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Truncatones A–D, benzo[*j*]fluoranthenes from *Annulohypoxylon* species (Xylariaceae, Ascomycota)

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ABSTRACT

From stromata (fruiting bodies) of three xylariaceous fungi belonging to the genus *Annulohypoxylon* (*A. leptascum*, *A.* cf. *truncatum* and an unidentified *Annulohypoxylon* sp.), truncatone A (**1**), three unprecedented derivatives named truncatones B–D (**2**–**4**) besides the known 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl (BNT, **5**), hypoxylonol C (**6**) and hypoxylonol F (**7**) were isolated. Planar structures of the new benzo[*j*]fluoranthene derivatives **2**–**4** were determined on the basis of NMR and HRESIMS data. While the relative configuration of **4** was demonstrated by ROESY NMR data and $^{1}H,^{1}H$ coupling constants, absolute configurations of **1**, **2**, **4** were determined by CD spectroscopy. Compounds **1**, **3** and **4** exhibited cytotoxicity against the mouse fibroblast cell line L929.

1. Introduction

The genus *Annulohypoxylon* (family Xylariaceae) comprises more than 40 known species worldwide.[1–3] The genus was segregated from the related *Hypoxylon* based on the morphological and molecular data and had previously been known as *Hypoxylon* sect. *Annulata*.[4] The stromata (fruiting bodies) of many species of *Hypoxylon* and phylogenetically related 'hypoxyloid'. Xylariaceae are rich in unique secondary metabolites, many of which are of chemotaxonomic significance.[3,5] From species that are now regarded to belong to *Annulohypoxylon*, azaphilones such as cohaerins and multiformins[6,7]; naphthalene derivatives such as urceolone and hypoxylone[8]; asterriquinones such as truncaquinones[9]; and benzo[*j*]fluoranthene derivatives like truncatone and hypoxylonols[10–12] have been previously reported. While most of these compounds expressed cytotoxic activities, hypoxylonols C–F were also reported to show antiangiogenic activity against endothelial cells.[12,13] In the course of our screening for novel natural products from xylariaceous fungi stromatal extracts of various *Annulohypoxylon* collections exhibited a variety of hitherto unknown compounds. We here describe the isolation and structure elucidation of three new benzo[*j*]fluoranthene derivatives from extracts of three *Annulohypoxylon* species.

2. Results and discussion

Stromata of *Annulohypoxylon* sp., *Annulohypoxylon leptascum* and *A.* cf. *truncatum* were extracted with acetone. Analyses with high-performance liquid chromatography coupled with a diode array detector and a mass spectrometer (HPLC-DAD/MS) showed that all extracts produced the ubiquitous BNT (**5**). The extract of *A. leptascum* contained truncatone A (**1**) as a major component and truncatone B (**2**) in minor amounts. Truncatone C (**3**) along with **1** was obtained from *Annulohypoxylon* sp. Truncatone D (**4**), hypoxylonol C (**6**) and hypoxyonol F (**7**) were isolated from *A.* cf. *truncatum*. The latter were confirmed by comparing the NMR spectroscopic data of the purified metabolites with those reported in the literature[12] (Fig. 1). The crude extracts were separated by column chromatography on Sephadex LH-20 or silica gel, followed by preparative reversed phase HPLC.

HRESIMS analysis of **1** revealed a molecular ion peak at *m*/*z* at 319.0978 with the molecular formula $C_{20}H_{14}O_4$. Detailed analysis of the 1D and 2D NMR data confirmed the structure of truncatone. Truncatone A was first isolated and described in 1998 by Hashimoto

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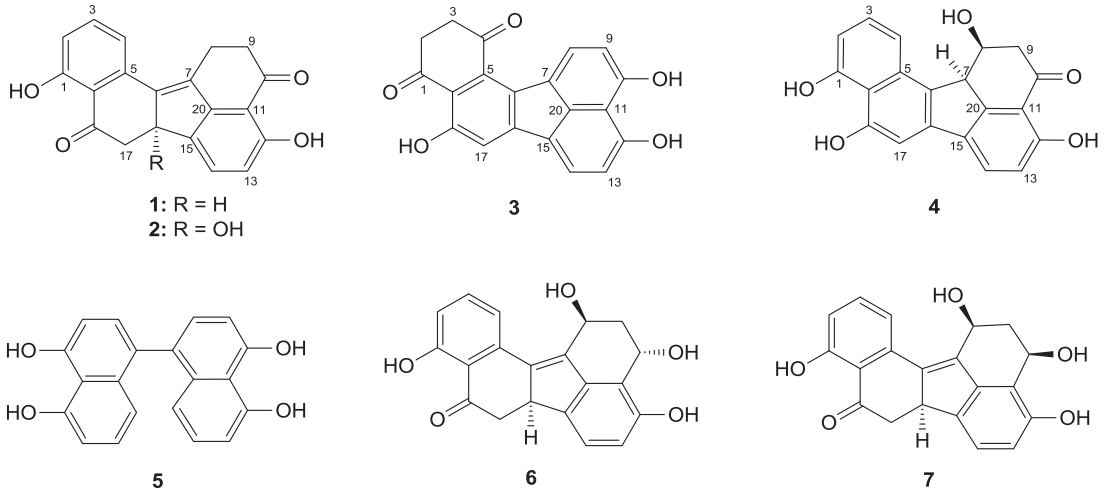

Fig. 1. Structures of secondary metabolites (**1**–**7**) from *Annulohypoxylon* species.

and Asakawa from *Hypoxylon truncatum* (identified later as *Annulohypoxylon annulatum*[1]).[11] Since we provide evidence of several derivatives, we propose to rename **1** to truncatone A. However, no comprehensive NMR data has been published so far nor stereochemistry has been assigned to the sole stereocentre C-16. To determine the configuration at C-16 a structural model of **1** was calculated by PM3 with HyperChem.

Subsequently, the absolute stereochemistry of truncatone A (**1**) was established on the basis of its CD spectrum and exciton chirality analysis.[14,15] Thus, a pair of exciton split Cotton effects at 285 (($\Delta\varepsilon$=−6.47) and 262 nm (($\Delta\varepsilon$=+6.29) in the CD spectrum highlighted a positive (+) torsion angle between its two chromophores according to the exciton chirality method, as shown in the structure model in Fig. 2. The absolute configuration of C-16 was deduced to be *R*.

Truncatone B (**2**) was isolated as a yellow solid and exhibited a molecular ion peak at *m*/*z* 335.0912 (calcd for $C_{20}H_{15}O_5$ 335.0914) which indicated the molecular formula of $C_{20}H_{14}O_5$ and thus the presence of an additional oxygen atom compared to **1**. Its UV/Vis spectrum was identical to that of truncatone A (**1**). The main difference in the ^{1}H NMR spectrum compared to **1** is the disappearance of the proton signal of H-16. The downfield shift of 83.8 ppm at C-16 in the ^{13}C NMR spectrum confirmed the presence of a hydroxyl group at C-16. Regarding its absolute stereochemistry, the negative first at 288 ($\Delta\varepsilon$=−4.7) and positive second Cotton effects at 258 nm ($\Delta\varepsilon$=+3.50) in the CD spectrum were in a strong agreement with those of **1**, confirming the *S* configuration at C-16.

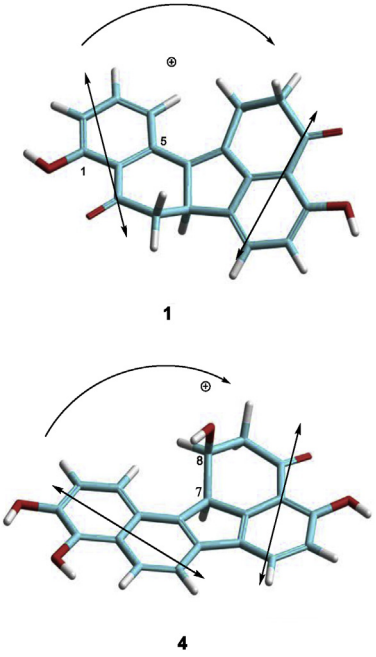

Fig. 2. Model conformation (calculated by pm3 with HyperChem) and exciton interaction between two chromophores of **1** and **4** with positive chirality.

Truncatone C (**3**) was obtained as a yellow solid and its molecular formula was determined to be $C_{20}H_{12}O_5$ by the molecular ion cluster $[M+H]^+$ at *m*/*z* 333.0756 (calcd for $C_{20}H_{13}O_5$ 333.0758) in its HRESIMS spectrum, indicating 15 degrees of unsaturation. The complete structure was elucidated by 1D and 2D NMR spectroscopy. The ^{1}H NMR spectrum displayed signals of two methylenes, five aromatic methines and 5 aromatic protons. The ^{13}C and DEPT NMR spectral data confirmed 20 carbons, including two carbonyl carbons (δ_C 198.8 and 204.5) and three phenol carbons (δ_C 158.0, 160.6 and 162.4). The ^{1}H,^{1}H COSY showed correlations between H-8/H-9, and H-13/H-14, which indicated the evidence of two pairs of ortho-coupled aromatic protons. This, in conjunction with the ^{1}H,^{13}C HMBC correlations (see Fig. 3) showed the presence of a 1,8-dihydroxynaphthalene system. Furthermore, the HMBC correlations of H-2 to C-4/C-19, H-3 to C-1/C-5, OH-18 to C-1/C-19/C-17 and H-17 to C-6/C-19 established the presence of a dihydronaphthoquinone system, which was connected to the naphthalene ring at C-6 and C-16, revealing HMBC correlations of H-8 to C-6 and H-14 to C-16, respectively. The NMR spectra of **3** are closely related to those of hortein,[16] with the only difference being the absence of a hydroxy function at position C-17.

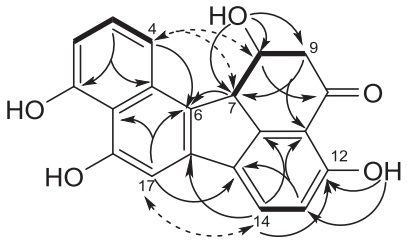

Fig. 3. Selected ^{1}H,^{1}H COSY (bold lines), ^{1}H,^{13}C HMBC (plain arrows) and ROESY (dashed arrows) correlations of **4**.

Truncatone D (**4**) was obtained as a light yellow solid. Its molecular formula was determined to be $C_{20}H_{14}O_5$ on the basis of its $[M+H]^+$ molecular ion cluster at *m*/*z* 335.0908 (calcd for $C_{20}H_{15}O_5$ 335.0914), implying 14 degrees of unsaturation. The ^{1}H NMR

spectrum of **4** exhibited 1 methylene, 2 methines and 6 aromatic protons. The ^{13}C and DEPT spectra showed 20 carbon signals including one carbonyl carbon (δ 203.2), one secondary alcohol (δ 70.9) and 3 phenol carbons (δ 155.9, 156.1 and 159.8). In the ^{1}H NMR spectrum, the methine signal for H-8 (δ 5.42) appeared abnormally low field, which is induced by the anisotropic effect of the conjugated ketone.[17] The dihydroxynaphthalene substructure was demonstrated by $^{1}H,^{1}H$ COSY correlations between H-3/H-2 and H-4 and $^{1}H,^{13}C$ HMBC correlations of H-3 to C-1 and C-5, of H-17 to C-6 and C-19 (Fig. 4). HMBC correlations of OH-8 to C-8/C-7/C-9, of OH-12 to C-12 and C-13 located the secondary alcohol at C-8 and the

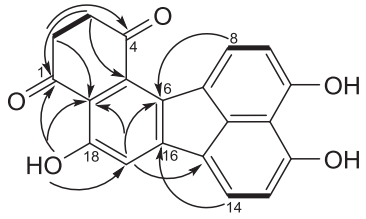

Fig. 4. Selected $^{1}H,^{1}H$ COSY (bold lines) and $^{1}H,^{13}C$ HMBC (arrows) correlations of **3**.

phenol at C-12, respectively.

HMBC correlations of H-8 to C-10, H-9 to C-7 and C-11, H-13 to C-11 and C-15, H-14 to C-12 and C-20 established the second tetralone moiety, which was connected to the naphthalene ring by HMBC correlations of H-7 to C-6, H-14 to C-16 and H-17 to C-15. Further support for this assignment was obtained through NOE correlations between H-4 and H-7/H-8 and between H-14 and H-17. The relative stereochemistry of H-7 and H-8 was identified to be *cis* configuration by utilizing coupling constants and ROESY data. The $^{1}H,^{1}H$ coupling constant $J_{7,8}$=2.5 Hz was supported by a strong NOE effect between H-7 and H-8, both indicating a *gauche* configuration. As a validation the stereo structure model of truncatone D (**4**) was calculated by pm3 using the Configuration Search module in Hyperchem. The small coupling constant was compatible with the calculated dihedral angle of 55.4° ($^{3}J_{HHcalcd.}$=3.9 Hz[†]) representing the relative conformation of the stereocenters. The structure shown in Fig. 2 represented a rigid molecule with the nearly planar naphthalene structure part and twist-boat conformation of cyclohexanone part. Furthermore, the CD spectrum exhibited a positive first exciton split Cotton effect at 328 nm ($\Delta\varepsilon$=+2.35) and a negative second CE at 244 nm ($\Delta\varepsilon$=−4.8*/1), forming a positive (+) torsion angle (positive chirality) between the naphthalene and tetralone chromophores of the molecule. Thus, the chirality between the two long axes of the chromophores is of the clockwise screw sense. Accordingly, the absolute configuration of compound **4** was deduced to be 7*R*, 8*R*.

Due to the low amount and instability of **2**, only compounds **1**, **3** and **4** were tested for antimicrobial and antifungal activity as well as for cytotoxicity against the mouse fibroblast cell line L929. While no activity against the tested bacteria, yeast and fungi were observed, compounds **1**, **3** and **4** exhibited moderate antiproliferative effects against L-929 with IC_{50} values of 3.2, 7.0 and 1.1 μM.

3. Conclusion

Natural occurring benzo[*j*]fluoranthene-based metabolites have been reported from different ascomycetes including *Bulgaria inquinans* (*Helotiales*),[18] *Cladosporium* cf. *cladosporioides* (*Capnodiales*)[19] and various *Xylariaceae* (Xylariales).[10–12,15,20] The presence of different benzo[*j*]fluoranthenes in the stromata of morphologically unrelated *Annulohypoxylon* species points towards a common biosynthetical pathway within the genus. It is proposed that such compounds are derived from 1,8-DHN pathway of the melanin biosynthesis where naphthol precursors are dimerized to form BNT and subsequent oxidation could establish the structure of truncatones.[21] This also explains the omnipresence of BNT in the studied extracts as it most likely functions as precursor of benzo[*j*]fluoranthenes. The structural variation of the stromatal constituents provides the base for chemotaxonomical analysis, a tool that is already frequently used to distinguish species of the genus *Hypoxylon*.[22] It is therefore important to investigate the occurrence of the new compounds within the stromata of *Annulohypoxylon* species to reveal their value as chemotaxonomical marker.

In summary, three new benzo[*j*]fluoranthenes, illustrated by truncatone B (**2**), truncatone C (**3**) and truncatone D (**4**) were isolated from stromata of different *Annulohypoxylon* species with the absolute stereochemistry being assigned for truncatone A (**1**), truncatone B (**2**) and truncatone D (**4**).

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured with a Perkin–Elmer 241 MC instrument, UV data were recorded on a Shimadzu UV–vis spectrophotometer UV-2450 using ethanol (UVASOL, Merck). CD spectra were recorded on JASCO spectropolarimeter, model J-815. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III-500 (^{1}H 500 MHz, ^{13}C 125.8 MHz) and III-700 (^{1}H 700.4 MHz, ^{13}C 176.1 MHz) spectrometer. Electrospray ionization mass spectrometry spectra were obtained with an ion trap MS (Amazon, Bruker), HRESIMS mass spectra were obtained with a maXis ESI-TOF mass spectrometer (maXis, Bruker) attached to an Agilent 1200 series: column 50×2.1 mm, 1.7 μm, C_{18} Acquity UPLC BEH (Waters), solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid in acetonitrile, gradient 5% B for 0.5 min increasing to 100% B in 19.5 min and maintaining 100% B for 5 min, flow rate 0.6 $mLmin^{-1}$; UV detection 200–500 nm. Analytical RP-HPLC was carried out with an Agilent 1260 HPLC system equipped with a UV diode-array detector and a Corona Ultra detector (Dionex); column 125×2 mm, Nucleodur 5 μm C18 (Macherey Nagel), solvent A: 5% acetonitrile in water, 5 $mmolL^{-1}$ NH_4Ac, 0.04 mLL^{-1} acetic acid, solvent B: 95% acetonitrile, 5 $mmolL^{-1}$ NH_4Ac, 0.04 mLL^{-1} acetic acid, gradient from 10% B to 100% B in 30 min, 10 min 100% B, flow rate 0.3 $mLmin^{-1}$.

4.2. Fungal material

A. leptascum was collected from Chiang Rai Province in Thailand in 2013 by E. Kuhnert. Identification of the species was based on morphological, phylogenetic (GenBank Acc. No. KU604580, KU604576) and chemotaxonomic data. Specimen and corresponding culture have been deposited at Mae Fah Luang University (Thailand, Chiang Rai; MFLU 13-0345, MFLUCC 13-0582). Stromata of *Annulohypoxylon* sp. were collected from Argentina (Jujuy, Depto. Ledesma, Calilegua National Park, Calilegua National Park) in 2014 by E. B. Sir (Sir & Hladki 021a, LIL,[‡] BAFC[§]). The species was originally identified as *Annulohypoxylon moriforme* based on morphological data, but molecular data derived from a culture of an identical specimen (Sir & Hladki 023, LIL, BAFC) deposited at the

[†] $^{3}J_{H,H}$ value was calculated according to C.A.G. Haasnoot, F.A.A.M. De Leeuw and C. Altona; *Tetrahedron* **36** (1980) 2783–2792.

[‡] Herbarium of Fundación Miguel Lillo (Argentina, Tucumán, San Miguel de Tucumán).

[§] Herbarium of University of Buenos Aires (Argentina, Buenos Aires).

Helmholtz Centre for Infection Research (Department Microbial Drugs, Braunschweig, Germany; STMA 14068) revealed a distant relationship. *Annulohypoxylon* cf. *truncatum* was obtained from the Jack Brooks Gregory Park, Texas (USA) in 2014 by E. Kuhnert. The material is morphologically very close to *A. truncatum* but the secondary metabolite composition of the stromata set the specimen apart from the latter. Parts of the collection have been deposited with the Staatliches Museum für Naturkunde (Germany, Karlsruhe; KR-M-0046705) and a corresponding culture was sent to CBS-KNAW Fungal Biodiversity Centre (The Netherlands, Utrecht; where it is deposited under acc no. CBS 140788).

4.3. Extraction and isolation

The stromata of *A. leptascum* (800 mg) were subjected to the extraction procedures described below resulting in a 300 mg crude extract. The acetone extract was separated by step gradient SiO_2 flash chromatography [cartridge 40 g, solvent A: dichloromethane, solvent B: dichloromethane/acetone (80/20, v/v), solvent C: dichloromethane/acetone/methanol (56/14/30, v/v/v), first gradient (AB system) from 0% B to 20% B in 20 min, second gradient (BC system) from 20% B to 60% C in 20 min and 60% C to 100% C in 10 min, flow 40 $mLmin^{-1}$, UV detection 260 nm)] to yield four fractions. Fraction 1 (32 mg) was further purified by RP-HPLC [column 21.2×250 mm, Nucleodur 100-10 C_{18} (Macherey-Nagel); solvent A: water, solvent B: acetonitrile, gradient from 30% B to 70% B in 60 min; flow 20 $mLmin^{-1}$, UV detection 260 nm] to give the compounds **1** (5.4 mg) and **2** (0.4 mg).

The stromata of *Annulohypoxylon* sp. (285 mg) were carefully detached from the woody substratum and extracted with acetone (3×100 mL) at room temperature. After removal of the solid material by filtration and concentration of solution in *vacuo*, the residue (137.6 mg) was redissolved in distilled water (100 mL), sonicated and extracted three times with EtOAc. The organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated in *vacuo* to yield 136 mg of crude extract. The extract was then dissolved in acetonitrile and separated by Sephadex LH-20 chromatography in 2-propanol (column 2.5×40 cm; flow 1 $mLmin^{-1}$; UV detection 360 nm). Fractions were collected and combined to the observed peaks. A fraction (15 mg) was further separated by RP-HPLC chromatography [column 10×250 mm, Nucleodur 100-10 C_{18} (Macherey-Nagel); solvent A: water+0.5% formic acid, solvent B: acetonitrile+0.5% formic acid, gradient from 40% B to 70% B in 60 min; flow 6 mL/min, UV detection 360 nm] to give pure amounts of compound **3** (1.04 mg).

The stromata of *Annulohypoxylon* cf. *truncatum* (1.5 g) and the corresponding extract were treated like those of *A. leptascum* to finally yield compound **4** (0.6 mg).

*4.3.1. Truncatone A (**1**).* Yellow solid; $[\alpha]_D^{20}=+13.5^\circ$ (*c* 0.2; $CDCl_3$); UV/VIS (EtOH): λ_{max} (log ε) 202 (4.24), 242 (4.07), 270 (4.02), 285 (4.00), 303 (3.88), 406 (3.95); CD (EtOH) λ_{max} (Δε) 224 (+8.2), 242 (−1.9), 262 (+6.3), 285 (−6.5), 304 (+1.1); NMR spectroscopic data (^1H NMR 500 MHz, ^{13}C 125.8 MHz, $CDCl_3$) see Table 1 and Table 2; HRESIMS: *m/z* for $C_{20}H_{15}O_4$ $[M+H]^+$ calcd 319.0978, found 319.0967.

*4.3.2. Truncatone B (**2**).* Yellow solid; $[\alpha]_D^{20}=\pm0^\circ$ (*c* 0.04; $CDCl_3$),; UV/VIS (EtOH): λ_{max} (log ε) 203 (4.01), 229 (3.81), 243 (sh, 3.80), 263 (3.67), 287 (3.62), 306 (3.55), 403 (3.55); CD (EtOH) λ_{max} (Δε) 246 (−1.8), 258 (+3.5), 288 (−4.7), 314 (0.4); NMR spectroscopic data (^1H NMR 700.4 MHz, ^{13}C 176.1 MHz, $CDCl_3$) see Tables 1 and 2; HRESIMS: *m/z* for $C_{20}H_{15}O_5$ $[M+H]^+$ calcd 335.0914, found 335.0912.

*4.3.3. Truncatone C (**3**).* Yellow solid; UV/Vis (EtOH): λ_{max} (log ε) 209 (4.29), 254 (4.15), 399 (4.20); NMR spectroscopic data (^1H

Table 1
^1H NMR data for truncatones A–D (**1**–**4**) (^1H 500 MHz, **1**; ^1H 700.4 MHz, **2**–**4**)

Pos.	**1**[a]	**2**[b]	**3**[b]	**4**[c]
1	—	—	—	—
2	6.95, dd (8.3, 0.9)	7.00, dd (8.3, 0.7)	3.23, t (6.8)	6.78, br. d (8.0)
3	7.55, dd (8.3, 7.4)	7.58, dd (8.3, 7.4)	3.21, t (6.8)	7.38, t (8.1)
4	7.12, d (7.4)	7.10, d (7.4)	—	7.63, d (8.1)
5	—	—	—	—
6	—	—	—	—
7	—	—	—	4.47, dt (2.5)
8	3.38, m	α: 3.29, m β: 3.40, m	8.94, d (7.4)	5.41, br. dd (5.5, 2.5)
9	2.94, m	2.92, m	6.99, d (7.4)	α:2.88, ddd (17.6, 5.5, 2.5) β: 3.27, dd (17.6, 2.5)
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
13	6.81, d (8.1)	6.83, d, (8.2)	7.04, d (5.8)	6.95, d (8.4)
14	7.53, d (8.1)	7.62, d, (8.2)	8.19, d (5.8)	7.88, d (8.4)
15	—	—	—	—
16	4.1, ddt (14.0, 5.6, 2.5)	—	—	—
17	α: 3.4, dd (16.4, 5.6) β: 2.4, dd (16.4, 14.0)	α: 3.49, d, (16.4) β: 2.68, d, (16.4)	7.81, s	7.20, s
18	—	—	—	—
19	—	—	—	—
20	—	—	—	—
OH-1	12.55, s	12.42, s	—	8.59, br. s
OH-8	—	—	—	3.28, s
OH-10	—	—	—	—
OH-12	10.62, s	10.60, s	8.11, s	10.70, s
OH-16	—	7.12, s	—	—
OH-18	—	—	13.24, s	8.59, br. s

^1H NMR spectra were recorded in [a]) chloroform-d_1, [b]) acetone-d_6, [c]) acetonitrile-d_3.

Table 2
^{13}C NMR data for truncatones A–D (**1**–**4**) (^{13}C 125.8 MHz, **1**;^{13}C 176.1 MHz, **2**–**4**)

Pos.	**1**[a]	**2**[a]	**3**[b]	**4**[c]
1	163.3, C	162.9, C	204.5, C	156.1, C
2	117.6, CH	117.9, CH	37.8, CH_2	109.6, CH
3	136.9, CH	136.6, CH	39.9, CH_2	128.9, CH
4	117.1, CH	118.0, CH	198.8, C	116.8, CH
5	137.6, C	134.9, C	131.4, C	135.3, C
6	138.6, C	139.8, C	129.5, C	130.8, C
7	132.4, C	131.9, C	126.1, C	51.6, CH
8	22.7, CH_2	22.2, CH_2	131.2, CH	70.9, CH
9	36.4, CH_2	36.1, CH_2	110.8, CH	47.9, CH_2
10	201.5, C	201.2, C	158.0, C	203.2, C
11	112.8, C	113.1, C	112.7, C	116.5; C
12	159.2, C	160.5, C	160.6, C	159.8, C
13	114.1, CH	114.8, CH	111.3, CH	116.6, CH
14	130.7, CH	130.0, CH	125.7, CH	128.9, CH
15	134.7, C	135.9, C	124.9, C	133.8, C
16	49.0, CH	83.8, C	149.6, C	153.1, C
17	42.5, CH_2	48.5, CH_2	114.1, CH	102.8, CH
18	203.9, C	202.7, C	162.4, C	155.9, C
19	114.5, C	114.5, C	116.5, C	114.3, C
20	150.1, C	147.5, C	139.1, C	142.2, C

^{13}C NMR spectra were acquired in [a]) chloroform-d_1, [b]) acetone-d_6, [c]) acetonitrile-d_3.

700.4 MHz, ^{13}C 176.1 MHz, CD_3COCD_3): see Tables 1 and 2; HRESIMS: *m/z* for $C_{20}H_{13}O_5$ $[M+H]^+$ calcd 333.0758, found 333.0756.

*4.3.4. Truncatone D (**4**).* Yellow solid; $[\alpha]_D^{20}=+61.7°$ (*c* 0.02; EtOH) UV/VIS (EtOH): λ_{max} (log ε) 216 (3.02), 273 (4.02), 282 (4.02), 345 (3.62), 364 (3.61); CD (EtOH) λ_{max} (Δε) 205 (+4.0), 244 (−4.8), 328 (+2.4); NMR spectroscopic data (^1H 700.4 MHz, ^{13}C 176.1 MHz, CD_3CN): see Tables 1 and 2; HRESIMS: *m/z* for $C_{20}H_{15}O_5$ $[M+H]^+$ calcd 335.0914, found 335.0908.

4.4. Biological assays

4.4.1. MIC assay. Minimum inhibitory concentrations (MICs) in μg/mL were determined in 96-well microtiter plates with EBS medium (0.5% peptone, 0.5% glucose, 0.1% meat extract, 0.1% yeast extract, 50 mM HEPES for bacteria and MYC medium for yeasts and fungi, respectively. A total of 2 μL and 10 μL of a 1 mg/ml concentration (67 and 33.3 μgmL^{-1}) were tested. Negative control wells were left blank. Compounds were dissolved in 2-propanol. Cell density was adjusted to about 5×10^6 mL^{-1}.

4.4.2. Cytotoxicity assay. Cytotoxicity (IC_{50}) was determined in vitro (50 μL from a concentration of 1 $mgmL^{-1}$ and 0.1 $mgmL^{-1}$; end concentration in the first well: 37.03 μgmL^{-1} and 3.7 μgmL^{-1}, respectively) against the mouse fibroblast cell line L929. Cell line L929 was cultured in DMEM (Lonza). All media were supplemented with 10% fetal bovine serum (Gibco) and incubated under 10% CO_2 at 37 °C. The MTT (2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used for the cytotoxicity assay on 96-well microplates.[23] 2-propanol was used as negative control.

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Appendix A. Supplementary data

Supplementary data (1D and 2D NMR spectra, UV spectra and CD spectra) related to this article can be found at http://dx.doi.org/10.1016/j.tet.2016.08.054.

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