Antileishmanial Metabolites from *Geosmithia langdonii*

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Supporting Information

ABSTRACT: Antileishmanial bioassay guided fractionation of *Geosmithia langdonii* has resulted in the isolation and identification of two new compounds (1 and 2) together with 10 known compounds (3–12). The structures of the isolated metabolites were elucidated based on comprehensive 1D and 2D NMR spectroscopic data as well as mass spectrometry. The absolute configuration at C4, C5, and C6 of 2 was determined as R using a modified Mosher esterification method and NOESY correlations. The extracts and the isolated metabolites were evaluated for their antileishmanial activities. Compounds 3, 9, 11, and 12 were found to be active against *Leishmania donovani* with IC₅₀ values of 6.9, 3.3, 8.5, and 9.2 μM, respectively, while compounds 1, 5, and 10 showed lower activities against *L. donovani* with IC₅₀ values of 13.0, 47.3, and 34.0 μM, respectively.

Leishmaniasis is a serious disease that affects human health, especially in developing countries.¹ According to a recent estimation by the World Health Organization (WHO), there are about 1.5–2 million new cases and 20,000 to 30,000 deaths annually. Leishmaniasis has been classified into three main clinical groups: The endemic visceral leishmaniasis (VL), also known as “Kala-azar”, is considered a life-threatening disease, as it interacts with HIV infection² and is accompanied by enlargement of the spleen and liver, weight loss, and anemia;¹,³ cutaneous leishmaniasis (CL) occurs in about 88 countries (66 countries in Africa, Europe, and Asia and 22 countries in North/South America and Oceania) and can cause ulcers and severe disability;¹,³ and mucocutaneous leishmaniasis (ML) can lead to destruction of mucous membranes in the nose, mouth, and throat.⁵,⁶ The currently used chemotherapeutic agents, both the first line of treatment such as the pentavalent antimonial compounds and the second line compounds including amphotericin B, have toxic side effects⁷ and become less effective due to the emergence of resistant strains.⁸,⁹ Thus, there is an urgent need for a new, effective, and inexpensive antileishmanial drug.

*Geosmithia langdonii* (Ascomycota: Hypocreales) is a filamentous fungus and was provided by Assiut University Mycological Center (Accession No. 6161). In this study, *G. langdonii* fungus was grown using three different media [tryptic soy broth, malt extract broth, and potato dextrose broth (PDB)]. Ethyl acetate extracts were evaluated for antileishmanial activity, and the extract obtained from cultures grown in PDB showed the highest activity against *Leishmania donovani* with an IC₅₀ value of 0.35 μg/mL, while extracts obtained by using tryptic soy and malt extract broth media exhibited IC₅₀ values of 5.75 and 36.03 μg/mL, respectively (Table 1). The fungus was then cultivated on a large scale using PDB medium, which led to the production of seven active antileishmanial metabolites. Among the produced metabolites, compounds 4-[2,4′-dihydroxy-6′-(hydroxymethyl)benzyl]benzene-1,2-diol (1) and (4R,S,R,6R)-4,5-dihydroxy-6-(6′-methylsalicylxylo)-2-methyl-2-cyclohexen-1-one (2) were found to be new. Ten known compounds had been identified: (+)-epiepoformin (3), (−)-dihydroepiepoformin (4), (4S,5S)-4,5-dihydroxy-2-methyl-cyclohex-2-enone (5), 6-methylsalicylic acid (6), gentisylquinone (7), 3,4-dihydroxytoluene (8), 2,5-dihydroxybenzaldehyde (9), 3-hydroxybenzyl alcohol (10), 2,5-dihydroxybenzyl alcohol (11), and 3-hydroxytoluene (12) (Figure 1).

RESULTS AND DISCUSSION

Compound 1 was obtained as white needles (CH₃OH) and had a molecular formula of C₁₆H₁₄O₅, which was determined on the basis of HRESIMS, showing eight degrees of unsaturation, suggesting the presence of two aromatic rings. The ¹³C NMR data (Table 2) showed five aromatic methines [δ 114.5 (C-5), 116.1 (C-5′), 116.8 (C-6), and 117.8 (C-3 and C-3′)], in addition to three quaternary and four oxygenated tertiary carbons [δ 126.9 (C-1′), 128.4 (C-6′), 129.5 (C-4′), 148.1 (C-1), 148.3 (C-2′), 148.8 (C-4′), and 150.9 (C-2')]]. Four of them are deshielded, suggesting their attachment to electron-
Table 1. Antileishmanial Activities of Ethyl Acetate Extracts of *Geosminthia langdonii* Obtained by Using Different Growth Media As Well As for the Isolated Metabolites, IC$_{50}$ and IC$_{90}$ (µM)

| extract/compound | Leishmania donovani IC$_{50}$ (µM) | Leishmania donovani IC$_{90}$ (µM) |
|-----------------|-----------------------------------|-----------------------------------|
| GL-PDB$^a$      | 0.35$^f$                          | 1.6$^f$                           |
| GL-T3B$^b$      | 5.73$^f$                          | 6.93$^f$                          |
| GL-MEB$^b$      | 36.03$^f$                         | >40$^f$                           |

$^a$IC$_{50}$ is the concentration that affords 50% inhibition of cell growth. $^b$IC$_{90}$ is the concentration that affords 90% inhibition of cell growth. $^c$Ethyl acetate extract obtained by using potato dextrose broth medium. $^d$Ethyl acetate extract obtained by using tryptic soy broth medium. $^e$Ethyl acetate extract obtained by using tryptic soy broth medium. $^f$Expressed in µg/mL. $^{1}H$ and $^{13}C$ NMR Spectroscopic Data of 1 and 2

| position | $\delta_{H}$ type | $\delta_{C}$ (j in Hz) | $\delta_{H}$ type | $\delta_{C}$ (j in Hz) |
|----------|-------------------|------------------------|-------------------|------------------------|
| 1        | 148.1, C          |                         | 194.4, C          |
| 2        | 150.9, C          |                         | 134.1, C          |
| 3        | 117.8, CH 6.50, br s |                         | 150.0, CH 7.04, br s |
| 4        | 129.5, C          |                         | 72.8, CH 5.00, d (8) |
| 5        | 114.5, CH 6.45, dd (3.2, 8.4) |                         | 77.5, CH 4.59, dd (8, 11.2) |
| 6        | 116.8, CH 6.59, d (8.4) |                         | 80.1, CH 6.23, d (11.6) |
| 7        | 116.1, CH 6.72, br s |                         | 15.7, CH$_{3}$ 1.84, s |
| 8        | 128.4, C          |                         | 160.0, C          |
| 9        | 117.8, CH 6.51, br s |                         | 115.6, CH 7.03, d (8) |
| 10       | 148.8, C          |                         | 133.5, CH 7.27, t (8.4, 7.2) |
| 11       | 116.1, CH 6.72, br s |                         | 122.9, CH 6.76, d (7.6) |
| 12       | 128.4, C          |                         | 140.6, C          |
| pentamidine | 3.2               |                         | 169.9, C          |
| amphotericin B | 0.2               |                         | 22.4, CH$_{3}$ 2.68, s |

$^{1}$H and $^{13}C$ NMR Spectroscopic Data of 1 and 2

$^{a}$Data for 1 and 2 are in CD$_{3}$OD and pyridine, respectively (400 MHz for $^{1}$H, 100 MHz for $^{13}C$, δ in ppm).

Figure 1. Structures of the isolated metabolites (1–12).

Withdrawing groups. The $^{1}$H NMR spectrum (Table 2) showed resonance for a diphenylmethene group (δ 3.73, s) as well as an oxygenated methylene group (δ 4.53, s). The presence of two aromatic rings connected by a methylene group was further confirmed by the $^{13}$C NMR data (Table 2) showed three oxymethines at δ 6.23 (d, j = 11.2 Hz)/δ 80.1, δ 5.00 (d, j = 8 Hz)/δ 72.8 and δ 4.59 (dd, j = 8, 11.2 Hz)/δ 77.5 and one sp$^{2}$ methine at δ 7.04 (br s)/δ 150.0; this was in addition to a carbonyl group at δ 194.4 and one methyl group at δ 1.84 (s)/δ 15.7. These signals were similar to those of 3 except for the downfield shifts of H-4/C-4 (from δ 4.52/62.8 in 3 to δ 5.00/72.8 in 2), H-5/C-5 (from δ 3.68/57.7 in 3 to δ 4.59/77.5 in 2), and H-6/C-6 (from δ 3.36/53.2 in 3 to δ 6.23/80.1 in 2). This in turn suggested the opening of the epoxide ring in 3, thus forming two hydroxy units in 2. In addition, the $^{1}$H and $^{13}C$ NMR spectra of 2 showed signals similar to those of 6-methylsalicylic acid (6), suggesting the presence of a similar moiety in the form of an ester. This was confirmed by the $^{13}$C NMR correlation of H-6 (δ 6.23, d) with the ester carbonyl carbon (δ 169.9) of the 6-methylsalicylic acid unit (SI), in addition to the downfield shifts of H-6/C-6 (δ 6.23/80.1). The structure was further confirmed by the $^{1}$J and $^{13}$C HMBC correlations (SI). H-3 (δ 7.04, br s) showed $^{1}$J HMBC correlations with C-5 (δ 77.5) and C-7 (δ 15.7); H-4 (δ 5.00, d) showed $^{1}$J HMBC correlation with C-2 (δ 134.1) and $^{1}$J HMBC correlations with C-5 (δ 77.5) and C-3 (δ 150.0); H-5 (δ 4.59, dd) showed $^{1}$J HMBC correlations with C-4 (δ 72.8) and C-6 (δ 80.1); H-6 (δ 6.23, d) showed $^{1}$J HMBC correlation with C-4 (δ 72.8) and $^{1}$J HMBC correlations with C-1 (194.4) and C-5 (δ 77.5); H-7 (δ 1.84, s) showed $^{1}$J HMBC correlations with C-1 (δ 194.4) and C-3 (δ 150.0) and $^{1}$J HMBC correlation with C-2 (δ 134.1); H-3′ (δ 7.03, d) showed $^{1}$J HMBC correlations with C-1′ (δ 116.1) and C-5′ (δ 122.9) and $^{13}$C HMBC correlation with C-2′ (δ 160.0) and C-6′ (δ 140.6); H-5′ (δ 6.76, d) showed $^{1}$J HMBC correlations with C-8′ (δ 22.4), C-3′ (δ 115.6), and C-1′ (δ 116.1); and H-8′ (δ 2.68, s) showed $^{1}$J HMBC correlations with C-1′ (δ 116.1) and C-5′ (δ 122.9) and $^{13}$C HMBC correlation of C-6′ (δ 140.6). By
comparing these spectral data with closely related published compounds, compound 2 was identified as 4,5-di-hydroxy-6-(6'-methylsalicyloxy)-2-methyl-2-cyclohexen-1-one.

The absolute configuration of compound 2 was determined as R, using the modified Mosher esterification method by preparing the diastereoisomeric esters separately with (R)- and (S)-α-methoxy-α-trifluoromethylphenacyl chloride (MTPA-Cl), followed by analyzing their 1H NMR chemical shift differences (\(\Delta\delta^R\)). Utilizing the acid chloride of Mosher's reagent rather than the free acid commonly used in the typical Mosher's method enabled running the reactions in the NMR tubes and rapidly acquiring the data. The 1H NMR \(\Delta\delta^R\) of MTPA esters of the compound (pyridine-\(\delta_2\), 400 MHz) were assigned for the following protons: H-5 (3.34 Hz), H-5 (2.4 Hz), and H-6 (2.4 Hz). Analysis of the above \(\Delta\delta^R\) values established that the C-5-OH group has an R-configuration. The absolute configurations of C-4-OH and C-6-OCO have been established to be R based on the NOESY correlations between H-3, H-4, and H-5.

Compound 3 displayed a deprotonated molecular ion \([M - H]^+\) at \(m/z\) 139.04099 (calcld 139.03951) in the negative mode HRESIMS, corresponding to a molecular formula of C7H8O3. The 1H and 13C NMR spectra showed the presence of three oxymethines; two are upfield shifted at \(\delta\ 3.36\) (\(J\ 3.6\) Hz)/\(\delta\ 3.32\) and \(\delta\ 3.68\) (\(J\ 0.8\) Hz)/\(\delta\ 5.77\), characterized for the exoepoxide, while the third oxymethine is at \(\delta\ 4.52\) (\(J\ 0.8\) Hz)/\(\delta\ 62.8\). Also, the spectra revealed the presence of one carbonyl at \(\delta\ 195.1\), suggesting the presence of a cyclohexenone, which was confirmed by the presence of an sp2 methine at \(\delta\ 6.37\) (\(J\ 1.2\) Hz)/\(\delta_c\ 139.8\), a singlet corresponding to a methyl group at \(\delta\ 1.70\) (3H, s)/\(\delta_c\ 15.7\), and one quaternary carbon at \(\delta\ 133.7\). The interpretation of the HMOC and HMBC correlations led us to the complete assignment of the structure. The absolute configuration of C-4 of compound 3 was determined as S, using the Mosher esterification method. The 1H NMR \(\Delta\delta^S\) of the MTPA esters of the compound (pyridine-\(\delta_2\), 400 MHz) were assigned for the following protons: H-5 (3.34 Hz), H-5 (2.4 Hz), and H-6 (2.4 Hz). Analysis of the above \(\Delta\delta^S\) values established that the C-4 has an S-configuration. By comparing these data with those of closely related published compounds, we concluded that compound 3 is (2R,3R,4S)-2,3-epoxy-4-hydroxy-6-methylcyclohex-5-enone [(+)-epiepioforin].

Nine known compounds had been identified: (−)-dihydroepiepioforin (4), (4S,5S,5)-4,5-dihydroxy-2-methylcyclohex-2-enone (5), 6-methylsalicylic acid (6), gentisylquinone (7), 3,4-dihydroxytoluene (8), 2,5-dihydroxybenzaldehyde (9), 3-hydroxybenzyl alcohol (10), 2,5-dihydroxybenzyl alcohol (11), and 3-hydroxytoluene (12). (+)-Epiepioforin, 2,5-dihydroxybenzaldehyde, 2,5-dihydroxybenzyl alcohol, and 3-hydroxytoluene were all found to have activities against L. donovani, with IC50 values of 6.9, 3.3, 8.5, and 9.2 μM, respectively, while compounds 1, (4S,5S)-4,5-dihydroxy-2-methylcyclohex-2-enone, and 10, 3-hydroxybenzyl alcohol have moderate activities against L. donovani, with IC50 values of 13.0, 47.3, and 34.0 μM, respectively (Table 1). All of the compounds were tested for antimicrobial activity, and only 3,4-dihydroxytoluene showed moderate antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA) and Staph. aureus, with IC50 values of 72.9 and 147.8 μM, respectively, [ciprofloxacin showed IC50 of 0.1 μg/mL against MRSA and Staph. aureus; IC50 = the concentration that affords 50% inhibition of growth (μg/mL)].

### EXPERIMENTAL SECTION

#### General Experimental Procedures

High-resolution mass spectra were measured using a Bruker BioApex spectrometer. 1D and 2D NMR spectra were recorded on a Varian AS 400 MHz spectrometer. Incubator shakers (New Brunswick Scientific, Innova 4430) were used for incubating fungi. Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan) and silica gel (60–120 mesh, Merck) were used for column chromatography (CC). Solid-phase extraction (SPE) cartridges (Supelco, silica, 2, 5, and 10 g) were used under vacuum. Fractions from CC were monitored using precoated aluminum sheets [silica 60 F254, 0.25 mm (Merck, Darmstadt, Germany)], with detection provided by UV light (254 and 366 nm) and by spraying with 1% vanillin–sulfuric acid reagent followed by heating for 5–10 min (105 °C). Diaion HP-20 (250 μm, Sigma-Aldrich) was used for the separation of metabolites from the liquid media.

#### Fungal Material

Geosmithia langdonii (Ascomycota: Hypocreales) was provided by Assiut University Mycological Center, Assiut, Egypt. The fungus has been identified by one of the coauthors, Dr. A. M. Moharram, at Assiut University Mycological Center based on its morphological characters and comparison with the literature (Accession No. 6161). This fungus was isolated from 100% cotton textiles, Assiut, Egypt. The cultures were rinsed with water followed by surface sterilization in 70% ethanol for 1 min, rinsed with sterilized water, cut into small pieces (2 cm in length and width), deposited on a Petri dish containing modified PDA medium (200 g of potato, 20 g of glucose, and 15 g of agar in 1 L of distilled water, supplemented with 100 mg/L chloramphenicol), and cultivated at 28 °C for 3 days. The hypha tips were observed and transferred to new PDA plates and subcultured until pure culture was obtained.

#### Culture Media

Fungi were grown on modified potato dextrose agar (PDA) plates at 28 °C for 14 days. Plates were kept in a refrigerator and used when needed. The fungus was grown on tryptic soy broth, malt extract, and PDB media.

#### Extraction and Isolation of Bioactive Metabolites

For small-scale extractions, the fungi were grown on a small scale using tryptic soy broth, malt extract broth, and PDB media. Fifty milliliters of each medium were placed in 125 mL conical flasks and incubated with small pieces of actively growing mycelium. The cultures were incubated at 30 °C under orbital agitation (160 rpm) for 14 days. After incubation, the contents of the flasks were filtered through sterile cotton using vacuum filtration, and then the filtrates were extracted exhaustively with ethyl acetate. The organic phase was vacuum concentrated to afford the extracts, which were then submitted for biological assays.

G. langdonii was grown in 2800 mL Erlenmeyer flasks containing 1 L of PDB medium (36 flasks), which had been prepared by dissolving 24 g of PDB in 1 L of distilled water and then autoclaved. Each flask was seeded by small fragments (~2–5 mm) of the mycelium. The fungi were incubated at 30 °C, using shakers (160 rpm), for 2 weeks. After the incubation period, the mycelia were filtered through sterile cotton using vacuum filtration, and the filtrates (40 L) were extracted with activated ion-exchange resin (Diaion HP-20) by adding 100 g of resin to each 1 L of filtrate before being returned to the shakers and left overnight. The contents of the flasks were then filtered, and the HP-20 was washed with distilled water to remove salts and sugars. Then, the resin was eluted with MeOH and acetone. The MeOH and acetone eluates were combined and dried under vacuum to yield a viscous residue, which was dissolved in water and successively extracted with n-hexane, DCM, and EtOAc (each 4 × 1 L). Each solvent was separately concentrated under vacuum to afford 2 g (n-hexane), 10 g (DCM), and 2 g (EtOAc). The crude DCM extract (10 g) was subjected to vacuum liquid chromatography (VLC) on flash silica gel (200 g, 15 × 15 cm) and eluted with gradients of n-hexane–EtOAc (80:20–100:0) and then with EtOAc–MeOH gradients (90:10–50:50) to afford nine fractions [A1–A9]. Fraction B (eluted with n-hexane–EtOAc, 4:1, 385 mg) was chromatographed over silica gel CC (9 g, 50 × 35 cm) and eluted with n-hexane–EtOAc gradients (100:0–80:20, 100 μL/fraction) to give five subfractions (B1–B5). Subfraction B3 (eluted with n-hexane–EtOAc, 93:7, 195 mg) was further purified on Sephadex LH-20 CC (10 g, 65 × 1.5 cm) with
MeOH—CHCl₃ (1:1) as eluent, yielding 9 (85 mg). Fraction C (eluted with n-hexane—EtOAc, 3:1, 460 mg) was subjected to solid-phase separation using a SPE cartridge (silica, 10 g) under vacuum and eluted with n-hexane—EtOAc with increasing polarities (95:5—50:50, 150 mL/fraction) to afford six subfractions (C₁—C₆). Subfraction C₃ (eluted with n-hexane—EtOAc, 17.3, 240 mg) was chromatographed over Sephadex LH-20 CC (15 g, 65 × 1.5 cm), using MeOH—CHCl₃ (50:50) as the eluent, to yield subfraction C₃-1 (170 mg) and 8 (35 mg). Subfraction C₃-1 (170 mg) was further purified by CC over silica gel (5 g, 25 × 1.5 cm) using n-hexane—EtOAc (95:5—85:15, 50 mL/fraction) to yield 3 (30 mg), 4 (1.5 mg), and 2 (5 mg). Fraction H (eluted with EtOAc—MeOH, 3:1, 2 g) was subjected to silica gel CC (50 g, 65 × 5 cm) using stepwise gradient elution of EtOAc and MeOH (90:10—70:30, 2 L/fraction) to yield three subfractions [H₁—H₃]. Subfraction H₁ (eluted with EtOAc—MeOH, 9:1, 200 mg) was further purified by Sephadex LH-20 CC (15 g, 65 × 1.5 cm) and eluted with MeOH—CHCl₃ (1:1) to afford 6 (4 mg). The EtOAc crude extract (2 g) was subjected to silica gel CC (50 g, 65 × 5 cm) and eluted with n-hexane—EtOAc in a manner of increasing polarities (100:0—0:100, 2 L/fraction) to afford 10 fractions [A—J]. Fraction B yielded 10 (45 mg). Fractions C, D, E, and F were further purified by Sephadex LH-20 CC (15 g, 65 × 1.5 cm) and eluted with MeOH—CHCl₃ (50:50), yielding 7 (3 mg), 11 (35 g), 5 (2.5 mg), and 1 (3.5 mg), respectively. The crude n-hexane (2 g) extract was subjected to silica gel CC (50 g, 65 × 5 cm) and eluted with n-hexane—EtOAc with increasing polarities (100:0—70:30, 1 L each) to afford five fractions [A—E]. Fraction C yielded 12 (2 mg).

4-(4′,6-Dihydroxy-6′-(hydroxymethyl)benzene-1,2-diyl (1): white needles (CH₂OH); UV (MeOH) λₘₐₓ (log ε) 298 (3.01) and 315 (3.24) nm; 1H NMR (CD₃OD, 400 MHz) see Table 2; 13C NMR (CD₃OD, 400 MHz) see Table 2; HRESIMS m/z 261.06980 [M – H]⁻ (calcld for C₁₅H₁₆O₆Na [2M + Na]⁺ and C₃₀H₃₂O₁₂Na 607.17916, respectively).

(4R,5R,6R)-4,5-Dihydroxy-6-(6-hydroxymethyl)methyl)benzene-1,2-diyl (1): white needles (CH₂OH); UV (MeOH) λₘₐₓ (log ε) 298 (3.01) and 315 (3.24) nm; 1H NMR (CD₃OD, 400 MHz) see Table 2; 13C NMR (CD₃OD, 400 MHz) see Table 2; HRESIMS m/z 261.06980 [M – H]⁻ (calcld for C₁₅H₁₆O₆Na [2M + Na]⁺ and C₃₀H₃₂O₁₂Na 607.17916, respectively).

Determination of the Absolute Configuration. In order to determine the absolute configuration, a modified Mosher esterification method was used, where the diastereomeric (R-) and (S)-α-methoxy-α-trifluoromethylphenacyl chloride (MTPA-Cl) esters of the compound were prepared in NMR tubes. First, the compound was methylated with (2S) and (2R)-MTPA-Cl separately in two different NMR tubes, in pyridine-δ₆ and MeOH; UV (MeOH) λₘₐₓ (log ε) 296 (3.40), 308 (3.51), and 318 (3.45) nm; 1H NMR (pyridine-δ₆, 400 MHz) see Table 2; 13C NMR (pyridine-δ₆, 400 MHz) see Table 2; HRESIMS m/z 315.0823 [M + Na]⁺ and 607.1724 [2M + Na]⁺ (calcld for C₁₅H₂₀O₁₃Na 315.08448 and C₃₀H₃₄Na₂O₃₃Na 607.1796, respectively).

Antileishmanial Bioassay. The antileishmanial activity was evaluated against a culture of L. donovani promastigotes grown in RPMI 1640 medium supplemented with 10% GIBCO fetal calf serum at 26 °C. Growth of leishmanial promastigotes was determined by the Alamar Blue assay (BioSource International, Camarillo, CA, USA). Standard fluorescence was measured by a FluorStar Galaxy plate reader (excitation wavelength, 544 nm; emission wavelength, 590 nm). The mixtures in the NMR tubes were warmed to 60−70 °C for 2−3 min, followed by acquiring the 1H NMR spectra for both the diastereoisomeric MTPA esters, and then their chemical shift differences [i.e., Δδ = δ(S-MTPA ester) − δ(R-MTPA ester)] were analyzed and calculated.10

Antimicrobial Bioassay. Pure compounds were tested for antimicrobial activity against the fungi Candida albicans ATCC 90028, C. glabrata ATCC 90030, C. krusei ATCC 6528, and Aspergillus fumigatus ATCC 90906, as well as the bacteria methicillin-resistant Staphylococcus aureus ATCC 35991, Cryptococcus neoformans ATCC 90113, Staphylococcus aureus ATCC 25923, Esherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853, and Mycobacterium intracellulare ATCC 23068. Amphotericin B (ICN Biomedicals, Aurora, Ohio) for fungal and ciprofloxacin (ICN Biomedicals) for bacterial bioassays were used as positive controls, respectively.
NOTE ADDED AFTER ASAP PUBLICATION

This article, published on Aug 1, 2014, had an error that has been corrected in the version posted on Sept 17, 2014. The authors referred to the Cahn–Ingold–Prelog rule being used to determine the absolute configuration. In fact this should have been by using the method reported by Su et al., and this has been reflected in the changed ref 11. Additional changes include corrections to the author affiliations and changes to address style issues.