Oxygenated Cyclohexene Derivatives and Other Constituents from the Roots of *Monanthotaxis trichocarpa*

Gasper Maeda, Jelle van der Wal, Arvind Kumar Gupta, Joan J. E. Munissi, Andreas Orthaber, Per Sunnerhagen, Stephen S. Nyandoro,* and Máte Erdélyi*

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**ABSTRACT:** Three new oxygenated cyclohexene derivatives, trichocarpeols A (1), B (2), and C (3), along with nine known secondary metabolites, were isolated from the methanolic root extract of *Monanthotaxis trichocarpa*. They were identified by NMR spectroscopic and mass spectrometric analyses, and the structure of trichocarpeol A (1) was confirmed by single-crystal X-ray diffraction. Out of the 12 isolated natural products, uvarétin (4) showed activity against the Gram-positive bacterium *Bacillus subtilis* with a MIC value of 18 μM. None of the isolated metabolites was active against the Gram-negative *Escherichia coli* at a ~5 mM (2000 μg/mL) concentration. Whereas 4 showed cytotoxicity at EC₅₀ 10.2 μM against the MCF-7 human breast cancer cell line, the other compounds were inactive or not tested.

**RESULTS AND DISCUSSION**

Using repeated silica gel gravity column chromatography, followed by Sephadex LH-20 gel filtration and preparative reverse-phase HPLC, twelve secondary metabolites were isolated from the roots of *M. trichocarpa*. The structures of the isolated metabolites were established based on their NMR spectroscopic, mass spectrometric, and single-crystal X-ray diffractometric analyses. In addition to the three new oxygenated secondary metabolites 1–3, nine known compounds previously isolated from other plants were identified. The structures of these known compounds, uvarétin (4),13 diuvarétin (5),13 1-(2-hydroxy-4-methoxyphenyl)-2-(4-hydroxyphenyl)propan-1-one (6)14 uvangoletin (7)13 2-methoxybenzyl benzoate (8),19 benzyl benzoate (9)13 cherrevenol H (10),16 and a mixture of stigmasterol (11) and sitosterol (12)16 (Supporting Information, p S2), were established by comparison of their observed and reported spectroscopic data (Supporting Information). The structures of compounds 1 and 4 were further confirmed by single-crystal X-ray analyses.

Compound 1 was obtained as white crystals from a MeOH–CH₂Cl₂ (1:1) solution. Its specific optical rotation, [α]D²⁴ ~−72.1 (c 0.14, MeOH), indicated it to be chiral. HRESIMS
analysis (m/z 417.1105, calc 417.1105 [M + H]+) suggested the molecular formula C_{22}H_{22}O_{7} and indicated 12 degrees of unsaturation. Its chlorine content was revealed by the isotope mass peaks at m/z 419.1100 and 417.1105 with a 1:3 intensity ratio (Figure S8, Supporting Information). The IR absorptions at 3675 and 3499 cm⁻¹ suggested the presence of hydroxy groups. The IR absorption at 1707 cm⁻¹ suggested 1 to possess a conjugated carbonyl system, and those at 1584 and 1601 cm⁻¹ the presence of carbon–carbon double bonds, whereas those at 2968 and 2901 cm⁻¹ indicated the prevalence of aliphatic C–H bonds. An aromatic system and an α,β-unsaturated carbonyl were indicated by the UV absorptions at 274 and 223 nm. ¹H NMR signals were observed (Figure S1, Supporting Information) corresponding to two pairs of protons with integrals of two each at δ_H 7.91 (H-2“/6”) and δ_H 7.98 (H-2“/6”), four protons at δ_H 7.37 (H-3“/5”, 3”/5”), and two overlapping protons at δ_H 7.50 (H-4“/4”) assigned to two aromatic rings (Table 1). The δ_H = 9.9 Hz indicated δ_H 6.00 (H-4) and δ_H 5.88 (H-5) to be cis-olefinic. The COSY correlations (Figure S3, Supporting Information) of H-3 (δ_H 4.73) and H-4 (δ_H 6.00), H-4 and H-5 (δ_H 5.88), H-5 and H-6 (δ_H 5.82), and H-6 and H-1 (δ_H 4.05) (Figure S3, Supporting Information) along with the HMBC cross-peaks (Figure S5, Supporting Information) of H-4 (δ_H 6.00) to C-2 (δ_C 75.6), C-3 (δ_C 80.1), and C-6 (δ_C 71.6) suggested 1 to possess a cyclohexene skeleton. This was confirmed by the TOCSY (Figure S6, Supporting Information) correlations of H-1 (δ_H 4.05), H-3 (δ_H 4.73), H-4 (δ_H 6.00), H-5 (δ_H 5.88), and H-6 (δ_H 5.82). The HMBC cross-peak of OCH₃-1 (δ_H 3.64) to C-1 (δ_C 79.2) indicated the position proposed for this methoxy group. A hydroxy group (δ_H 2.96) was located at C-2 (δ_C 75.6), based on its HMBC cross-peak to C-1 (δ_C 66.6) and the cross-peak of the oxymethylene protons CH₂-1 (δ_H 4.77) to C-2 (δ_C 75.6). Furthermore, HMBC cross-peaks of CH₂-1 and H-2“/6” (δ_H 7.91) to the benzoyl carbonyl C-7” (δ_C 166.8) revealed the linkage of one of the benzoxyl moieties to the cyclohexene skeleton through an oxymethylene unit. The substitution of another benzoxyl functionality to the cyclohexene core was established based on the HMBC cross-peak of H-6 (δ_H 5.82) to C-7” (δ_C 160.0). NOESY cross-peaks (Figure S7, Supporting Information) of δ_H 4.05 (H-1) and δ_H 4.77 (H-1’) indicated their suprafacial orientation. Similarly, the NOEs of H-3 (δ_H 4.73) to OH-2 (δ_H 2.96) and H-6 (δ_H 5.82) and of OH-2 to OCH₃-1 (δ_H 3.64) revealed their syn orientation. The absolute configuration of 1 has been determined by single-crystal X-ray analysis (Figure 1) and was in agreement with the above NMR observations. It indicated that the cyclohexene ring of 1 exists in a chair-chair conformation, with an unexpectedly low trans-axial 〈J_H,H₆〉 = 5.5 Hz. The above spectroscopic and crystallographic evidence revealed compound 1 to be the new chlorinated secondary metabolite trichocarpeol A, which was characterized as ((15S,25,35,6R)-6-benzoxyl-3-chloro-2-hydroxy-1-methoxycyclohex-4-en-2-yl)methyl benzoate. Similar chlorinated cyclohexene derivatives have been previously reported from Piper hookeri,¹⁷ P. nigripes,¹⁸ Dasymaschalon sootepense,¹⁹ Cleistochlamys kirkii,¹⁰ and some Uvaria species.²⁰⁻²²

Compound 2 was isolated as a white solid. It showed the specific rotation [α]²⁴_D = −88.2 (c 0.14, MeOH) and was assigned the molecular formula C_{22}H_{22}O_{7} based on HR-ESIMS ([M + H]+ m/z 399.1453, calc 399.1444, Figure S16, Supporting Information) and NMR data analyses. Its IR absorbance at 3464 cm⁻¹ suggested the presence of a hydroxy group, and that at 1715 cm⁻¹ a conjugated carbonyl functionality and those at 1584 and 1601 cm⁻¹ aromatic moiety. UV absorptions at 274, 228, and 206 nm were in agreement with an aromatic system. Compound 2 showed similar spectroscopic features to compound 1 (Figures S9–S15, Supporting Information), suggesting it to be a cyclohexene derivative, with the only difference being at C-3, which was substituted by a hydroxy group instead of a chlorine atom. The presence of a methoxy group at C-1 (δ_C 80.1) was confirmed by its HMBC cross-peak of δ_H 3.62 (OCH₃-1) (Table 2 and Figure S13, Supporting Information). Similarly to 1, trichocarpeol B (2) is benzoxyl substituted at C-6, as confirmed by the HMBC cross-peak of H-6 (δ_H 5.73) to carbonyl C-7” (δ_C 166.3). As no single crystals suitable for X-ray analysis were obtained, the absolute configuration of 2 could not be determined. However, its most plausible configuration could be established by comparison of its NMR data to that of the structurally closely related 1. The 〈J_H,H₆〉 = 4.5 Hz of H-1 (δ_H 3.92) and H-6 (δ_H 5.73) suggest their cis axial–equatorial configuration. The magnitude of this coupling is very similar to that observed for 1 (5.5 Hz, Table 1), which has a trans diaxial H-1–H-6 configuration (Figure 1).

Table 1. ¹H and ¹³C NMR Spectroscopic Data for Compound 1 (600 MHz, CDCl₃)

| position | δ_C (ppm) | δ_H (J in Hz) | HMBC |
|----------|-----------|---------------|------|
| 1        | 79.2, CH   | 4.05 d (5.5)  | C-1’, C-3, C-5, OCH₃-1, C-2, C-6 |
| OCH₃-1   | 60.3, CH₃ | 3.64 s        | C-1 |
| OH-2     | 75.6, C-O | 2.96 br s     | C-1, C-1’, C-2, C-3 |
| 3        | 58.1, CH   | 4.73 d (3.6)  | C-1, C-1’, C-2, C-4, C-5 |
| 4        | 129.5, CH  | 6.00 ddd (9.9, 3.1, 1.4) | C-2, C-3, C-5, C-6 |
| 5        | 126.4, CH  | 5.88 ddd (9.9, 3.1) | C-1, C-3, C-4 |
| 6        | 71.6, CH   | 5.82 ddd (5.5, 3.1, 1.4) | C-1, C-4, C-5, C-7” |
| 1’       | 66.6, CH₂  | 4.77 d (AA’) (12.6) | C-1, C-2, C-3, C-7 |
| 1”       | 129.58, C  |              |     |
| 2’/6”    | 129.7, CH  | 7.91 dd (8.1, 1.3) | C-7, C-3”, C-4”, C-6” |
| 3”/5”    | 128.5, CH  | 7.37 dd (8.1, 1.3) | C-1’, C-2”, C-4’, C-5’ |
| 4”       | 133.2, CH  | 7.50 tt (7.6, 1.3) | C-2”, C-3”, C-5”, C-6” |
| 7”       | 166.8, C=O |              |     |
| 1”’      | 129.63, C  |              |     |
| 2”/6”    | 129.8, CH  | 7.98 dd (7.6, 1.6) | C-8, C-3”, C-4”, C-6” |
| 3”/5”    | 128.6, CH  | 7.37 dd (7.6, 1.6) | C-1”, C-2”, C-4’, C-5’ |
| 4”       | 133.2, CH  | 7.50 tt (7.6, 1.6) | C-2”, C-3”, C-5”, C-6” |
| 7”       | 166.0, C=O |              |     |

Figure 1: Structure of trichocarpeol A (1). A new chlorinated secondary metabolite from Cleistochlamys kirkii. J. Nat. Prod. 2020, 83, 210–215.
Figure 1. Solid state structures of trichocarpeol A (1) to the left and uvarelan (4) to the right, shown as thermal ellipsoids with 50% probability levels.

Table 2. $^1$H and $^{13}$C NMR Spectroscopic Data for Compound 2 (400 MHz, CD$_2$Cl$_2$)

| position | $\delta_\mathrm{C}$, type | $\delta_\mathrm{H}$ (J in Hz) | HMBC |
|----------|----------------|-----------------|------|
| 1        | 80.1, CH       | 3.92 d (4.5)    | C-2, C-6, C-5, C-3, OCH$_3$-1, C-1$'$ |
| OCH$_3$-1| 60.4, CH$_3$   | 3.62 d (1.7)    | C-1 |
| OH-2     | 76.0, C        | 2.87 br s       | C-1', C-2, C-5, C-4 |
| 3        | 70.5, CH       | 4.39 d (3.4)    | C-1', C-2, C-5, C-4 |
| OH-3     |                | 2.79 br s       | C-3, C-6, C-2 |
| 4        | 131.7, CH      | 5.98 ddd (102.2, 3.4, 1.7) | C-3, C-6, C-2 |
| 5        | 126.2, CH      | 5.83 dd (102.2, 3.4) | C-3, C-6, C-1 |
| 6        | 71.5, CH       | 5.73 ddd (48.4, 3.4, 1.7) | C-4, C-5, C-1, C-7$''$ |
| 1$'$     | 65.9, CH$_2$   | 4.79 d (11.9)   | C-2, C-1, C-3, C-7$''$ |
| 2$''$/5$''$| 129.9, CH       | 7.87 m         | C-2$''$/5$''$, C-3$''$/5$''$, C-1$''$, C-7$''$, C-7$''$ |
| 3$''$/5$''$| 128.8, CH       | 7.34 m         | C-3$''$/5$''$, C-2$''$/6$''$, C-1$''$, C-4$''$ |
| 4$''$    | 130.2, CH      | 7.51 m         | C-3$''$/5$''$, C-2$''$/6$''$ |
| 7$''$    | 167.3, C=C=O   |                | C-1$''$, C-2$''$, C-7$''$ |
| 1$''$    | 133.7, C       |                | C-1$''$, C-2$''$, C-7$''$ |
| 2$''$/6$''$| 130.0, CH       | 7.94 m         | C-2$''$/6$''$, C-3$''$/5$''$, C-1$''$, C-4$''$, C-1$''$ |
| 3$''$/5$''$| 128.9, CH       | 7.34 m         | C-2$''$/6$''$, C-3$''$/5$''$, C-1$''$, C-4$''$, C-1$''$ |
| 4$''$    | 130.2, CH      | 7.51 m         | C-3$''$/5$''$, C-2$''$/6$''$ |
| 7$''$    | 166.3, C=C=O   |                | C-1$''$, C-2$''$, C-7$''$ |

1). However, in contrast to 1, a strong NOE correlation (Figure S15, Supporting Information) of H-1 ($\delta_\mathrm{H} 3.92$) and H-6 ($\delta_\mathrm{H} 5.73$) was observed, indicating these protons to be syn-oriented. The NOEs between H-1 ($\delta_\mathrm{H} 3.92$) and H-1$'$ ($\delta_\mathrm{H} 4.57$ and 4.79) indicated these to be syn-oriented as well and thus revealed the relative configuration of C-2. The NOE of H-3 ($\delta_\mathrm{H} 4.39$) and OH-2 ($\delta_\mathrm{H} 2.87$) as well as the absence of an NOE between H-6 and H-3 suggested C-3 to have the opposite relative configuration as compared to the C-3 of 1. Based on the above spectroscopic evidence, this new compound, trichocarpeol B (2), was characterized as (6β-(benzoyloxy)-2β,3α-dihydroxy-1β-methoxy-cyclohex-4-en-2-yl)methyl benzoate.

Compound 3 was obtained as a white solid and showed a specific rotation of $[\alpha]_D^21 −75.7$ (c 0.14, MeOH). It was assigned the molecular formula C$_{22}$H$_{22}$O$_4$, based on the analyses of HRESIMS ([M + H]$^+$ m/z 413.1572, calcd 413.1600, Figure S24, Supporting Information) and NMR data (Figures S17–S23, Supporting Information) and Table 3). Its IR spectrum suggested the presence of hydroxy (3471 cm$^{-1}$), carbonyl (1716 cm$^{-1}$), and aromatic (1601 and 1584 cm$^{-1}$) groups, with the latter being confirmed by the UV absorptions at 274, 230, and 205 nm. Its NMR data (Table 3, Figures S17–S23, Supporting Information) showed high similarities to those of trichocarpeol B (2), with the only difference being the alkylation of the OH-3 of the latter to a methoxy group. This was indicated by the HMBC cross-peaks of OCH$_3$-3 ($\delta_\mathrm{C} 3.52$) to C-3 ($\delta_\mathrm{C} 79.4$) and of H-3 ($\delta_\mathrm{H} 3.96$) to OCH$_3$-3 ($\delta_\mathrm{C} 59.1$). The relative configuration of H-1 ($\delta_\mathrm{H} 3.88$) and H-6 ($\delta_\mathrm{H} 5.70$) was established as cis axial-equatorial, based on the observed $^3$J$_{\mathrm{H},\mathrm{H}} = 4.0$ Hz, which was similar to trichocarpeol B (2) (4.5 Hz, Table 2) and corroborated by the NOE (Figure S23, Supporting Information) of H-1 ($\delta_\mathrm{H} 3.88$) and H-6 ($\delta_\mathrm{H} 5.70$). Overall, the NOE correlations of compound 3 resembled those
The isolated natural products were evaluated against theGram-positive bacteriumB. subtilisand Gram-negativeE. coli, and for cytotoxicity using human MCF-7 cells. WhereasB. subtilis is nonpathogenic and can serve as a proxy for the microbiological investigation of Gram-positive species, manyE. colistrains are human pathogens and are medically relevant. Compound 4 was active againstB. subtilis with a MIC value of 18.0 μM, and it also inhibited MCF-7 cells with an EC_{50} value of 10.2 μM. All other compounds were either inactive or not tested for their cytotoxicity. None of the isolated metabolites were active against the Gram-negativeEscherichia coli. at ~5 mM (2000 μg/mL) concentration. The bioactivities of compound 4 corroborate the previously reported antimicrobial and anticancer activities of some chalcones.23,24 This is the compound of 10.2 μM.

### EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were determined using an Autopol II Rudolph Research Analytical WLG polarimeter at 589 nm, at 241 °C. UV absorptions were determined using a Shimadzu UV-1650PC ultraviolet visible (UV/vis) spectrophotometer. IR spectra were measured on a PerkinElmer Spectrum One FTIR spectrometer. NMR spectra were acquired on an Agilent MR400-DD2 (400 MHz), Bruker Avance NEO 500 and 600 MHz, or Bruker Avance III HD 800 MHz spectrometers and were processed using the software MestreNova (v12.0.3). Chemical shifts were indirectly referenced to tetramethylsilane, by referencing to the residual solvent signal. Structural assignments were based on 1H, 13C, COSY, TOCSY, NOESY, HSCQ, and HMBC spectra. LC-ESIMS were acquired on an API SCIEX 150 EX PerkinElmer ESIMS (30 eV) spectrometer attached to a PerkinElmer gradient pump system and a 5 mm RP-C18 110 Å column (Gemini), utilizing acetonitrile and 1% formic acid in Milli-Q water (gradient elution using 5−95% acetonitrile over 4 min) as mobile phases. HRESIMS were obtained with a Q-TOF-LC/MS spectrometer with a lockmass-ESI source (Stenhagen Analysis Lab AB, Gothenburg, Sweden) using a 2.1 × 30 mm 1.7 μm RP-C18 column and a H2O−CH3CN gradient (5:95 to 95:5, with 0.2% HCOOH). The isolation process was monitored using analytical thin-layer chromatography (TLC), performed on silica gel 60 F254 (Merck) precoated aluminum plates, and visualized under UV light (254 and 365 nm). Following elution, the TLC plates were sprayed with 4-anisaldehyde reagent followed by heating for identification of UV-negative compounds and for detection of color change of the UV-positive spots. The latter reagent was prepared by mixing 3.5 mL of 4-anisaldehyde with 2.5 mL of concentrated H2SO4 and 5 mL of glacial HOAc, and 90 mL of MeOH. Gravity column chromatography was carried out using silica gel 60 (230−400 mesh). Gel filtration was done using Sephadex LH-20 (Pharmacia) suspended in CH2Cl2−MeOH (1:1). Preparative HPLC was performed on a Waters 600E system using the Chromulan (Pikron Ltd.) software and an RP-C8 Komasil column (250 mm × 25 mm) with a H2O−MeOH gradient (70:30 to 100:0) for 20−40 min with a flow rate of 7 mL/min.

**Plant Material.** Roots of Monanthotaxis trichocarpa were collected in March 2017 from the coastal forest at Mkindani ya Leo, in the Lindi Rural District, Lindi Region, Tanzania, at GPS location 37L 0577335 UTM 8871102. The plant was identified by Mr. F. M. Mbago, a senior taxonomist of the Herbarium, Botany Department, University of Dar es Salaam, where a voucher specimen (FMM-3793) was deposited.

**Extraction and Isolation.** The root bark of M. trichocarpa was air-dried for 2 weeks and then powdered to obtain 581.2 g of plant material. The ground material was then soaked in MeOH for 3 days twice consecutively. The filtrate was concentrated in vacuo on a rotary evaporator at 40 °C to obtain 55 g of root bark crude extract. Gravity column chromatography of the crude extract (55 g) was performed, by adsorbing the extract on silica gel and by gradient elution ranging from 5% ethyl acetate−isohexane to 10% ethyl acetate−MeOH. Altogether, 759 fractions of ca. 100 mL were collected and, based on TLC analysis, were pooled into 102 subfractions. Fractions 21−22, obtained with 5% ethyl acetate−isohexane, were combined and subjected to preparative HPLC, collecting benzyl benzoate (9.10.6 mg) with 90:10 H2O−MeOH. Fractions 51 and 52, obtained with 5% ethyl acetate−isohexane, were combined and purified by preparative HPLC to obtain 2-methoxybenzyl benzoate (8, 1.8 mg), collected with 90:10 H2O−MeOH. Combined fractions 125−127, eluted with 5% ethyl acetate−isohexane, followed by precipitation from MeOH, gave a mixture of stigmasterol (11) and sitosterol (12) (10.0 mg). Fractions 276−285, obtained with 20% ethyl acetate−isohexane, precipitated from the eluent. Washing this precipitate with isohexane afforded colorless needles of trichocarpel A (1, 57.5 mg). Subsequently, the combined fractions 292−331 were separated on a Sephadex column eluting with 1:1 MeOH−CH2Cl2, which resulted in 11 fractions of ca. 1 mL each. Upon standing, fraction 322 crystallized from MeOH−CH2Cl2, to give uvangoletin (7,5.9 mg). Combined fractions 390−396, obtained with 25% ethyl acetate−isohexane, were purified by preparative HPLC, utilizing 90:10 H2O−MeOH, yielding trichocarpel D (3, 6.3 mg) and diuvaretin (5, 4.6 mg), with a 12.9 and 19.8 min elution time, respectively. Fraction 397, obtained with 25% ethyl acetate−isohexane, crystallized from MeOH−CH2Cl2, to give uvaretin (4, 2.2 mg), while fraction 413, obtained with the same gradient, precipitated from MeOH−CH2Cl2 and gave additional uvaretin (4, 3.0 mg). The combined fractions 592−599 were subjected to HPLC utilizing 90:10 H2O−MeOH, yielding trichocarpel C (3, 15.5 mg). HPLC purification of fractions 489−501 yielded cherrevenol H (10, 1.1 mg).

**Trichocarpel A (1):** white crystals (isohexane); [α]D−72.1 (c 0.14, MeOH); UV (MeOH) λmax (log ε) 274 (2.92), 223 (6.40) nm; IR νmax 3675, 3499, 2968, 2901, 1707, 1601, 1584, 1449, 1393, 1381, 1360, 1317, 1278, 1253, 1179, 1140, 1096, 1072, 1027, 950, 966, 927 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 417.1105 [M + H]+ (calcd for C24H25ClO3, 417.1105).

**Trichocarpel B (2):** white solid; [α]D+88.2 (c 0.14, MeOH); UV (MeOH) λmax (log ε) 274 (2.32), 228 (3.36), 206 (3.28) nm; IR νmax 3446, 3056, 1781, 1473, 1423, 1265, 110, 940, 738 cm−1; 1H and 13C NMR data, see Table 2. HRESIMS m/z 399.1453 [M + H]+ (calcd for C23H24O3, 399.1444).

**Trichocarpel C (3):** white solid; [α]D−75.7 (c 0.14, MeOH); UV (MeOH) λmax (log ε) 274 (1.96), 230 (2.93), 205 (3.11) nm; IR νmax 3471, 2934, 1716, 1601, 1584, 1451, 1315, 1272, 1096, 955, 710 cm−1; 1H and 13C NMR data, see Table 3; HRESIMS m/z 413.1572 [M + H]+ (calcd for C24H24O4, 413.1600).

**X-ray Diffraction Analysis of Trichocarpel A (1) and Uvaretin (4).** Single crystals of 1 and 4, obtained from isohexane, were collected on a Bruker D8 Apex-II equipped with a CCD camera using Mo Kα radiation (λ = 0.71073 Å). Crystals were mounted on a fiber loop and fixated using Fomblin oil. Data reduction was performed with SADABS.29 Structures were solved by direct methods and refined by least-squares methods on F2 using the SHELX and the OLEX2 software suites.27,28 The data for 3 were completed at 235 K.27,28 Hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained in geometrical positions to their parent atoms. Anomalous dispersion was used to determine the absolute structure of 1 with a Flack parameter of 0.06(2).29,30 One phenyl ring containing C17 was...
modeled with a positional disorder with partial occupancy of 20% to 80%. The solid-state structure of compound 4 was previously reported from room-temperature measurements without thermal displacement parameters.23,24 The X-ray structure ( cif ) data of 1 (CCDC 1906380) and 4 (CCDC 1908381) have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

**Antibacterial Assays.** The antibacterial activity of the isolated compounds was determined against *Escherichia coli* and *Bacillus subtilis*. The cultures were first redissolved at 10 mg/mL in 100% DMSO, then further diluted 30× in H2O and stored at −20 °C. *E. coli* and *B. subtilis* were cultured as previously described by Mueller and Hintong2,3 and Doyle.3,24 For in vitro determination of antibacterial activity, a culture of bacterial cells was grown to OD 600 μL = 0.5. The culture was diluted 10× with prewarmed medium, and the substances to be tested were added to the culture medium for a final concentration of 30 μg/mL, each at 100 μL in a 96-well microtiter plate. Cultures with substances to be tested were then incubated at 37 °C without agitation for 18 h. To measure cell health, we used the resazurin-based assay as described previously.1,2 Next, 12 μL of AlamarBlue solution (commercial name of resazurin solution, ThermoFisher) was added to each well, and incubation at 37 °C was continued for 1 h. Then, fluorescence was measured using a POLARStar Omega microplate reader from BMG Labtech with the excitation filter set to 544 nm and emission filter to 590 nm. Cells exposed to an equivalent concentration of DMSO only were used as negative control. Before setting up the assay in microtiter format, bleed-through of fluorescence from resorufin between wells in the microtiter plate fluorescence reader was measured and found to be <1% between adjacent wells. To check for quenching of fluorescence by any of the investigated compounds, normally grown bacterial cultures were mixed after 1 h of incubation with resazurin and the compound of interest at the highest concentration to be investigated, and the immediately measured fluorescence was compared with samples without compound added. All tests of compound activity were performed in three independent replicates. All compounds where a reduction of fluorescence by at least 50% relative to the solvent control was observed in any of the species were followed up by additional tests for more accurate determination of the degree of antibacterial activity.

**Cytotoxicity Assay.** The cytotoxicity of the isolated compounds was evaluated against human MCF-7 cells grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and kept in exponential growth as previously reported.3,24 Before the assay, cells were reseeded into 96-well microtiter plates at a density allowing continued exponential growth and allowed to settle for 24 h. The isolated compounds were added from a stock solution in DMSO, for a final concentration of 0.3% v/v of the solvent in the culture medium. After 24 h of incubation in the presence of the compound, cell viability was assayed using PrestoBlue cell viability reagent (ThermoFisher) according to the manufacturer’s instructions. A Polar Star Omega plate reader (BMG Lab Tech) was used to measure resorufluence at 544 nm excitation/590 nm emission. Survival was expressed as percentage of the solvent-only control. EC50 values for each compound were calculated, from three independent replicate experiments, using 2-fold dilution intervals.

The original NMR spectra along with the corresponding NMReDATA36 for the new compounds 1−3 are freely available on Zenodo as DOI: 10.5281/zenodo.3592334.
