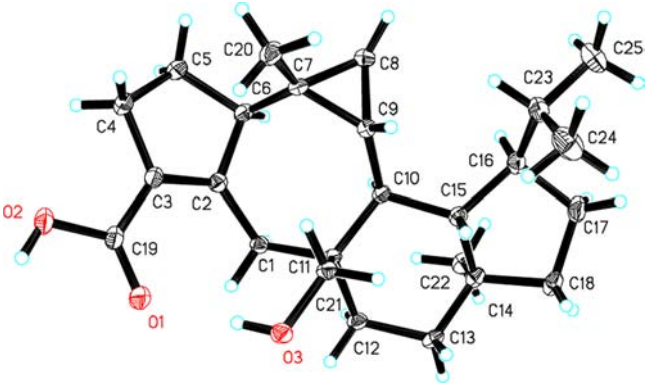


**Figure 2.** Perspective ORTEP drawing for Asperterpenoid A (**1**).

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(8) Asperterpenoid A (**1**): colorless cubic crystals (MeOH); Mp 225−227 °C; $[\alpha]^{25}_D$ −12.6 (*c* 0.20, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon \approx$ 4.1): 236 nm; IR (KBr): 3403, 2955, 2863, 1677, 1641, 1463, 1382, 1252, 1234, 1200, and 1027 $cm^{-1}$; for $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS 386 $[M]^+$; HREIMS *m*/*z* 386.2815 $[M]^+$ (calcd for $C_{25}H_{38}O_3$, 386.2821).

**Table 1.** $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) NMR Data of **1** in $CDCl_3$ ($\delta$ in ppm, $J$ in Hz)[a]

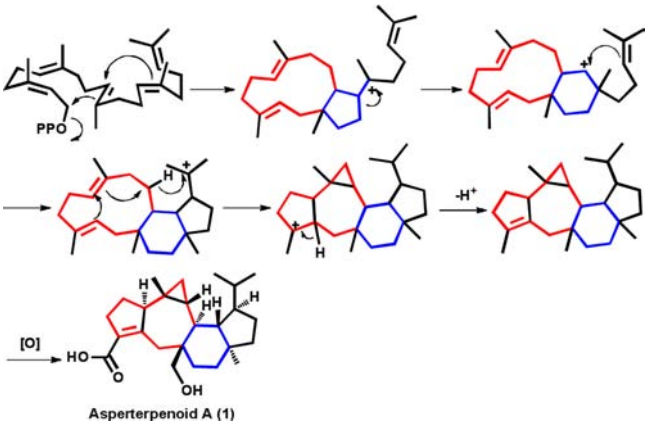
| position | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1a | 3.50 (1H, d, 6.3) | 43.1, $CH_2$ |
| 1b | 1.59 (1H, d, 6.3) | |
| 2 | | 161.0, C |
| 3 | | 127.1, C |
| 4 | 2.62 (2H, br d, 6.8) | 32.9, $CH_2$ |
| 5a | 2.02 (1H, br d, 6.8) | 26.2, $CH_2$ |
| 5b | 1.96 (1H, m) | |
| 6 | 2.33 (1H, br d, 8.2) | 57.1, CH |
| 7 | | 21.6, C |
| 8a | 0.68 (1H, dd, 8.3, 4.2) | 26.1, $CH_2$ |
| 8b | 0.38 (1H, br t, 4.2) | |
| 9 | 0.16 (1H, m) | 28.9, CH |
| 10 | 1.36 (1H, m) | 47.9, CH |
| 11 | | 44.8, C |
| 12a | 2.14 (1H, dt, 13.6, 3.1) | 29.9, $CH_2$ |
| 12b | 1.22 (1H, m) | |
| 13a | 1.40 (1H, m) | 39.8, $CH_2$ |
| 13b | 1.02 (1H, m) | |
| 14 | | 42.8, C |
| 15 | 1.23 (1H, m) | 51.2, CH |
| 16 | 1.75 (1H, tt, 10.2, 3.7) | 45.5, CH |
| 17a | 1.63 (1H, m) | 22.2, $CH_2$ |
| 17b | 1.47 (1H, m) | |
| 18a | 1.50 (1H, m) | 35.5, $CH_2$ |
| 18b | 1.35 (1H, m) | |
| 19 | | 169.8, C |
| 20 | 0.92 (3H, s) | 20.7, $CH_3$ |
| 21 | 3.61 (2H, s) | 61.4, $CH_2$ |
| 22 | 0.77 (3H, s) | 17.8, $CH_3$ |
| 23 | 2.24 (1H, m) | 28.4, CH |
| 24 | 0.85 (3H, d, 6.9) | 23.1, $CH_3$ |
| 25 | 0.76 (3H, d, 6.9) | 15.0, $CH_3$ |

[a] Assignments based on $^{1}H-^{1}H$COSY, HSQC, and HMBC data.

These HMBC correlations not only confirmed the existence of a seven-membered ring (ring B), tethering rings C and D, but also enabled localization of the α,β-unsaturated carboxylic acid at C-2. The α,β-unsaturated carboxylic acid fragment was found to be connected with C-4 ($\delta_C$ 32.9) to form a five-membered ring (ring A). Thus, the planar structure of **1** was defined as shown in Figure 1.

The finalized structure of asperterpenoid A (**1**) was subsequently confirmed by single-crystal X-ray diffraction experiments using Cu Kα radiation (Figure 2).[9] The absolute configuration of **1** was established as 6*S*, 7*R*, 9*R*, 10*R*, 11*S*, 14*S*, 15*S*, and 16*R* through refinement of Flack's parameter [$x$ = 0.02(4)].[10] Thus, the structure of **1** with its new skeleton was fully elucidated.

**Scheme 1.** Plausible Biosynthetic Pathway for **1**




Asperterpenoid A (**1**) has a carbon skeleton consisting of five fused rings which, to the best of our knowledge, is a new skeleton. Its biosynthetic pathway is rationally derivable from geranylfarnesyl diphosphate (GFPP) and may involve cyclization, migration, and oxidation processes, as shown in Scheme 1. We postulate that initial head-to-tail cyclization of GFPP produces an 11/5 fused ring system, followed by migration of a σ bond to generate an 11/6 fused ring system. Further cyclization of the 11/6 ring system may afford an 11/6/5 fused tricyclic intermediate. H-migration and cyclization of the tricyclic intermediate is envisioned to result in the elaborate sesquiterpene precursor, which is subsequently oxidized to **1**.

In this study, asperterpenoid A (**1**) was investigated for its inhibitory activity against *m*PTPB. The results showed that **1** is a strong inhibitor of *m*PTPB with an $IC_{50}$ value of 2.2 μM. Asperterpenoid A (**1**) exhibited inhibitory activity against *m*PTPB and significantly revealed that **1** is a potential antituberculosis drug and/or lead compound for constructing an antituberculosis compound library.

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**Supporting Information Available.** NMR, MS, IR spectra, and the X-ray crystallographic data (CIF file) of **1** as well as *m*PTPB inhibition bioassay. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(9) Crystallographic data for Asperterpenoid A (**1**): $C_{26}H_{42}O_4$, M+MeOH = 418.60; orthorhombic, space group $P2_12_12_1$; a = 6.1880(2) Å, b = 10.5272(4) Å, c = 36.9541(14) Å, α = β = γ = 90°, V = 2407.28 (15) $Å^3$, Z = 4, $D_x$ = 1.155 mg/$m^3$, Crystal dimensions: 0.39 × 0.30 × 0.26 mm were used for measurement on an Oxford Gemini S Ultra diffractometer with Cu Kα radiation (λ = 1.54178 Å). The total number of reflections measured was 4295 (Rint = 0.0360), I > 2σ (I). The final $R_1$ values were 0.0354, $wR_2$ = 0.0867. The crystal structure of compound **1** was solved by direct method SHELXS-97 and expanded using difference Fourier techniques, refined by the program SHLXL-97 and the full-matrix least-squares calculations.

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