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Syntheses and evaluation of macrocyclic engelhardione analogs as antitubercular and antibacterial agents

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Abstract

The natural product engelhardione is an underexplored chemotype for developing novel treatments for bacterial infections; we therefore explored this natural product scaffold for chemical diversification and structure-activity relationship studies. Macrocyclic engelhardione and structural regioisomers were synthesized using a series of aldol condensations and selective hydrogenations to generate the 1,7-diarylheptan-3-one derivatives, followed by microwave-assisted intramolecular Ullmann coupling to afford a series of macrocyclic diaryl ether analogs. An extended macrocyclic chemical library was then produced by oxime formation, reductive amination, and *O*-alkylation. Antibacterial evaluation revealed that the reductive amination derivatives **7b** and **7d** showed moderate activities (minimum inhibitory concentrations: 12.5–25 $\mu\text{g ml}^{-1}$) against *Mycobacterium tuberculosis* and Gram-positive pathogens, as well as anti-Gram-negative activity against an efflux impaired *E. coli* strain. These results provide validated leads for further optimization and development.

Keywords

antibacterial; antitubercular; diarylheptanoid; engelhardione; macrocycle; pterocarine

INTRODUCTION

Due to the emergence and spread of drug resistant *Mycobacterium tuberculosis* and other pathogenic bacterial infections, there is an urgent need to discover new chemotype antitubercular and antibacterial agents with novel mechanisms of action.^{1, 2} Only five novel chemical classes of antibiotics, exemplified by linezolid (Zyvox®), daptomycin (Cubicin®), retapamulin (Altabax®), fidaxomicin (Difcid®), and bedaquiline (Sirturo™), have been introduced into the clinic since the early 1960s.³ Among antibacterial discovery strategies, whole cell-based phenotypic screens of small molecule and/or natural product-like libraries, followed by target deconvolution and identification remain an attractive and efficient approach.⁴

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Supplementary information is available

Natural products represent one of the most valuable sources for novel bioactive molecules and chemical diversity in drug discovery.⁵ Indeed most antibiotics in clinical use are natural products, semisynthetic and/or natural product-inspired derivatives.⁶ Notably, most clinically used natural product antibiotics are derived from microorganisms such as bacteria and fungi; and no plant-derived antibacterial agents have been used clinically.³ Macrocyclic diarylheptanoids belong to a chemical class of bioactive naturally occurring phytochemicals, which display a characteristic diphenyl ether motif linked by a seven carbon bridge.⁷ Since acerogenin A, the first member in the cyclic diarylheptanoid class, was isolated and reported by the Nagai group in 1976,⁸ diverse diarylheptanoids⁹⁻¹⁶ have been isolated and found to mediate a variety of biological activities (Figure 1). One such example, engelhardione was recently isolated from the roots of *Engelhardia roxburghiana* and reported to show potent antituberculosis activity with a minimum inhibitory concentration (MIC) of 0.2 $\mu\text{g ml}^{-1}$.¹⁷

As our continued effort to develop natural products-derived novel antibacterial agents, we have been interested in chemical modification of emerging natural product scaffolds. Inspired by the reported potent antitubercular activity of engelhardione and the limited attention given to exploring this macrocyclic molecule, we directed medicinal chemistry efforts toward this promising natural product scaffold. Consequently, we recently reported the first total synthesis of the published structure (**1a**) of engelhardione and this effort led to its structural revision to that of pterocarine (**1b**, Fig. 1).¹⁸ The structural revision was also subsequently confirmed by the Natarajan group⁹ and the Chen group¹⁹. To further improve the efficiency of macrocyclization, we developed an efficient and modular microwave-assisted macrocyclization via intramolecular Ullmann coupling and investigated the scope and generality of a panel of substrates with different linkers, ring sizes, and substitution patterns.²⁰ To extend the medicinal chemistry effort of this work and to investigate if this cyclic diarylheptanoid architecture possesses any tractable antibacterial activity, herein we report the synthesis, antibacterial evaluation, and preliminary structure-activity relationships (SAR) of this class of macrocyclic diarylheptanoids against *M. tuberculosis* and a broad panel of Gram-positive and Gram-negative pathogens. This work constitutes the first systematic report describing the antitubercular and antibacterial evaluation of synthetic engelhardione, pterocarine, and related structural analogs. Our preliminary mechanistic study identified that lead compounds inhibited several key macromolecular processes (DNA, RNA, and protein).

RESULTS AND DISCUSSION

Chemistry

As illustrated in scheme 1, starting from 1,7-diarylhepten-3-one **2a-c**,¹⁸ the proposed structure (**1a**) of engelhardione, pterocarine (**1b**), and their regioisomer **1c** were synthesized by a series of cross aldol condensations and selective hydrogenations, affording linear 1,7-diaryl-3-ketones as key intermediate **3a-c**, followed by intramolecular Ullmann reactions to give the macrocyclic architectures **4a-c** and final *O*-demethylation (Scheme 1).

To evaluate the influence of the carbonyl group on the antibacterial activity, compounds **5-7** were then designed and synthesized. First, the carbonyl group of engelhardione dimethyl ether **4a** was reduced to the racemic secondary alcohol **5** by reaction with NaBH_4 in quantitative yield (Scheme 2). Second, reaction of **4a** with different substituted hydroxylamine hydrochloride in the presence of pyridine in ethanol at room temperature afforded the oxime derivatives **6a-c**.²¹ In the cases of **6b** and **6c**, based on their ^1H NMR spectra, a mixture of *E* and *Z* oxime isomers in an approximate ratio of 1:1 and 1:2 was obtained, respectively. Initial attempts to prepare Schiff base imines from the reaction of **4a** with amines were unsuccessful due to the facile decomposition of imine products during the

work-up and purification. The one-pot syntheses of the secondary amine analogs **7a-e** were achieved by reacting **4a** with an array of different substituted amines, followed by NaBH₄-mediated reduction in a sealed tube under solvent-free conditions. In all cases, the reactions proceeded smoothly to give products **7a-e** in fair to good yields (47-78%).

Next, synthesis of different substituted engelhardione *O*-alkyl analogs **13a-f** was carried out by employing an orthogonal protecting strategy and adapting the similar synthetic scheme as used for **4a** (Scheme 3). The conjugated diarylhepten-3-one **2d** was obtained by Claisen-Schmidt condensation of **8** with MOM-protected benzaldehyde **10**,²² followed by chemoselective hydrogenation to give the 1,7-diphenylheptan-3-one **3d**.²³ Intramolecular macrocyclic Ullmann reaction was then performed under microwave irradiation to give the MOM-protected macrocycle **11** in good yield (85%). The MOM-deprotected macrocyclic phenol **12** was then prepared by deprotection of **11** with concentrated HCl in methanol at room temperature in high yield. Finally, in the presence of appropriate bases, **12** was subjected to the reactions with different alkyl bromides to afford a series of *O*-alkylated macrocycles **13a-f** in 47-94% yields.

Biology

To evaluate the potential for antituberculosis and broad spectrum antibacterial activities, all the linear 1,7-diarylheptanoid intermediates and macrocyclic engelhardione analogs were tested against *M. tuberculosis* and a panel of Gram-positive and Gram-negative pathogens including *Enterococcus faecalis*, *Staphylococcus aureus* (MSSA), *Staphylococcus aureus* (MRSA), *Escherichia coli* (K12), *Escherichia coli* (Δ TolC), *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. The results are shown in Table 1 and preliminary SARs are summarized below.

Most linear chain diarylheptanoid compounds **2** and **3** were inactive. The engelhardione analogs **1a-c** and their corresponding methyl ether derivatives **4a-c** only showed marginal antibacterial activities. Unfortunately, both the originally published structure¹⁷ (**1a**) of engelhardione and pterocarine (**1b**) only showed weak antituberculosis activity (200 μ g ml⁻¹) in our assay. These results are consistent with a recent report by Reddy and co-workers that corniculatolides, close structural lactone analogs of engelhardione/pterocarine diarylheptanoids, did not inhibit the growth of *M. tuberculosis* at 100 μ g ml⁻¹.²⁴

The oxime derivative **6a** showed slight activity against *S. aureus* (ATCC 29213) (MSSA) (MIC = 100 μ g ml⁻¹). Improved activities were obtained for the reductive amination products **7b-e**; in particular, compounds **7b** and **7d** containing secondary amine functionality exhibited antibacterial activities against *M. tuberculosis*, *E. faecalis*, *S. aureus* MSSA and MRSA with MIC values of 12.5-25 μ g ml⁻¹. Interestingly, compounds **7b-e** also showed moderate activity against an *E. coli* strain that is deficient in TolC, a critical component of the AcrAB-TolC efflux system that pumps antibiotics out the periplasm.²⁵ In contrast, compound **7a** with an additional morpholine motif in this *N*-substituted amine series was not active possibly due to the increased polarity and decreased cellular membrane penetration. It is worthwhile to note that compounds **7b-e** were not active toward wild-type *E. coli* strain. This result suggests that these compounds may be subject to efflux pump mediated resistance mechanism and explains their lack of activity against Gram-negative bacteria.

The *O*-alkylated cyclic derivative **13e** with an isopropyl group showed moderate antituberculosis activity (MIC = 25 μ g ml⁻¹), but all the other compounds in this series were inactive.

To explore the basis for the antibacterial activities of selected compounds, their effects on the biosynthesis of key macromolecules were examined against the *E. coli* TolC deletion

mutant. These effects were examined at low and elevated concentrations (i.e. 1 and $8 \times$ the MIC) to determine concentration dependent effects that may point to a specific process as the main antibacterial target. Interestingly, the biosynthesis of DNA, RNA and protein were all highly inhibited by the compounds **7b**, **7c**, and **7d** at 1 and $8 \times$ their MICs (Figure 2), which may be indicative of a non-specific mode of action, possibly at the cell envelope. This is similar to the effects observed in cells treated with the macrocycles daptomycin and telavancin that target the bacterial envelope and disrupts multiple processes in cells.^{26, 27}

In summary, a small focused library of diarylheptanoid engelhardione and derivatives bearing the oxime, *N*- and *O*-alkylated functionalities were designed and synthesized. Antituberculosis and antibacterial evaluation revealed that the *N*-substituted secondary amine derivatives demonstrated moderate antitubercular and antibacterial activities. In particular, compounds **7b** with a phenethylamine substituent and **7d** with a *n*-hexylamino moiety demonstrated the most potent antibacterial activity against *M. tuberculosis*, *E. faecalis*, and *S. aureus* with MICs ranging from 12.5-25 $\mu\text{g ml}^{-1}$. Based on existing SARs, further synthesis and optimization are ongoing in an effort to obtain compounds with improved antibacterial potency and therapeutic potential.

EXPERIMENTAL

Chemistry

All reagents and solvents obtained from commercial sources were used without further purification. Reactions were monitored either by HPLC with a Shimadzu LC-20A series high performance liquid chromatography (HPLC) system. Hydrogenation reactions were done using domnick hunter NITROX UHP-60H hydrogen generator, USA. Reactions in sealed tube were carried out using Q-TubeTM pressure tube reactors from Q Lab tech, USA. Microwave-assisted synthesis was performed by Biotage Initiator 8 Exp Microwave System. Flash column chromatography was performed using a Biotage Isolera One system and a Biotage SNAP cartridge. Proton and carbon nuclear magnetic resonance (¹H and ¹³C NMR) and 2D HSQC, COSY, HMBC spectra were recorded employing a Bruker AM-400 spectrometer. Chemical shifts were expressed in parts per million (ppm), J values were in Hertz. Mass spectra were recorded on a Varian 500-MS IT mass spectrometer using ESI. The purity of compounds was determined by analytical HPLC using a Gemini, 3 μm , C18, 110 Å column (50 mm $\times$ 4.6 mm, Phenomenex) and a flow rate of 1.0 ml min⁻¹. Gradient conditions: solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile): 0-2.00 min 100% A, 2.00-7.00 min 0-100% B (linear gradient), 7.00-8.00 min 100% B, UV detection at 254 and 220 nm.

Syntheses of engelhardione (**1a**), pterocarine (**1b**), regioisomer **1c** and their corresponding synthetic precursors **2-4** were described previously.^{18, 20} Syntheses of **2d**, **3d**, **5**, **10-12** were described in the supporting information.

Compound 6a: To a suspension of hydroxylamine hydrochloride (13.6 mg, 0.196 mmol) in ethanol (2 ml), 15.8 μL (0.196 mmol) of pyridine was added dropwise, followed by the addition of engelhardione dimethyl ether **4a** (16.5 mg, 0.049 mmol). The reaction was stirred at room temperature for 7 h. The ethanol was evaporated to give a white powder which was washed with water and ethyl acetate to give the product as a white powder (91%). ¹H NMR (DMSO-*d*₆, 400 MHz): δ = 10.34 (1H, s), 7.10 (1H, d, *J* = 8.3 Hz), 7.05 (1H, d, *J* = 8.3 Hz), 6.97 (1H, dd, *J* = 8.3 and 2.1 Hz), 6.82 (1H, dd, *J* = 8.3 and 2.0 Hz), 6.61 (1H, d, *J* = 2.1 Hz), 6.23 (1H, d, *J* = 2.0 Hz), 3.86 (3H, s), 3.81 (3H, s), 2.85-2.82 (2H, m), 2.51-2.48 (2H, m), 2.40-2.37 (2H, m), 1.77-1.72 (2H, m), 1.56-1.49 (2H, m), 1.19-1.11 (2H, m) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 158.6, 150.3, 147.7, 147.6, 145.4, 134.9, 133.2, 125.6, 122.7, 121.2, 118.0, 114.0, 113.9, 56.3, 56.2, 35.5, 31.9, 30.9, 29.5, 28.0, 22.2 ppm.

ESI-MS: calc. for $C_{21}H_{26}NO_4[M+H]^+$: 356.2, found: 356.3. HPLC purity: 98.4% (254 nm), t_R : 6.85 min; 97.7% (220 nm), t_R : 6.86 min.

Compound **6b**: **6b** was prepared as described above for compound **6a**, from **4a** (20 mg, 0.059 mmol) with methoxylamine hydrochloride (19.7 mg, 0.24 mmol). After ethanol was removed, the solid was dissolved in dichloromethane and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and then filtered. Solvent was removed by evaporation to give the crude product, which was further purified by flash column chromatography on silica gel (hexanes/ethyl acetate, 88:12) to give 18.2 mg white powder as a mixture of *Z*- and *E*-oxime isomers (approximately 1:1 ratio based on 1H NMR spectrum). (84%). 1H NMR ($CDCl_3$, 400 MHz): δ = 6.92-6.86 (3H, m), 6.76-6.71 (2H, m), 6.43 (0.5H, s), 6.30 (0.5H, s), 3.92 (1.5H, s), 3.90 (1.5H, s), 3.88 (1.5H, s), 3.86 (1.5H, s), 3.81 (1.5H, s), 3.71 (1.5H, s), 2.83-2.80 (2H, m), 2.52-2.49 (2H, m), 2.45-2.39 (2H, m), 2.08 (1H, t, J = 7.4 Hz), 1.80-1.76 (1H, m), 1.56-1.53 (1H, m), 1.48-1.41 (1H, m), 1.25-1.18 (1H, m), 1.17-1.09 (1H, m) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 161.1, 158.7, 150.4, 149.4, 148.4, 148.0, 147.7, 146.6, 146.0, 134.3, 134.0, 133.6, 133.1, 125.4, 125.2, 123.3, 122.3, 121.2, 118.9, 118.6, 118.2, 112.9, 112.8, 112.5, 112.4, 61.2, 61.0, 56.1, 35.4, 33.0, 32.2, 32.0, 31.5, 30.2, 29.2, 28.9, 28.2, 28.0, 23.7, 22.4 ppm. ESI-MS: calc. for $C_{22}H_{28}NO_4[M+H]^+$: 370.2, found: 370.3. HPLC purity: 98.4% (254 nm), t_R : 7.51 min; 99.4% (220 nm), t_R : 7.50 min.

Compound **6c**: **6c** was prepared as described above for compound **6b**, from **4a** (18 mg, 0.053 mmol) with benzyloxylamine hydrochloride (33.9 mg, 0.212 mmol). The reaction mixture was purified by flash column chromatography on silica gel (hexanes/ethyl acetate, 90:10) to give 21.4 mg white powder as a mixture of *Z*- and *E*-oxime isomers (approximately 1:2 ratio based on 1H NMR spectrum) (91%). 1H NMR ($CDCl_3$, 400 MHz): δ = 7.36-7.29 (3H, m), 7.26-7.22 (3H, m), 7.12-7.10 (2H, m), 6.92-6.88 (3H, m), 6.83 (1H, d, J = 8.2 Hz), 6.79 (0.5H, d, J = 8.4 Hz), 6.75 (0.5H, d, J = 8.2 Hz), 6.70 (1H, d, J = 8.0 Hz), 6.63-6.15 (1.5H, m), 6.28 (1.5H, s), 5.05 (1H, s), 4.94 (1H, s), 3.93 (1.5H, s), 3.92 (3H, s), 3.88 (3H, s), 3.85 (1.5H, s), 2.85-2.79 (3H, m), 2.52-2.49 (1H, m), 2.47-2.44 (4H, m), 2.42-2.39 (1H, m), 2.36-2.33 (4H, m), 2.15 (2H, t, J = 6.8 Hz), 1.80-1.76 (1H, m), 1.58-1.52 (1H, m), 1.25-1.22 (3H, m), 1.09-1.04 (2H, m) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 161.4, 158.6, 150.4, 148.9, 148.7, 147.1, 146.9, 138.5, 138.3, 134.2, 134.0, 133.8, 133.1, 128.6, 128.3, 128.2, 128.1, 127.7, 127.6, 125.2, 124.9, 123.7, 122.3, 121.4, 118.8, 118.1, 117.3, 112.8, 112.7, 112.4, 112.2, 75.6, 75.5, 56.1 (2C), 35.5, 33.2, 32.0, 31.9, 31.3, 29.6, 29.1, 28.8, 28.3, 28.0, 24.1, 22.3 ppm. ESI-MS: calc. for $C_{28}H_{32}NO_4[M+H]^+$: 446.2, found: 446.3. HPLC purity: 98.5% (254 nm), t_R : 7.93 min; 98.9% (220 nm), t_R : 7.94 min.

Compound **7a**: A mixture of engelhardione dimethyl ether **4a** (15.0 mg, 0.044 mmol) and 2-morpholinoethanamine (60 μ l, 0.46 mmol) in a sealed tube was heated at 70 °C for overnight. $NaBH_4$ (16.7 mg, 0.44 mmol) was then added, and the mixture was stirred at 70 °C for 10 min. The reaction mixture was cooled to room temperature, and water (2 ml) was added carefully, and the resulting mixture was extracted with ethyl acetate (2 $\times$ 10 ml). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 . Solvent was removed by evaporation to give the crude product, which was further purified by flash column chromatography on silica gel (DCM/MeOH, 91:9) to give 12 mg of white powder (60%). 1H NMR ($CDCl_3$, 400 MHz): δ = 6.89-6.85 (3H, m), 6.78 (1H, dd, J = 8.2 and 1.4 Hz), 6.62 (1H, s), 6.58 (1H, d, J = 1.7 Hz), 3.89 (6H, 2s), 3.73 (4H, t, J = 4.6 Hz), 2.88-2.70 (4H, m), 2.66-2.56 (3H, m), 2.48-2.44 (4H, m), 2.42-2.30 (2H, m), 1.87-1.80 (1H, m), 1.72-1.61 (1H, m), 1.59-1.38 (4H, m), 1.23-1.15 (1H, m), 0.94-0.85 (1H, m) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 149.5, 149.0, 147.7, 147.2, 133.6, 132.3, 124.4, 124.3, 118.2, 118.0, 112.6 (2C), 67.0, 56.5, 56.1 (2C), 54.5, 53.5, 42.1, 33.1, 32.9, 32.2, 29.8, 28.1, 23.4 ppm. ESI-MS: calc. for $C_{27}H_{39}N_2O_4[M+H]^+$: 455.3, found: 455.4. HPLC purity: 98.5% (254 nm), t_R : 5.36 min; 98.8% (220 nm), t_R : 5.36 min.

Compound **7b**: **7b** was prepared as described above for compound **7a**, from **4a** (18.2 mg, 0.054 mmol) with 2-phenylethanamine to give 12 mg of product (50%). ¹H NMR (CDCl₃, 400 MHz): δ = 7.36-7.32 (2H, m), 7.29-7.21 (3H, m), 6.86 (1H, d, J = 8.2 Hz), 6.79 (1H, d, J = 8.2 Hz), 6.75 (1H, dd, J = 8.3 and 1.2 Hz), 6.63 (1H, d, J = 1.4 Hz), 6.54 (1H, d, J = 1.0 Hz), 6.44 (1H, d, J = 8.1 Hz), 3.89 (3H, s), 3.88 (3H, s), 2.99-2.90 (1H, m), 2.85-2.70 (3H, m), 2.66-2.61 (1H, m), 2.58-2.52 (1H, m), 2.41-2.32 (2H, m), 2.02 (1H, t, J = 9.6 Hz), 1.75-1.68 (1H, m), 1.58-1.37 (4H, m), 1.24-1.09 (2H, m), 0.91-0.81 (1H, m) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 149.4, 148.7, 147.7, 147.1, 140.0, 133.7, 133.2, 128.8, 128.5, 126.3, 124.5, 124.0, 118.9, 117.4, 112.4 (2C), 56.1 (2C), 53.9, 48.1, 36.3, 33.9, 33.5, 33.1, 29.9, 27.9, 23.5 ppm. ESI-MS: calc. for C₂₉H₃₅NO₃ [M+H]⁺: 446.3, found: 446.4. HPLC purity: 96.5% (254 nm), t_R : 6.06 min; 98.5% (220 nm), t_R : 6.06 min.

Compound **7c**: **7c** was prepared as described above for compound **7a**, from **4a** (15.0 mg, 0.044 mmol) with benzylamine to give 9 mg of product (47%). ¹H NMR (CDCl₃, 400 MHz): δ = 7.32-7.24 (5H, m), 6.87 (1H, d, J = 8.2 Hz), 6.83 (1H, d, J = 8.2 Hz), 6.75 (1H, dd, J = 8.2 and 1.4 Hz), 6.68 (1H, dd, J = 8.2 and 1.5 Hz), 6.64 (1H, d, J = 1.5 Hz), 6.49 (1H, d, J = 1.3 Hz), 3.90 (3H, s), 3.89 (3H, s), 3.81 (1H, d, J = 13.0 Hz), 3.54 (1H, d, J = 13.0 Hz), 2.73 (1H, t, J = 4.2 Hz), 2.69 (1H, t, J = 4.2 Hz), 2.66-2.58 (1H, m), 2.42-2.34 (1H, m), 2.13 (1H, t, J = 9.8 Hz), 1.81-1.72 (1H, m), 1.54-1.37 (4H, m), 1.25-1.09 (2H, m), 0.94-0.83 (1H, m) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 149.3, 148.7, 147.5, 147.2, 133.8, 133.6, 128.4, 126.9, 124.6, 124.0, 118.8, 117.7, 112.5 (2C), 56.2 (2C), 53.0, 51.1, 34.0 (2C), 33.2, 30.1, 28.0, 23.7 ppm. ESI-MS: calc. for C₂₈H₃₄NO₃ [M+H]⁺: 432.3, found: 432.3. HPLC purity: 96.0% (254 nm), t_R : 5.92 min; 96.4% (220 nm), t_R : 5.92 min.

Compound **7d**: **7d** was prepared as described above for compound **7a**, from **4a** (15.1 mg, 0.044 mmol) with hexylamine to give 14 mg of product (74%). ¹H NMR (CDCl₃, 400 MHz): δ = 6.98 (1H, dd, J = 8.2 and 1.2 Hz), 6.87 (1H, d, J = 8.2 Hz), 6.86 (1H, d, J = 8.2 Hz), 6.75 (1H, dd, J = 8.2 and 1.2 Hz), 6.61 (1H, s), 6.50 (1H, s), 3.89 (3H, s), 3.87 (3H, s), 2.94-2.59 (5H, m), 2.46-2.36 (2H, m), 1.82-1.74 (2H, m), 1.67-1.43 (6H, m), 1.33-1.25 (6H, m), 1.21-1.13 (1H, m), 0.94-0.85 (4H, m) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 149.7, 148.9, 147.6, 147.5, 133.4, 132.4, 125.1, 124.0, 118.8, 118.0, 112.7, 112.6, 56.2, 56.1, 54.8, 45.9, 33.1, 32.3, 32.1, 31.4, 29.8, 28.0, 27.4, 26.7, 23.5, 22.5, 14.0 ppm. ESI-MS: calc. for C₂₇H₄₀NO₃[M+H]⁺: 426.3, found: 426.4. HPLC purity: 99.5% (254 nm), t_R : 6.15 min; 99.4% (220 nm), t_R : 6.15 min.

Compound **7e**: **7e** was prepared as described above for compound **7a**, from **4a** (15.2 mg, 0.045 mmol) with cyclohexylamine to give 14.7 mg of product (78%). ¹H NMR (CDCl₃, 400 MHz): δ = 6.98 (1H, d, J = 8.1 Hz), 6.89 (1H, d, J = 8.4 Hz), 6.88 (1H, d, J = 8.3 Hz), 6.77 (1H, dd, J = 8.1 and 1.2 Hz), 6.61 (1H, s), 6.52 (1H, d, J = 1.2 Hz), 3.90 (3H, s), 3.89 (3H, s), 3.05-3.01 (1H, m), 2.76-2.66 (3H, m), 2.53-2.42 (2H, m), 2.10-2.07 (1H, m), 1.88-1.84 (2H, m), 1.79-1.62 (6H, m), 1.53-1.43 (3H, m), 1.26-1.17 (5H, m), 0.94-0.84 (1H, m) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 149.7, 148.9, 147.6, 147.5, 133.4, 132.3, 125.3, 124.0, 118.8, 118.3, 112.6 (2C), 56.2, 56.1, 55.4, 52.5, 33.2, 32.8, 32.7, 32.3, 32.2, 29.8, 28.2, 25.1, 25.0 (2C), 23.7 ppm. ESI-MS: calc. for C₂₇H₃₈NO₃[M+H]⁺: 424.3, found: 424.3. HPLC purity: 100% (254 nm), t_R : 5.98 min; 99.8% (220 nm), t_R : 5.99 min.

Compound **13a**: The mixture of ketophenol **12** (18.6 mg, 0.057 mmol), benzyl bromide (9.5 μ l, 0.08 mmol), and potassium carbonate (15.7 mg, 0.114 mmol) in acetone (5 ml) was refluxed for 6 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. Dichloromethane (20 ml) was added to the residue, and the solution was washed with water (2 $\times$ 30 ml), the organic layer was filtered using a Biotage ISOLUTE® phase separator, evaporated and then purified by flash column chromatography on silica gel (hexanes/ethyl acetate = 90/10) to give the product as a white powder (17.5 mg, yield:

74%). ^1H NMR (CDCl_3 , 400 MHz): δ = 7.31-7.30 (2H, m), 7.28-7.27 (3H, m), 6.92 (1H, d, J = 8.0 Hz), 6.91 (1H, d, J = 8.4 Hz), 6.83 (1H, dd, J = 8.2 and 2.2 Hz), 6.78 (1H, dd, J = 8.2 and 2.0 Hz), 6.53 (1H, d, J = 2.0 Hz), 6.34 (1H, d, J = 2.0 Hz), 5.18 (2H, s), 3.91 (3H, s), 2.85-2.82 (2H, m), 2.60-2.57 (2H, m), 2.52-2.49 (2H, m), 2.19 (2H, t, J = 7.4 Hz), 1.56-1.53 (2H, m), 1.32-1.29 (2H, m) ppm. ^{13}C NMR (CDCl_3 , 100 MHz): δ = 211.0, 148.7, 148.5, 147.4, 147.3, 137.4, 134.6, 133.6, 128.4, 127.7, 127.4, 124.6, 123.0, 119.1, 118.9, 116.5, 113.1, 56.3, 43.7, 41.6, 33.0, 29.2, 27.8, 21.3 ppm. ESI-MS: calc. for $\text{C}_{27}\text{H}_{29}\text{O}_4[\text{M}+\text{H}]^+$: 417.2, found: 417.2. HPLC purity: 99.6% (254 nm), t_{R} : 7.52 min; 99.5% (220 nm), t_{R} : 7.52 min.

Compound **13b**: **13b** was prepared as described above for compound **13a**, from **12** (21 mg, 0.064 mmol) with geranyl bromide to give 13 mg of product (47%). ^1H NMR (CDCl_3 , 400 MHz): δ = 6.91 (1H, d, J = 8.4 Hz), 6.88-6.85 (2H, m), 6.76 (1H, dd, J = 8.2 and 1.8 Hz), 6.51 (1H, d, J = 1.8 Hz), 6.37 (1H, d, J = 1.8 Hz), 5.48 (1H, t, J = 6.0 Hz), 5.07 (1H, t, J = 6.2 Hz), 4.63 (2H, d, J = 6.3 Hz), 3.89 (3H, s), 2.86-2.83 (2H, m), 2.61-2.58 (2H, m), 2.51-2.48 (2H, m), 2.20 (2H, t, J = 7.5 Hz), 2.12-2.01 (4H, m), 1.69-1.59 (9H, m), 1.57-1.52 (2H, m), 1.34-1.26 (2H, m) ppm. ^{13}C NMR (CDCl_3 , 100 MHz): δ = 211.0, 148.9, 148.6, 147.4, 147.2, 140.1, 134.0, 133.6, 131.7, 124.5, 123.9, 122.9, 120.3, 119.0, 118.9, 115.8, 113.0, 66.6, 56.3, 43.7, 41.6, 39.5, 33.1, 29.2, 27.8, 26.3, 25.7, 21.3, 17.7, 16.6 ppm. ESI-MS: calc. for $\text{C}_{30}\text{H}_{39}\text{O}_4[\text{M}+\text{H}]^+$: 463.3, found: 463.4. HPLC purity: 98.7% (254 nm), t_{R} : 8.13 min; 99.4% (220 nm), t_{R} : 8.13 min.

Compound **13c**: **13c** was prepared as described above for compound **13a**, from **12** (21 mg, 0.064 mmol) with 1-bromohexane to give 20.5 mg of product (94%). ^1H NMR (CDCl_3 , 400 MHz): δ = 6.92-6.86 (3H, m), 6.75 (1H, d, J = 8.2 Hz), 6.51 (1H, s), 6.33 (1H, s), 4.02 (2H, t, J = 6.8 Hz), 3.90 (3H, s), 2.86-2.83 (2H, m), 2.60-2.58 (2H, m), 2.51-2.48 (2H, m), 2.19 (2H, t, J = 7.5 Hz), 1.80-1.73 (2H, m), 1.58-1.51 (2H, m), 1.43-1.36 (2H, m), 1.33-1.27 (6H, m), 0.85 (3H, t, J = 6.6 Hz) ppm. ^{13}C NMR (CDCl_3 , 100 MHz): δ = 211.1, 149.3, 148.4, 147.6, 146.9, 133.9, 133.6, 124.7, 122.8, 119.2, 118.7, 115.3, 113.0, 69.7, 56.3, 43.7, 41.6, 33.0, 31.6, 29.3, 29.2, 27.8, 25.6, 22.6, 21.3, 14.0 ppm. ESI-MS: calc. for $\text{C}_{26}\text{H}_{35}\text{O}_4[\text{M}+\text{H}]^+$: 411.3, found: 411.3. HPLC purity: 96.5% (254 nm), t_{R} : 7.93 min; 99.0% (220 nm), t_{R} : 7.93 min.

Compound **13d**: **13d** was prepared as described above for compound **13a**, from **12** (20 mg, 0.06 mmol) with 1-bromooctane to give 25 mg of product (93%). ^1H NMR (CDCl_3 , 400 MHz): δ = 6.93-6.86 (3H, m), 6.75 (1H, d, J = 8.2 Hz), 6.51 (1H, s), 6.34 (1H, s), 4.02 (2H, t, J = 6.8 Hz), 3.90 (3H, s), 2.86-2.83 (2H, m), 2.61-2.58 (2H, m), 2.51-2.48 (2H, m), 2.20 (2H, t, J = 7.5 Hz), 1.81-1.73 (2H, m), 1.58-1.51 (2H, m), 1.43-1.36 (2H, m), 1.33-1.23 (10H, m), 0.86 (3H, t, J = 6.6 Hz) ppm. ^{13}C NMR (CDCl_3 , 100 MHz): δ = 211.1, 149.3, 148.4, 147.6, 146.9, 133.9, 133.5, 124.7, 122.8, 119.2, 118.7, 115.3, 113.0, 69.7, 56.3, 43.7, 41.6, 33.0, 31.8, 29.4, 29.3, 29.2, 27.8, 25.9, 22.7, 21.3, 14.1 ppm. ESI-MS: calc. for $\text{C}_{28}\text{H}_{39}\text{O}_4[\text{M}+\text{H}]^+$: 439.3, found: 439.3. HPLC purity: 97.3% (254 nm), t_{R} : 8.25 min; 98.5% (220 nm), t_{R} : 8.25 min.

Compound **13e**: 2-bromopropane (45 μl , 0.48 mmol) and *N,N*-diisopropylethylamine (DIPEA, 25 μl , 0.14 mmol) were added to the solution of **12** (15.7 mg, 0.048 mmol) in acetonitrile (10 ml). The reaction mixture was heated under reflux overnight. After the reaction was complete (monitored by HPLC), the solvent was removed by evaporation. The residue was dissolved in EtOAc (10 ml) and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and then filtered. The filtrate was concentrated *in vacuo* to yield the crude residue, which was further purified by flash column chromatography on silica gel (hexanes/ethyl acetate = 91/9) to give 16 mg of product (90%). ^1H NMR (CDCl_3 , 400 MHz): δ = 6.93 (1H, d, J = 8.2 Hz), 6.89 (1H, d, J = 8.2 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.75

(1H, d, J = 8.2 Hz), 6.50 (1H, s), 6.33 (1H, d, J = 2.1 Hz), 4.56-4.46 (1H, m), 3.90 (3H, s), 2.86-2.83 (2H, m), 2.60-2.58 (2H, m), 2.51-2.48 (2H, m), 2.19 (2H, t, J = 7.5 Hz), 1.58-1.51 (2H, m), 1.33-1.24 (8H, m) ppm. ^{13}C NMR (CDCl_3 , 100 MHz): δ = 211.1, 148.5, 148.2, 148.1, 147.6, 134.7, 133.7, 124.7, 122.8, 119.3, 118.8 (2C), 113.2, 72.7, 56.4, 43.7, 41.6, 33.0, 29.3, 27.8, 22.3, 21.3 ppm. ESI-MS: calc. for $\text{C}_{23}\text{H}_{29}\text{O}_4[\text{M}+\text{H}]^+$: 369.2, found: 369.2. HPLC purity: 97.8% (254 nm), t_R : 7.34 min; 99.0% (220 nm), t_R : 7.34 min.

Compound **13f**: At 0 °C, NaH (60% in mineral oil, 4.4 mg, 0.11 mmol) was added to a solution of **12** (18 mg, 0.055 mmol) in DMF (2 ml). To the resultant suspension, 3,3-dimethylallyl bromide (9.5 μl , 0.083 mmol) was added at 0 °C. The mixture was then warmed up to room temperature and stirred for 2 h. The reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 , filtered and evaporated.

Purification of the crude product by flash column chromatography on silica gel (hexanes/ethyl acetate = 91/9) to give 12.6 mg of product (58%). ^1H NMR (CDCl_3 , 400 MHz): δ = 6.92 (1H, d, J = 8.3 Hz), 6.90-6.85 (2H, m), 6.76 (1H, d, J = 8.2 Hz), 6.50 (1H, s), 6.37 (1H, s), 5.48 (1H, t, J = 6.5 Hz), 4.60 (2H, d, J = 6.6 Hz), 3.89 (3H, s), 2.86-2.83 (2H, m), 2.61-2.58 (2H, m), 2.51-2.48 (2H, m), 2.20 (2H, t, J = 7.5 Hz), 1.73 (3H, s), 1.69 (3H, s), 1.58-1.52 (2H, m), 1.34-1.25 (2H, m) ppm. ^{13}C NMR (CDCl_3 , 100 MHz): δ = 211.1, 148.9, 148.5, 147.3, 147.2, 137.1, 134.0, 133.5, 124.5, 122.9, 120.4, 119.0, 118.8, 115.7, 112.9, 66.5, 56.2, 43.7, 41.5, 33.1, 29.2, 27.8, 25.8, 21.4, 18.2 ppm. ESI-MS: calc. for $\text{C}_{25}\text{H}_{31}\text{O}_4[\text{M}+\text{H}]^+$: 395.2, found: 395.2. HPLC purity: 96.8% (254 nm), t_R : 7.56 min; 98.5% (220 nm), t_R : 7.56 min.

Biological studies

MIC determination—MIC values were determined against *M. tuberculosis* (H37Rv) and other bacteria using the standard microbroth dilution method exactly as previously described,²⁸ which is based on the methods by the Clinical and Laboratory Standards Institute.²⁹ The maximum test concentration used against *M. tuberculosis* and other pathogens was 200 $\mu\text{g ml}^{-1}$.

Macromolecular synthesis—The effects of selected active compounds **7b**, **7c**, and **7d** (at 1 and 8 $\times$ MIC) on DNA, RNA and protein were determined on mid-logarithmic *E. coli* ΔTolC (OD_{600} = 0.4), by measuring the incorporation of the radiolabeled precursors [methyl- ^3H]thymidine, [5,6- ^3H]uridine and [4,5- ^3H]leucine into macromolecular fractions as previously described.^{30, 31} Briefly, cells were mixed with tested compound for 5 min before the addition of precursors at 1 $\mu\text{Ci ml}^{-1}$. After an additional 20 min, cells were lysed with cold 10% TCA and precipitated macromolecules collected on GF/C filters. After washing with 95% ethanol, GF/C filters were analyzed by liquid scintillation counting. The relative % activity in cells exposed to compounds was determined. Ciprofloxacin (DNA), rifampicin (RNA) and streptomycin (protein) were used as positive controls.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Chemical structures of bioactive diarylheptanoids with a cyclic diphenyl ether moiety.

Figure 2.

Inhibition of macromolecular synthesis by **7b**, **7c**, and **7d**. Indicated are the SEMs from three or more biologically independent experiments. SM = streptomycin (MIC, 1.6 $\mu\text{g ml}^{-1}$); CPFX = ciprofloxacin (MIC, 0.40 $\mu\text{g ml}^{-1}$); RFP = rifampicin (MIC, 12.5 $\mu\text{g ml}^{-1}$).

Scheme 1.

Synthesis of engelhardione **1a** and its regioisomers **1b-c**. Reagents and conditions: (a) 5-10% Pd/C, H₂, Ph₂S (0.05 equiv), CHCl₃, 7-18 h, 78-90%; (b) CuO, K₂CO₃, pyridine, 175 °C, 4.5-5 h, 52-58%; (c) AlCl₃, CH₂Cl₂, reflux, 11-25 h, 31-62%.

Scheme 2.

Synthesis of engelhardione derivatives **5-7**. Reagents and conditions: (a) NaBH₄, MeOH, 0 °C to rt, 20 min, 100%; (b) Hydroxylamine hydrochloride, NaOH, MeOH, reflux, 6 h or R¹ONH₂·HCl, pyridine, EtOH, rt, 78-91%; (c) i) R²NH₂, solvent-free, 70 °C, overnight; ii) NaBH₄, 70 °C, 10 min, 47-78%.

Scheme 3.

Synthesis of engelhardione *O*-alkylated derivatives **13a-f**. Reagents and conditions: (a) methyl chloromethyl ether (MOMCl), DIPEA, CH₂Cl₂, 0 °C to rt, overnight, 94%; (b) 10% NaOH, EtOH, rt, overnight, 62%; (c) 10% Pd/C, H₂, Ph₂S (0.05 equiv), CHCl₃, 18 h, 78%; (d) CuO, K₂CO₃, pyridine, MW, 220 °C, 85%; (e) conc. HCl, MeOH, rt, overnight, 94%; (f) for **13a-d**: RBr, K₂CO₃, acetone, reflux, 47-94%; for **13e**: DIPEA, CH₃CN, reflux, overnight, 90%; for **13f**: NaH, DMF, 0 °C to rt, 2 h, 58%.

Table 1

Antitubercular and antibacterial activities ($\mu\text{g ml}^{-1}$) of linear diarylheptanoid intermediates and macrocyclic engelhardone analogs

Compound	<i>M. tuberculosis</i> (H37Rv)	<i>E. faecalis</i> (ATCC 33186)	<i>S. aureus</i> (ATCC 29213) (MSSA)	<i>S. aureus</i> (NRS 70) (MRSA)	<i>E. coli</i> (K12)	<i>E. coli</i> (TolC)	<i>K. pneumoniae</i> (ATCC 33495)	<i>P. aeruginosa</i> (PAO1)
2a	>200	>200	>200	>200	ND ^a	ND	>200	ND
2b	>200	>200	>200	>200	ND	ND	>200	ND
2c	>200	>200	>200	>200	ND	ND	>200	ND
2d	>200	>200	>200	>200	>200	50	>200	>200
3a	>200	>200	>200	>200	ND	ND	>200	ND
3b	>200	>200	>200	>200	ND	ND	>200	ND
3d	>200	>200	>200	>200	>200	25	>200	>200
4a	>200	>200	>200	200	ND	ND	>200	ND
4b	>200	>200	>200	200	ND	ND	>200	ND
4c	>200	>200	>200	>200	ND	ND	>200	ND
1a	200	>200	>200	>200	ND	ND	>200	ND
1b (engelhardone/ pterocaraine)	200	200	>200	>200	ND	ND	>200	ND
1c	200	>200	>200	>200	ND	ND	>200	ND
5	>200	>200	>200	>200	>200	50	>200	>200
6a	>200	>200	100	>200	>200	>200	>200	>200
6b	>200	>200	>200	>200	>200	>200	>200	>200
6c	>200	>200	>200	>200	>200	>200	>200	>200
7a	200	>200	>200	>200	>200	200	>200	>200
7b	25	25	25	25	>200	12.5	>200	>200
7c	25	100	25	50	>200	25	>200	>200
7d	25	25	25	12.5	>200	12.5	>200	>200
7e	50	100	100	100	>200	25	>200	>200
11	>200	>200	>200	>200	>200	50	>200	>200
12	200	>200	>200	200	>200	50	>200	>200
13a	>200	>200	>200	>200	>200	>200	>200	>200
13b	>200	>200	>200	>200	>200	>200	>200	>200

Compound	<i>M. tuberculosis</i> (H37Rv)	<i>E. faecalis</i> (ATCC 33186)	<i>S. aureus</i> (ATCC 29213) (MSSA)	<i>S. aureus</i> (NRS 70) (MRSA)	<i>E. coli</i> (K12)	<i>E. coli</i> (TolC)	<i>K. pneumoniae</i> (ATCC 33495)	<i>P. aeruginosa</i> (PAO1)
13c	> 200	>200	>200	>200	>200	>200	>200	>200
13d	>200	>200	>200	>200	>200	>200	>200	>200
13e	25	>200	>200	>200	>200	>200	>200	>200
13f	> 200	>200	>200	>200	>200	>200	>200	>200
Vancomycin	0.032-0.064 (INH as control)	0.78	0.39	<0.2	100	100	>200	>200

^a ND indicates not determined.