# Supplementary Materials for

# Bio-Activity and Dereplication Based Discovery of Ophiobolins and Other Fungal Secondary Metabolites Targeting Leukemia Cells

**Contents**


1. Strains tested in the initial screening against chronic lymphocytic leukemia (CLL) ......... S4
2. Strains found active against chronic lymphocytic leukemia (CLL) ......... S6
3. Penicillic acid isolated from *Penicillium pulvillorum* (IBT 22393) ......... S8
4. Viridicatumtoxin isolated from *P. brasilianum* (IBT 22244) ......... S9
5. Emestrin A isolated from *Aspergillus* sp. (*Emericella*-like state) (IBT 22838) ......... S10
6. Neosolaniol monoacetate isolated from *Fusarium compactum* (IBT 9034) ......... S12
7. Ophiobolins ......... S13

7.1 Potential ophiobolin producing strains from the *Aspergilli* section *Usti* ......... S13

7.2 NMR data for ophiobolin U, ophiobolin K, 6-epiophiobolin K, ophiobolin C, ophiobolin H, 6-epiophiobolin H, and 6-epiophiobolin G ......... S14

7.3 Ophiobolin U isolated from *A. insuetus* (IBT 28266) ......... S16

7.4 Ophiobolin H isolated from *A. insuetus* (IBT 28266) ......... S18

7.5 6-epiophiobolin N isolated from *A. insuetus* (IBT 28266) ......... S20

7.6 Ophiobolin K isolated from a new sp. in the *Aspergillus* section *Usti* (IBT 18591) ......... S22

7.7 6-epiophiobolin K isolated from a new sp. in the *Aspergillus* section *Usti* (IBT 18591) ......... S24

7.8 Ophiobolin C isolated from *A. calidoustus* (IBT 25726) ......... S26

7.9 6-epiophiobolin G isolated from *A. calidoustus* (IBT 25726) ......... S28

7.10 Activity of ophiobolin A, B, C and K + 6-epiophiobolin K towards healthy fibroblasts (Wi-38) cells ......... S30

7.11 Apoptosis induction in CLL cells by different ophiobolins. ......... S31

**List of Figures**


Figure S1. $^{1}$H-NMR spectrum of penicillic acid in DMSO-$d_6$ at 500 MHz..................................... S8
Figure S2. $^{1}$H-NMR spectrum of viridicatumtoxin in DMSO-$d_6$ at 400 MHz................................. S9
Figure S3. $^{1}$H-NMR spectrum of emestrin A in DMSO-$d_6$ at 500 MHz....................................... S10
Figure S4. $^{1}$H-NMR spectrum of neosolaniol monoacetate in DMSO-$d_6$ at 500 MHz.................. S12
Figure S5. $^{1}$H-NMR spectrum of ophiobolin U in $CDCl_3$.at 800 MHz.......................................... S16
Figure S6. $^{13}$C-NMR spectrum of ophiobolin U in $CDCl_3$.at 200 MHz. ....................................... S16
Figure S7. $^{1}$H-NMR spectrum of ophiobolin H in $CDCl_3$.at 800 MHz.......................................... S18
Figure S8. $^{13}$C-NMR spectrum of ophiobolin H in $CDCl_3$.at 200 MHz. ....................................... S18
Figure S9. $^{1}$H-NMR spectrum of 6-epiophiobolin N in $CDCl_3$.at 800 MHz. ................................ S20
Figure S10. $^{13}$C-NMR spectrum of 6-epiophiobolin N in $CDCl_3$.at 200 MHz. ............................. S20
Figure S11. $^{1}$H-NMR spectrum of ophiobolin K in $CDCl_3$.at 800 MHz........................................ S22
Figure S12. $^{1}$H-NMR spectrum of 6-epiophiobolin K in $CDCl_3$.at 800 MHz. .............................. S24
Figure S13. $^{13}$C-NMR spectrum of 6-epiophiobolin K in $CDCl_3$.at 200 MHz............................... S24
Figure S14. $^{1}$H-NMR spectrum of ophiobolin C in $CDCl_3$.at 800 MHz........................................ S26
Figure S15. $^{1}$H-NMR spectrum of 6-epiophiobolin G in $CDCl_3$.at 800 MHz. .............................. S28
Figure S16. $^{13}$C-NMR spectrum of 6-epiophiobolin G in $CDCl_3$.at 200 MHz. ............................. S28
Figure S17. Activity of ophiobolin A, B, C and K + 6-epiophioboln K towards healthy fibroblasts (Wi-38)................................................................................................ S30
Figure S18. CLL cells cultured in DMEM were treated for 24 hours with two different concentrations of ophiobolin A, ophiobolin B, ophiobolin C and ophiobolin K. In order to analyze apoptosis induction, cells of each well were divided into two parts and analyzed by flow cytometry using two different staining strategies. (a) cell survival was analyzed by gating on cells that were negative for Annexin V-phycoerythrin (PE) and 7-aminoactinomycin (7-AAD). Relative survival compared to DMSO control (0.1%) is depicted as mean values +SD of four independent CLL samples. (b) Caspase-3 activity is depicted as mean values +SD of four independent CLL samples relative to DMSO control (0.1%). ........................................................................................ S31

**List of Tables**

Table S1. Strains tested in the initial screening.................................................................................. S4
Table S2. Strains found active in the initial screening .................................................................... S6
Table S3. $^{1}$H and $^{13}$C-NMR for penicillic acid in DMSO at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C. .......................................................................................................................... S8
Table S4. $^{1}$H-NMR for viridicatumtoxin in DMSO at 500 MHz. .................................................. S9
Table S5. $^{1}$H and $^{13}$C-NMR for emestrin A in DMSO at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C.. S11
Table S6. $^{1}$H-NMR for neosolaniol monoacetate in DMSO at 500 MHz. ..................................... S12
Table S7. *Aspergilli* section *Usti*. .......................................................................................... S13
Table S8. $^{13}$C-NMR data for ophiobolin U, ophiobolin K, 6-epiophiobolin K, ophiobolin C, ophiobolin H, 6-epiophiobolin H, and 6-epiophiobolin G. (*125 MHz in dimethyl sulfoxide (DMSO)-$d_6$, †125 MHz in $CDCl_3$, or ‡200 MHz in $CDCl_3$, $\delta_C$). .................. S14
Table S9. $^{1}$H-NMR data for ophiobolin U, ophiobolin K, 6-epiophiobolin K, ophiobolin C, ophiobolin H, 6-epiophiobolin H, and 6-epiophiobolin G. (*500 MHz in DMSO-$d_6$, †500 MHz in $CDCl_3$, or ‡ 800 MHz in $CDCl_3$, $\delta_H$ mult. ($J$ (Hz)))........................... S15
Table S10. $^{1}$H and $^{13}$C-NMR for ophiobolin U in $CDCl_3$.at 800 MHz for $^{1}$H and 200 MHz for $^{13}$C.................................................................................................................. S17
Table S11. $^{1}$H and $^{13}$C-NMR for ophiobolin H in $CDCl_3$.at 800 MHz for $^{1}$H and 200 MHz for $^{13}$C.................................................................................................................. S19
Table S12. $^{1}$H and $^{13}$C-NMR for 6-epiophiobolin N in $CDCl_3$.at 800 MHz for $^{1}$H and 200 MHz for $^{13}$C. ............................................................................................................ S21
Table S13. $^{1}$H and $^{13}$C-NMR for ophiobolin K in DMSO-$d_6$.at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C. ............................................................................................................ S23
Table S14. $^{1}$H and $^{13}$C-NMR for 6-epiophiobolin K in DMSO-$d_6$.at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C.................................................................................................... S25
Table S15. $^{1}$H and $^{13}$C-NMR for ophiobolin C in $CDCl_3$.at 800 MHz for $^{1}$H and 200 MHz for $^{13}$C. .............................................................................................................27
Table S16. $^{1}$H and $^{13}$C-NMR for 6-epiophiobolin G in $CDCl_3$.at 800 MHz for $^{1}$H and 200 MHz for $^{13}$C. ............................................................................................................ S29

## 1. Strains tested in the initial screening against chronic lymphocytic leukemia (CLL)

**Table S1.** Strains tested in the initial screening.

| Isolate number | Species | Isolate number | Species |
|---|---|---|---|
| 21947 | *Aspergillus cystallinus* | 22516, 22523 | *Penicillium faroense* |
| 3234, 5265 | *Aspergillus cavernicola* | 21051 | *Penicillium cluniae* |
| 24813 | *Aspergillus granulosus* | 11843 | *Penicillium decumbens* |
| 24666 | *Aspergillus conjunctus* | 26291 | *Penicillium bialowiezense* |
| 10526, 18590 | *Aspergillus caespitosus* | 18329 | *Penicillium brevicompactum* |
| 22551 | *Aspergillus diversus* | 22244 | *Penicillium brasillianum* |
| 22564, 24812 | *Aspergillus funiculosis* | 22393 | *Penicillium pulvillorum* |
| 11054 | *Aspergillus deflectus* | 23856 | *Penicillium svalbardense* |
| 22568 | *Aspergillus varians* | 23667 | *Penicillium lapatayae* |
| 4537, 28161 | *Aspergillus pseudoustus* | 16536 | *Penicillium alpinium* |
| 14906, 23076 | *Aspergillus subvesicolor* | 14084 | *Penicillium caseium* |
| 10525 | *Aspergillus neocaespitos* | 16545 | *Penicillium pinicola* |
| 23282 | *Aspergillus microcysticus* | 22760 | *Penicillium aquamarinum* |
| 24752, 22153 | *Aspergillus karnakataense* | 22662 | *Penicillium groenlandense* |
| 26386, 22274 | *Aspergillus janus* | 24414 | *Penicillium algidum* |
| 17337 | *Aspergillus aureolatus* | 24411 | *Penicillium jamesonlandense* |
| 11835 | *Aspergillus sydowii* | 22544 | *Penicillium eidiense* |
| 10127 | *Aspergillus quasivesicolor* | 22356 | *Penicillium monticola* |
| 18591 | New sp. in the *Aspergillus* section *Usti* | 22663 | *Penicillium floccosum* |
| 21781, 22558 | *Aspergillus brevis* | 17760 | *Penicillium artemision* |
| 13670, 13691 | *Aspergillus neopuniceus* | 14073 | *Penicillium wyomingense* |
| 20587 | *Aspergillus neoustus* | 13954 | *Penicillium minitum* |
| 16756 | *Aspergillus panamensis* | 24420 | *Penicillium rivulorum* |
| 11860, 11847 | *Aspergillus protuberus* | 29798 | *Penicillium diversicolor* |
| 11821, 25061 | *Aspergillus vesicolor* | 16537 | *Penicillium ribeum* |
| 23160 | *Aspergillus subsessilis* | 16625 | *Penicillium turcosum* |
| 22554, 25041 | *Aspergillus ustus* | 22779 | *Penicillium jugorum* |
| 17674 | *Aspergillus pseudoversicolor* | 30014 | *Penicillium galathea* |
| 4332 | *Aspergillus neovesicolor* | 30013 | *Penicillium nassarsuaquense* |
| 18288 | *Aspergillus arizonae* | fel 248, cml 832 | *Pestalotiopsis* |
| 13989 | *Aspergillus aculeatus* | fel 64 | *Arthrinium phaeospermum* |
| 22838 | *Aspergillus sp. (Emericella-like state)* | fel 192 | *Ascochyta* |

**Table S1.** *Cont.*

| Isolate number | Species | Isolate number | Species |
|---|---|---|---|
| 9194 | *Cylindrocarpon olidum* | fell 01 | *Acremonium* |
| 8893 | *Fusarium poae* | fel 240 | *Paraconiothyrium* |
| 8988 | *Fusarium avenaceum* | cml 1707 | *Nodulisporium* |
| 8979, 9077, 9115 | *Fusarium equiseti* | fel 89, fel 355, fel 364 | *Phomopsis* |
| 8977 | *Fusarium tricinctum* | fel 302 | *Cytospora* |
| 8945, 7785 | *Epicoccum nigrum* | fel 159 | *Bipolaris sp.* |
| 9034 | *Fusarium compactum* | fel 06 | *Glomerella cingulata* |
| 9044 | *Fusarium culmorum* | fel 307, fel 308 | *Lecanicillium pasalliotae* |
| 9063 | *Fusarium cerealis* | fel 17, fel 42 | *Paraconiothyrium sporulosum* |
| 9085 | *Fusarium oxysporum* | fel 05, fel 30, cml 1716 | *Periconia* |
| 9096 | *Fusarium redolens* | fel 299A | *Curvularia* |
| 9089 | *Fusarium merismoides* | fel 142 | *Tubercularia sp.* |
| 9087 | *Fusarium torulosum* | fel 315 | *Verticillium leptobactum* |
| 9086, 9206 | *Fusarium solani* | cml 1692 | *Lasiodiplodia theobromae* |
| 9103 | *Fusarium flocciferum* | cml 1709 | *Collototrichum crassipes* |
| 9112, 9121 | *Fusarium pallidoroseum* | cml 1681 | *Cuvularia prasadii* |
| 9107 | *Fusarium proliferatum* | cml 1671 | *Libertella* |
| 9117 | *Fusarium verticillioides* | fel 58, cml 1702, cml 1703, cml 1690 | *Microsphaeropsis* |
| 1807 | *Fusarium sambucinam* | cml 1670A | *Clonostachys* |
| 9184 | *Fusarium chlamydosporum* | cml 1693 | *Spegazzinia deightonii* |
| 9181 | *Fusarium neohelle* | cml 1695 | *Virgatospora echinofibrosa* |
| 9182 | *Fusarium neochlam* | fel 09, fel 241 | *Paraconiothyrium brasiliense* |

## 2. Strains found active against chronic lymphocytic leukemia (CLL)

**Table S2.** Strains found active in the initial screening.

| Isolate number | Species | Maximum activity observed on media |
|---|---|---|
| 9103 | *Fusarium occiferum* | YES |
| 9034 | *Fusarium compactum* | YES |
| 22838 | *Aspergillus sp. (Emericella-like state)* | YES |
| 13989 | *Aspergillus aculeatus* | CYA |
| 23105 | *Aspergillus pseudovesicolor* | YES |
| 3235 | *Aspergillus cavernicola* | YES |
| 18950 | *Aspergillus caespitosus* | YES |
| 22564 | *Aspergillus funiculosis* | YES |
| 22551 | *Aspergillus diversus* | YES |
| 14906 | *Aspergillus subvesicolor* | CYA |
| 23282 | *Aspergillus microcysticus* | CYA |
| 24752 | *Aspergillus karnakataense* | YES |
| 18951 | New sp. in the *Aspergillus* section *Usti* | YES |
| 20587 | *Aspergillus neoustus* | YES |
| 23160 | *Aspergillus subsessilis* | CYA |
| 26291 | *Penicillium bialowienze* | YES |
| 18329 | *Penicillium brevicompactum* | YES |
| 21051 | *Penicillium cluniae* | YES |
| 22393 | *Penicillium pulvillorum* | YES |
| 22244 | *Penicillium brasilianum* | YES |
| 11843 | *Penicillium decumbens* | YES |
| 18288 | *Aspergillus arizonae* | CYA |
| 16545 | *Penicillium pinicola* | YES |
| 22760 | *Penicillium aquamarinum* | CYA |
| 22523 | *Penicillium faroense* | YES |
| 22662 | *Penicillium groenlandense* | ALK |
| 24411 | *Penicillium jamesonlandense* | CYA |
| 22544 | *Penicillium eidense* | YES |
| 22356 | *Penicillium monticola* | CYA |
| 22663 | *Penicillium occosum* | CYA |
| 17760 | *Penicillium artemision* | YES |
| 29798 | *Penicillium diversicolor* | YES |
| 16537 | *Penicillium ribeum* | YES |
| 16625 | *Penicillium turcosum* | ALK |

**Table S2**. *Cont*.

| **Isolate number** | **Species** | **Maximum activity observed on media** |
|---|---|---|
| 22779 | *Penicillium jugorum* | CYA |
| 30014 | *Penicillium galathea* | CYA |
| fel 248 | *Pestalotiopsis* | MEA |
| fel 64 | *Arthrinium phaeospermum* | MEA |
| fel 355 | *Phomopsis* | MEA |
| fel 302 | *Cytospora* | MEA |
| fel 159 | *Bipolaris sp* | YES |
| fel 308 | *Lecanicillium psalliotae* | YES |
| fel 240 | *Paraconiothyrium brasiliense* | YES |
| fel 17 | *Paraconiothyrium sporulosum* | YES |
| fel 05, fel 30 and cml 1716 | *Periconia* | YES |
| fel 299A | *Curvularia* | YES |
| fel 142 | *Tubercularia* | YES |
| cml 1692 | *Lasiodiplodia theobromae* | YES |
| cml 1709 | *Collototrichum crassipes* | YES |
| cml 1692 | *Libertella* | YES |
| cml 1703, cml 1690 | *Microphaeropsis* | YES |
| cml 1707 | *Nodulisporium* | YES |
| cml 1670A | *Clonostachys* | YES |
| cml 1693 | *Spegazzinia deightonii* | YES |
| cml 1695 | *Virgatospora echinofibrosa* | YES |

## 3. Penicillic acid isolated from *Penicillium pulvillorum* (IBT 22393)

**Figure S1.** $^{1}H$-NMR spectrum of penicillic acid in DMSO-$d_6$ at 500 MHz

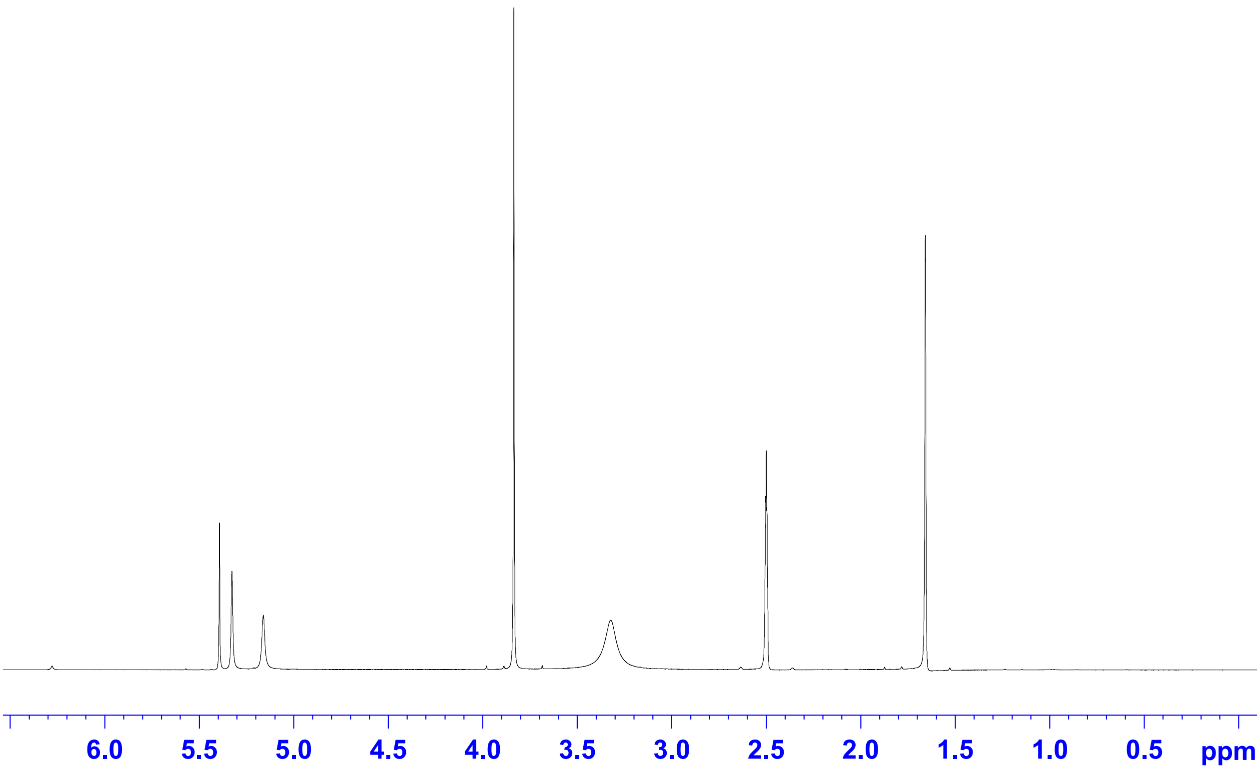
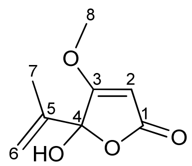



**Table S3.** $^{1}H$ and $^{13}C$-NMR for penicillic acid in DMSO at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$.




| | $\delta_H$ mult. ($J$ (Hz)) | $\delta_C$ * |
|---|---|---|
| 1 | | 169.8 |
| 2 | 5.39 1 H, s | 89.6 |
| 3 | | 178.6 |
| 4 | | 106.3 |
| 4-OH | 3.33 1 H, br.s. | |
| 5 | | 140.4 |
| 6a | 5.16 1 H, s | 115.4 |
| 6b | 5.33 1 H, br.s. | 115.4 |
| 7 | 1.65 3 H, s | 16.9 |
| 8 | 3.84 3 H, s | 58.5 |

* $^{13}C$-NMR chemical shifts determined from HSQC and HMBC experiments.

## 4. Viridicatumtoxin isolated from *P. brasilianum* (IBT 22244)

**Figure S2.** $^{1}$H-NMR spectrum of viridicatumtoxin in DMSO-$d_6$ at 400 MHz.

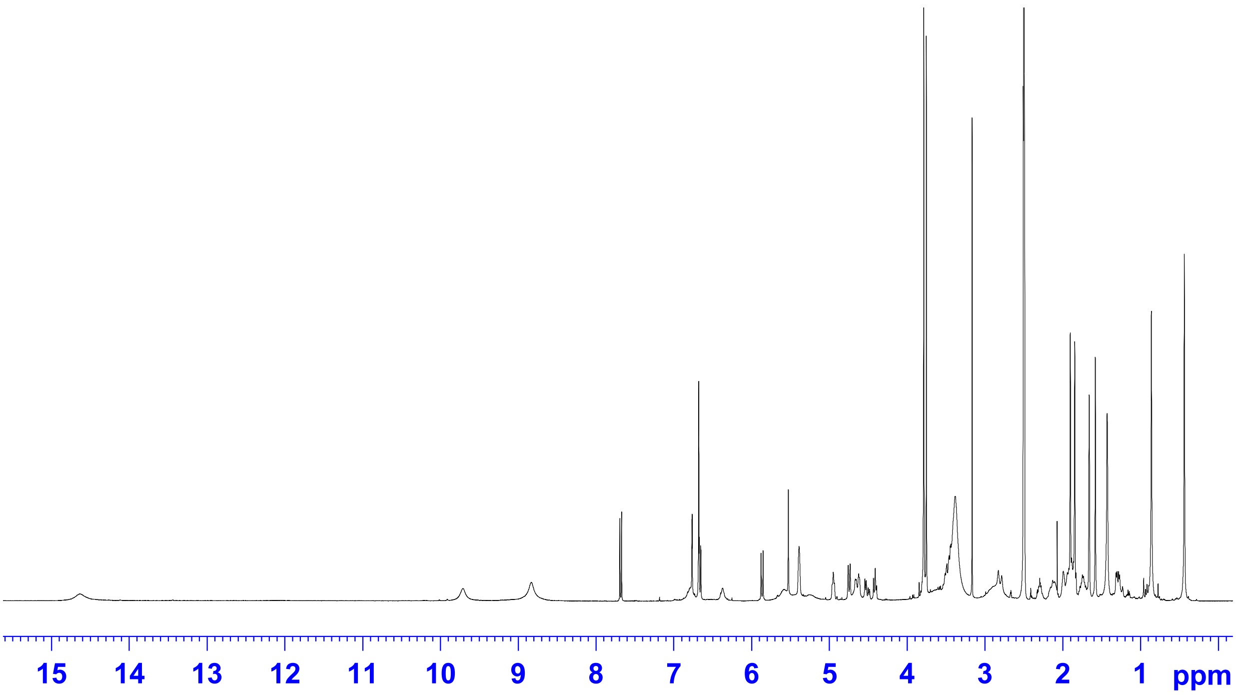
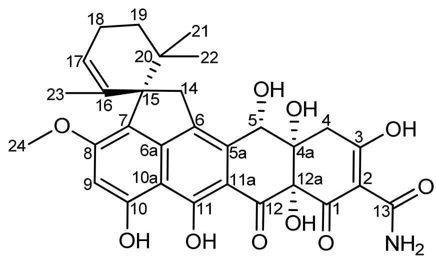



**Table S4.** $^{1}$H-NMR for viridicatumtoxin in DMSO at 500 MHz.

| | $\delta_H$ mult. (*J* (Hz)) |
|---|---|
| 4α | 2.55 (1H, m) |
| 4β | 2.81 (1H, m) |
| 5 | 4.66 (1H, br.s.) |
| 9 | 6.68 (1H, s) |
| 14α | 2.91 (1H, m) |
| 14β | 3.17 (1H, m) |
| 17 | 5.40 (1H, br.s.) |
| 18α | 1.98 (1H, m) |
| 18β | 2.13 (1H, m) |
| 19α | 1.30 (1H, dd, 5.6, 12.8) |
| 19β | 1.75 (1H, m) |
| 21 | 0.45 (3H, s) |
| 22 | 0.87 (3H, s) |
| 23 | 1.43 (3H, s) |
| 24 | 3.79 (3H,s) |
| OH | 5.59 (1H, br.s.) |
| OH | 6.79 (1H, br.s.) |
| OH | 8.84 (1H, br.s.) |
| OH | 9.73 (1H, br.s.) |
| OH | 14.64 (1H, br.s.) |



## 5. Emestrin A isolated from *Aspergillus* sp. (*Emericella*-like state) (IBT 22838)

**Figure S3.** $^1$H-NMR spectrum of emestrin A in DMSO-$d_6$ at 500 MHz.

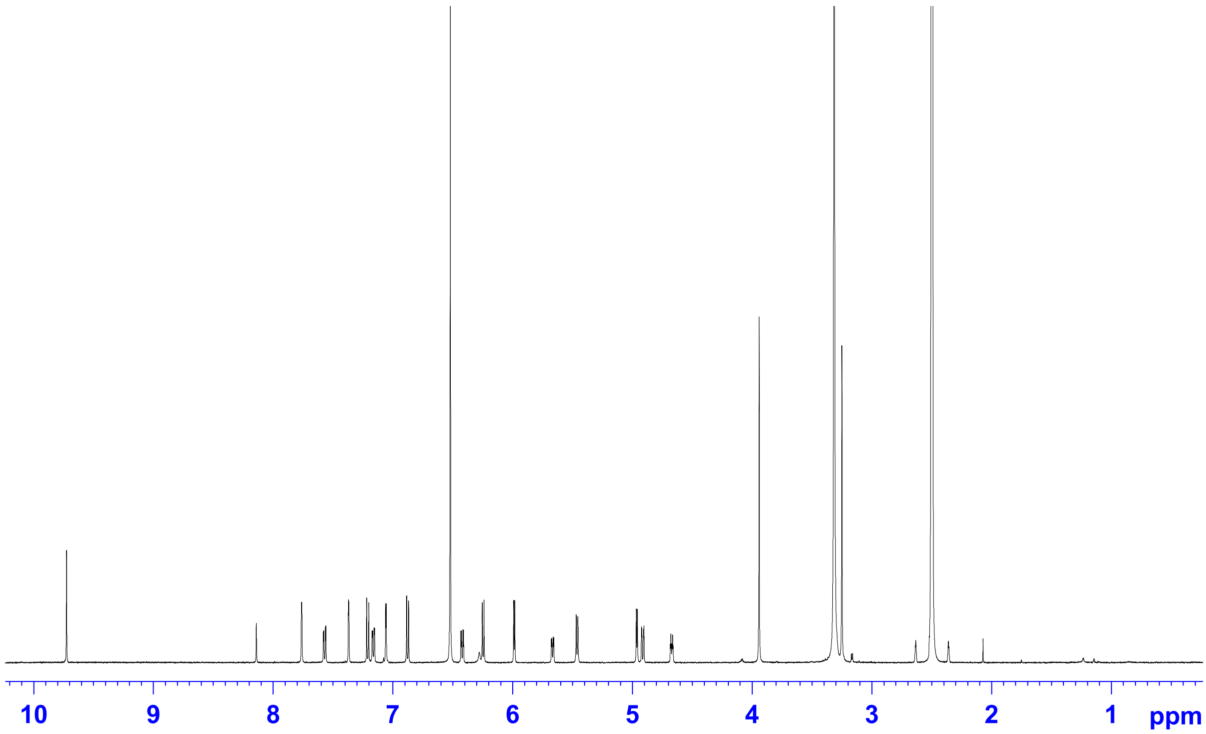
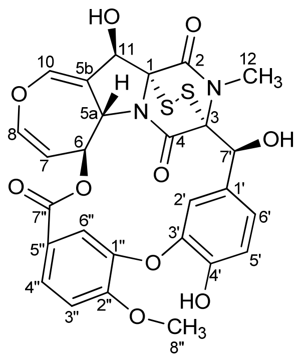


**Table S5.** $^{1}$H and $^{13}$C-NMR for emestrin A in DMSO at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C.

| | $\delta_H$ mult. (*J* (Hz)) | $\delta_C$ * |
|---|---|---|
| 1 | | 80.8 ‡ |
| 2 | | 164.2 † |
| 3 | | 80.8 ‡ |
| 4 | | 164.2 † |
| 5a | 5.67 1 H, dd (2.5, 7.4) | 59.8 |
| 5b | | 160.2 |
| 6 | 4.67 1 H, ddd (2.0, 2.5, 7.4) | 74.6 |
| 7 | 4.91 1 H, dd (2.0, 8.5) | 106.2 |
| 8 | 6.42 1 H, dd (2.5, 8.5) | 137.1 |
| 10 | 7.06 1 H, d (2.5) | 141.1 |
| 11 | 5.46 1 H, d (7.2) | 72.6 |
| 11-OH | 6.25 1 H, d (7.2) | |
| 12 | 3.25 3 H, s | 27.1 |
| 1' | | 127.2 |
| 2' | 7.76 1 H, d (1.9) | 122.5 |
| 3' | | 143.6 |
| 4' | | 149.0 |
| 4'-OH | 9.73 1 H, s | |
| 5' | 6.88 1 H, d (8.3) | 115.2 |
| 6' | 7.16 1 H, dd (2.0, 8.3) | 124.8 |
| 7' | 4.96 1 H, d (4.6) | 74.6 |
| 7'-OH | 5.99 1 H, d (4.6) | |
| 1" | | 145.6 |
| 2" | | 153.4 |
| 3" | 7.21 1 H, d (8.6) | 112.1 |
| 4" | 7.57 1 H, dd (1.9, 8.6) | 123.8 |
| 5" | | 122.0 |
| 6" | 7.37 1 H, d (1.9) | 120.0 |
| 7" | | 164.3 |
| 8" | 3.94 3 H, s | 55.7 |




* $^{13}$C-NMR chemical shifts determined from HSQC and HMBC experiments; ‡ Not possible to distinguish between C1 and C3; † Not possible to distinguish between C2 and C4.

## 6. Neosolaniol monoacetate isolated from *Fusarium compactum* (IBT 9034)

**Figure S4.** $^1$H-NMR spectrum of neosolaniol monoacetate in DMSO-$d_6$ at 500 MHz.

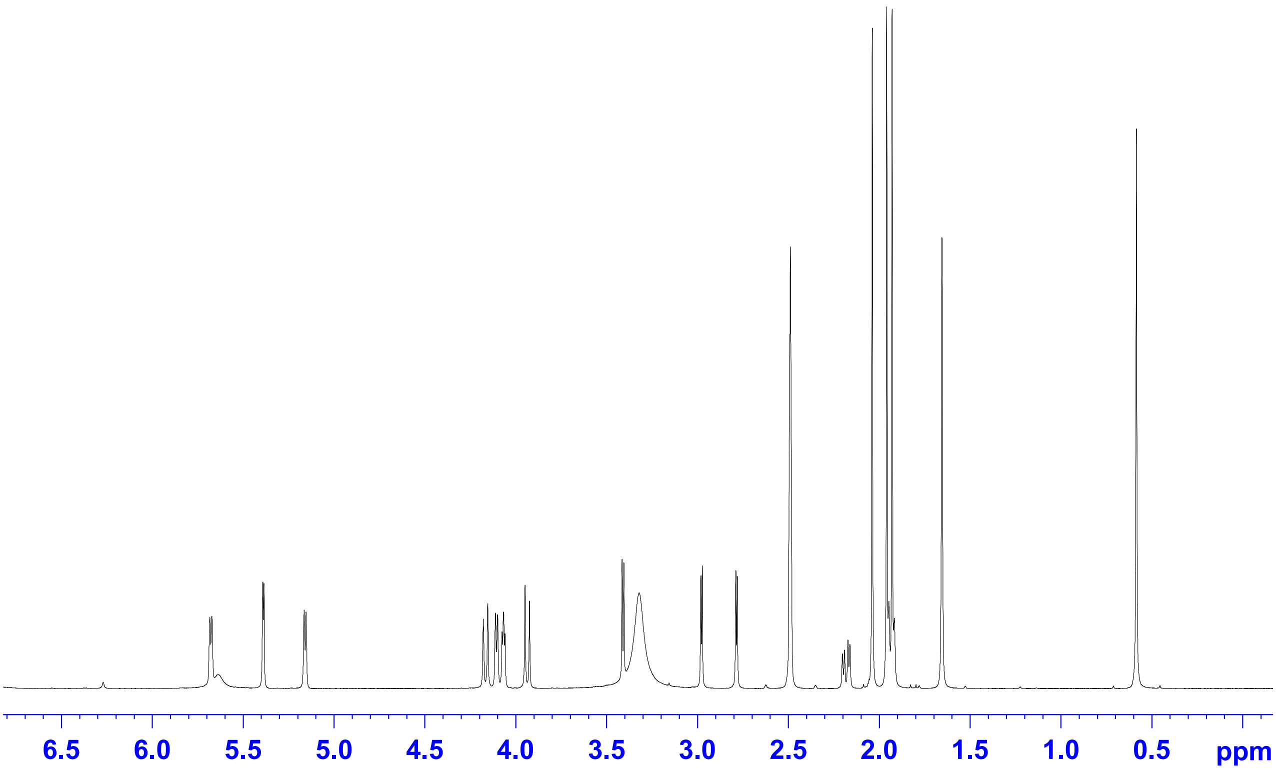
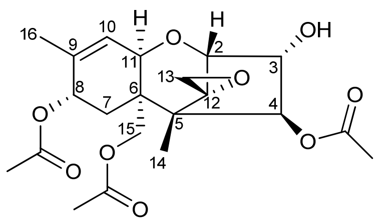



**Table S6.** $^1$H-NMR for neosolaniol monoacetate in DMSO at 500 MHz.

| | $\delta_H$ mult. ($J$ (Hz)) |
|---|---|
| 2 | 3.41 1H, d (5.0) |
| 3 | 4.07 1H, dd, (3.0; 5.0) |
| 4 | 5.39 1H, d (3.2) |
| 5 | |
| 6 | |
| 7a | 2.18 1H, dd (5.3; 15.7) |
| 7b | 1.94 1H, m |
| 8 | 5.15 1H, d, (5.3) |
| 9 | |
| 10 | 5.67 1H, d (5.7) |
| 11 | 4.1 1H, d (5.7) |
| 12 | |
| 13a | 2.78 1H, d (3.9) |
| 13b | 2.98 1H, d (3.9) |
| 14 | 0.59 3H, s |
| 15a | 3.94 1H, d (12.2) |
| 15b | 4.16 1H, d (12.2) |
| 16 | 1.66 3H, s |
| C=O C4 | |
| C=O C8 | |
| C=O C15 | |
| CH3 C4 | 1.96 3H, s |
| CH3 C8 | 2.04 3H, s |
| CH3 C15 | 1.93 3H, s |



## 7. Ophiobolins

### 7.1 Potential ophiobolin producing strains from the *Aspergilli* section *Usti*

**Table S7.** *Aspergilli* section *Usti*.

| IBT number | Species |
|---|---|
| 4537 | *Aspergillus ustus* |
| 10619 | |
| 20587 | |
| 22554 | |
| 25041 | |
| 10524 | *Aspergillus keveii* |
| 24673 | |
| 24708 | |
| 28266 | *Aspergillus insuetus* |
| 28267 | |
| 13091 | *Aspergillus calidoustus* |
| 25726 | |

## 7.2 NMR data for ophiobolin U, ophiobolin K, 6-epiophiobolin K, ophiobolin C, ophiobolin H, 6-epiophiobolin H, and 6-epiophiobolin G

**Table S8.** $^{13}$C-NMR data for ophiobolin U, ophiobolin K, 6-epiophiobolin K, ophiobolin C, ophiobolin H, 6-epiophiobolin H, and 6-epiophiobolin G. (* 125 MHz in dimethyl sulfoxide (DMSO)-$d_6$, † 125 MHz in $CDCl_3$, or ‡ 200 MHz in $CDCl_3$, $\delta_C$).

| Ophiobolin | U ‡ | K * | 6-epi-K * | C † | H ‡ | 6-epi-N ‡ | 6-epi-G † |
|---|---|---|---|---|---|---|---|
| **1** | 35.2 | 34.5 | 41.0 | 36.0 | 35.7 | 45.6 | 45.8 |
| **2** | 51.0 | 49.2 | 49.6 | 50.8 | 50.9 | 49.3 | 49.2 |
| **3** | 81.9 | 76.2 | 74.7 | 76.6 | 80.2 | 179.8 | 178.1 |
| **4** | 53.4 | 53.9 | 54.7 | 54.6 | 50.8 | 130.0 | 130.2 |
| **5** | 73.1 | 217.0 | 216.1 | 217.5 | 116.0 | 209.6 | 208.2 |
| **6** | 50.5 | 48.3 | 48.2 | 48.4 | 52.8 | 49.9 | 50.0 |
| **7** | 142.1 | 140.6 | 141.5 | 141.5 | 138.5 | 140.1 | 139.9 |
| **8** | 164.1 | 157.6 | 159.9 | 163.8 | 123.6 | 157.3 | 158.1 |
| **9** | 25.6 | 24.3 | 30.0 | 24.7 | 25.0 | 31.0 | 30.9 |
| **10** | 54.0 | 53.0 | 43.1 | 53.4 | 55.0 | 43.1 | 43.8 |
| **11** | 44.1 | 43.3 | 44.9 | 43.6 | 43.6 | 45.0 | 45.4 |
| **12** | 42.0 | 42.2 | 44.4 | 42.6 | 43.0 | 44.5 | 44.3 |
| **13** | 26.7 | 25.4 | 27.1 | 22.8 | 26.8 | 27.1 | 27.8 |
| **14** | 47.4 | 46.2 | 51.4 | 45.2 | 47.2 | 51.1 | 52.1 |
| **15** | 35.9 | 34.4 | 31.9 | 32.7 | 35.5 | 31.8 | 32.6 |
| **16** | 137.7 | 136.9 | 136.0 | 36.8 | 138.0 | 37.1 | 135.7 |
| **17** | 122.3 | 121.7 | 123.3 | 26.0 | 121.7 | 25.6 | 124.0 |
| **18** | 120.2 | 119.9 | 120.1 | 124.3 | 120.4 | 124.4 | 120.0 |
| **19** | 135.9 | 134.6 | 135.3 | 131.0 | 135.2 | 131.6 | 136.6 |
| **20** | 26.3 | 25.7 | 25.2 | 25.4 | 25.4 | 17.4 | 17.3 |
| **21** | 198.1 | 193.1 | 194.7 | 195.9 | 71.5 | 193.0 | 193.0 |
| **22** | 18.6 | 18.2 | 22.8 | 19.0 | 18.7 | 23.1 | 22.9 |
| **23** | 20.6 | 19.6 | 21.1 | 16.4 | 20.4 | 18.6 | 21.3 |
| **24** | 18.3 | 18.0 | 17.9 | 17.6 | 18.2 | 17.7 | 18.2 |
| **25** | 26.7 | 25.9 | 26.1 | 25.6 | 26.6 | 25.7 | 26.5 |

**Table S9.** [1]H-NMR data for ophiobolin U, ophiobolin K, 6-epiophiobolin K, ophiobolin C, ophiobolin H, 6-epiophiobolin H, and 6-epiophiobolin G. (* 500 MHz in DMSO-$d_6$, † 500 MHz in $CDCl_3$, or ‡ 800 MHz in $CDCl_3$, $\delta_H$ mult. (*J* (Hz))).

| Ophiobolin | U ‡ | K * | 6-epi-K * | C † | H ‡ | 6-epi-N ‡ | 6-epi-G † |
|---|---|---|---|---|---|---|---|
| **1a** | 1.03 m | 1.14 m | 1.58 m | 1.26 m | 1.36 m | 1.18 m | 1.15 m |
| **1b** | 1.58 m | 1.58 m | 1.65 m | 1.81 m | 1.42 m | 2.04 dd (3.7; 13.2) | 2.03 m |
| **2** | 2.30 m | 2.25 m | 1.91 m | 2.38 m | 2.26 m | 2.71 m | 2.66 m |
| **3-OH** | | | 6.51 br.s. | | | | |
| **4a** | 1.87 dd (4.1, 15.1) | 2.35 m | 2.21 m | 2.49 m | 2.10 m | 6.11 s | 6.04 s |
| **4b** | 2.68 dd (7.9, 15.1) | 2.50 m | 2.77 d (16.0) | 2.80 m | 2.18 m | | |
| **5** | 4.91 dd (4.7, 7.9) | | | | | | |
| **6** | 3.02 d (9.6) | 3.22 d (9.8) | 3.04 d (10.8) | 3.26 m | 3.17 d (9.8) | 3.54 d (3.7) | 3.40 m |
| **8** | 6.94 t (8.5) | 7.02 t (8.5) | 6.96 m | 7.21 m | 5.64 br.s. | 6.86 dd (2.0; 6.3) | 6.80 m |
| **9a** | 2.24 m | 2.04 m | 2.27 m | 2.31 m | 1.70 m | 2.25 m | 2.20 m |
| **9b** | 2.89 dd (8.5, 12.5) | 2.69 m | 2.65 m | 2.45 m | 2.50 dd (8.6; 13.8) | 2.71 m | 2.93 m |
| **10** | 1.55 m | 1.56 m | 2.46 m | 1.67 m | 1.60 m | 2.72 m | 2.63 m |
| **12a** | 1.38 m | 1.33 m | 1.38 m | 1.41 m | 1.40 dd (7.6; 11.7) | 1.42 m | 1.43 m |
| **12b** | | 1.35 m | 1.44 m | 1.44 m | 1.57 m | 1.51 m | 1.52 m |
| **13a** | 1.58 m | 1.54 m | 1.22 m | 1.46 m | 1.54 m | 1.23 m | 1.25 m |
| **13b** | 1.78 m | 1.69 m | 1.57 m | 1.55 m | 1.76 m | 1.58 m | 1.67 m |
| **14** | 2.09 m | 2.09 m | 1.87 m | 2.36 m | 2.05 m | 1.75 m | 1.89 m |
| **15** | 2.72 m | 2.72 m | 2.61 m | 1.65 m | 2.68 m | 1.42 m | 2.55 m |
| **16a** | 5.21 t (10.0) | 5.23 t (9.5) | 5.25 t (9.3) | 1.18 m | 5.20 m | 0.99 m | 5.11 t (1.3) |
| **16b** | | | | 1.24 m | | 1.45 m | |
| **17a** | 6.03 m | 5.99 m | 6.11 m | 1.95 m | 5.99 m | 1.94 m | 6.10 m |
| **17b** | | | | 2.00 m | | 2.07 m | |
| **18** | 6.00 m | 6.04 m | 6.09 m | 5.09 m | 5.98 m | 5.12 t (7.0) | 6.00 m |
| **20** | 1.26 s | 1.19 s | 1.26 s | 1.36 s | 1.24 s | 2.10 s | 2.06 s |
| **21a** | 9.26 s | 9.14 s | 9.12 s | 9.23 s | 4.48 br.s. | 9.30 s | 9.26 s |
| **21b** | | | | | 4.59 d (12.2) | | |
| **22** | 0.99 s | 0.91 s | 0.77 s | 0.90 s | 0.90 s | 0.86 m | 0.85 s |
| **23** | 0.91 d (6.7) | 0.85 d (6.6) | 0.92 d (6.6) | 0.78 d (6.8) | 0.88 d (6.7) | 0.91 d (6.4) | 0.97 d (6.8) |
| **24** | 1.74 s | 1.68 s | 1.70 s | 1.61 s | 1.73 s | 1.61 s | 1.76 s |
| **25** | 1.82 s | 1.76 s | 1.79 s | 1.69 s | 1.80 s | 1.69 s | 1.83 s |

### 7.3 Ophiobolin U isolated from *A. insuetus* (IBT 28266)

**Figure S5.** $^{1}$H-NMR spectrum of ophiobolin U in $CDCl_3$ at 800 MHz.

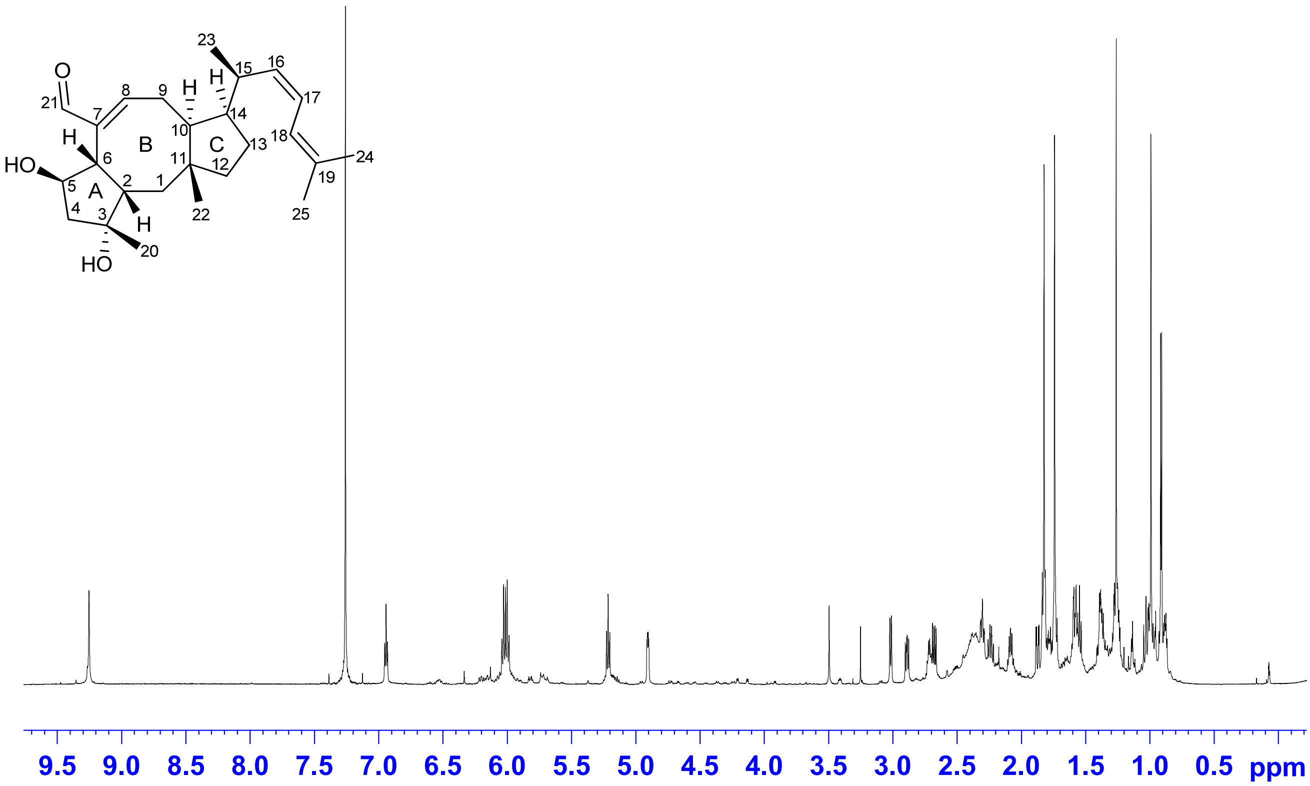



**Figure S6.** $^{13}$C-NMR spectrum of ophiobolin U in $CDCl_3$ at 200 MHz.

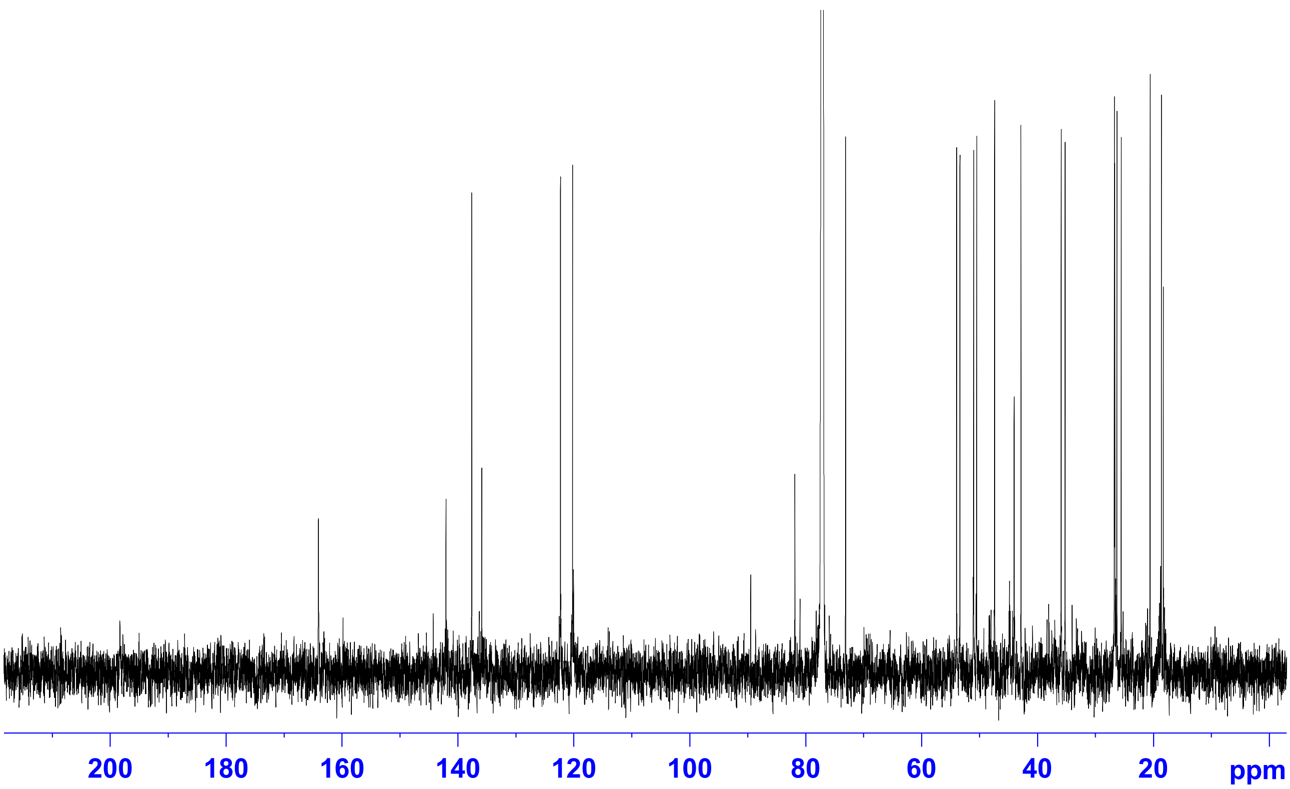


**Table S10.** $^1$H- and $^{13}$C-NMR for ophiobolin U in $CDCl_3$ at 800 MHz for $^1$H and 200 MHz for $^{13}$C.

| | $\delta_H$ mult. (*J* (Hz)) | $\delta_C$ | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.03 (1H, m) | 35.2 | 2, 3, 10, 11, 22 | 1b |
| 1b | 1.58 (1H, m) | 35.2 | 3, 10, 12 | 1a, 2, 15, 20, 22 |
| 2 | 2.30 (1H, m) | 51.0 | 1, 3, 6, 7 | 6, 20, 22 |
| 3 | | 81.9 | | |
| 4a | 1.87 (1H, dd, 4.1, 15.1) | 53.4 | 3, 5 | 2, 4b, 20 |
| 4b | 2.68 (1H, dd, 7.9, 15.1) | 53.4 | 2, 3, 6 | 4a, 5 |
| 5 | 4.91 (1H, dd, 4.7, 7.9) | 73.1 | 3, 6, 7 | 4b, 6 |
| 6 | 3.02 d (1H, d, 9.6) | 50.5 | 2, 3, 5, 7, 8, 21 | 2, 5, 9a, 22 |
| 7 | | 142.1 | - | - |
| 8 | 6.94 t (1H, d, 8.5) | 164.1 | 6, 9, 21 | 9a, 9b, 10, 21 |
| 9a | 2.24 (1H, m) | 25.6 | 7, 8, 10, 11 | 6, 8, 9b, 22 |
| 9b | 2.89 (1H, dd, 8.5, 12.5) | 25.6 | 7, 8, 10, 11, 14 | 8, 9a, 10, 14, 15, 16 |
| 10 | 1.55 (1H, m) | 54.0 | 8, 9, 11, 14, 15, 22 | 8, 9b, 14 |
| 11 | | 44.1 | - | - |
| 12 | 1.38 (2H, m) | 42.0 | 10, 11, 13, 14, 22 | 13a, 13b, 22 |
| 13a | 1.58 (1H, m) | 26.7 | 11, 14, 15 | 12, 13b, 22, 23 |
| 13b | 1.78 (1H, m) | 26.7 | 10, 11, 14, 15 | 12, 13a, 14 |
| 14 | 2.09 (1H, m) | 47.4 | 10, 11, 13, 15, 16, 23 | 9b, 10, 13b, 15, 16, 23 |
| 15 | 2.72 (1H, m) | 35.9 | 14, 16, 17, 23 | 9b, 1b, 14, 16, 18, 22, 23 |
| 16 | 5.21 (1H, t, 10.0) | 137.7 | 14, 15, 18, 23 | 9b, 14, 15, 17, 23 |
| 17 | 6.03 (1H, m) | 122.3 | 15, 19 | 16, 24 |
| 18 | 6.00 (1H, m) | 120.2 | 24, 25 | 15, 25 |
| 19 | | 135.9 | | |
| 20 | 1.26 (3H, s) | 26.3 | 2; 3; 4 | 1b, 2, 4a |
| 21 | 9.26 (1H, s) | 198.1 | 6, 7, 8 | 5, 8 |
| 22 | 0.99 s (3H, s) | 18.6 | 1, 10, 11, 14 | 1b, 2, 6, 9a, 12, 13a, 15 |
| 23 | 0.91 (2H, d, 6.7) | 20.6 | 14, 15, 16 | 13a, 14, 15, 16 |
| 24 | 1.74 (3H, s) | 18.3 | 18, 19, 25 | 17 |
| 25 | 1.82 (3H, s) | 26.7 | 18, 19, 24 | 18 |

## 7.4 Ophiobolin H isolated from *A. insuetus* (IBT 28266)

**Figure S7.** $^{1}$H-NMR spectrum of ophiobolin H in $CDCl_3$ at 800 MHz.

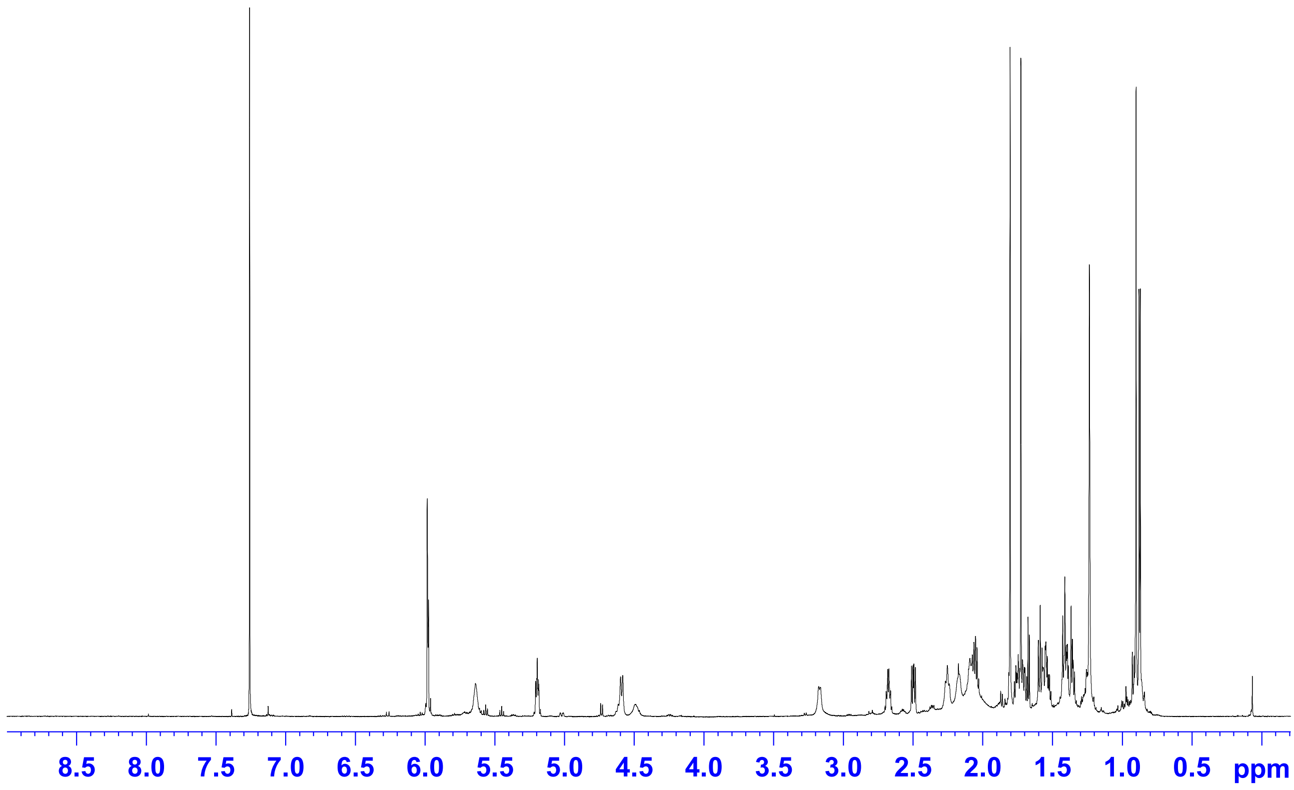



**Figure S8.** $^{13}$C-NMR spectrum of ophiobolin H in $CDCl_3$ at 200 MHz.

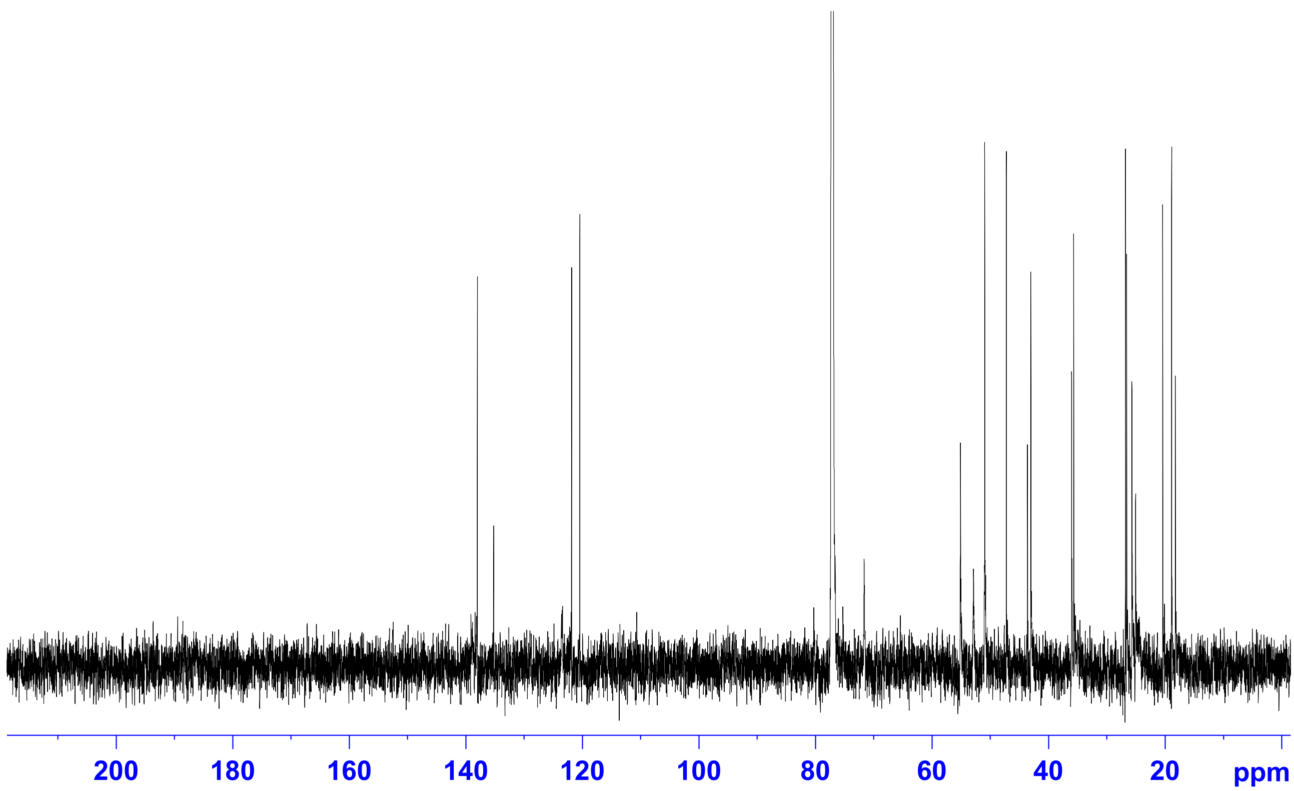


**Table S11.** $^1$H- and $^{13}$C-NMR for ophiobolin H in $CDCl_3$ at 800 MHz for $^1$H and 200 MHz for $^{13}$C.

| | $\delta_H$ mult. (*J* (Hz)) | $\delta_C$ | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.36 m | 35.7 | 2, 6, 10, 11, 22 | 1b |
| 1b | 1.42 m | 35.7 | | 1a, 2, 8, 20 |
| 2 | 2.26 m | 50.9 | | 1b, 6, 20, 22 |
| 3 | | 80.2 | | |
| 4a | 2.10 m | 50.8 | | 4b, 20 |
| 4b | 2.18 m | 50.8 | | 4a |
| 5 | | 116.0 | | |
| 6 | 3.17 d (9.8) | 52.8 | | 2, 9a, 22 |
| 7 | | 138.5 | | |
| 8 | 5.64 br.s. | 123.6 | | 1b, 9b |
| 9a | 1.70 m | 25.0 | 8, 10, 11 | 6, 9b, 22 |
| 9b | 2.50 dd (8.6; 13.8) | 25.0 | 7, 8, 10, 11, 14 | 8, 9a, 10, 15, 16 |
| 10 | 1.60 m | 55.0 | 8, 9, 11, 14, 15, 22 | 9b, 14 |
| 11 | | 43.6 | | |
| 12a | 1.40 dd (7.6; 11.7) | 43.0 | 10, 11, 14, 22 | 12b, 13a, 22 |
| 12b | 1.57 m | 43.0 | 13, 15 | 12a, 13b |
| 13a | 1.54 m | 26.8 | 12, 14, 15 | 12a, 13b, 23 |
| 13b | 1.76 m | 26.8 | 10, 11, 14 | 12b, 13a, 14 |
| 14 | 2.05 m | 47.2 | 10, 11, 13, 15, 16, 23 | 10, 13b, 16, 23 |
| 15 | 2.68 m | 35.5 | 14, 16, 23 | 9b, 18, 22 |
| 16 | 5.20 m | 138.0 | 18 | 9b, 14, 17, 23 |
| 17 | 5.99 m | 121.7 | 15, 19 | 16, 24 |
| 18 | 5.98 m | 120.4 | 16, 24, 25 | 15, 23, 25 |
| 19 | | 135.2 | | |
| 20 | 1.24 s | 25.4 | 2, 3 | 1b, 2, 4a |
| 21a | 4.48 br.s. | 71.5 | | |
| 21b | 4.59 d (12.2) | 71.5 | | |
| 22 | 0.90 s | 18.7 | 1, 10, 11 | 2, 6, 9a, 12a, 15 |
| 23 | 0.88 d (6.7) | 20.4 | 14, 15, 16 | 13a, 14, 16, 18 |
| 24 | 1.73 s | 18.2 | 18, 19, 25 | 17 |
| 25 | 1.80 s | 26.6 | 18, 19, 24 | 18 |

### 7.5 6-epiophiobolin N isolated from *A. insuetus* (IBT 28266)

**Figure S9.** $^{1}$H-NMR spectrum of 6-epiophiobolin N in $CDCl_3$ at 800 MHz.

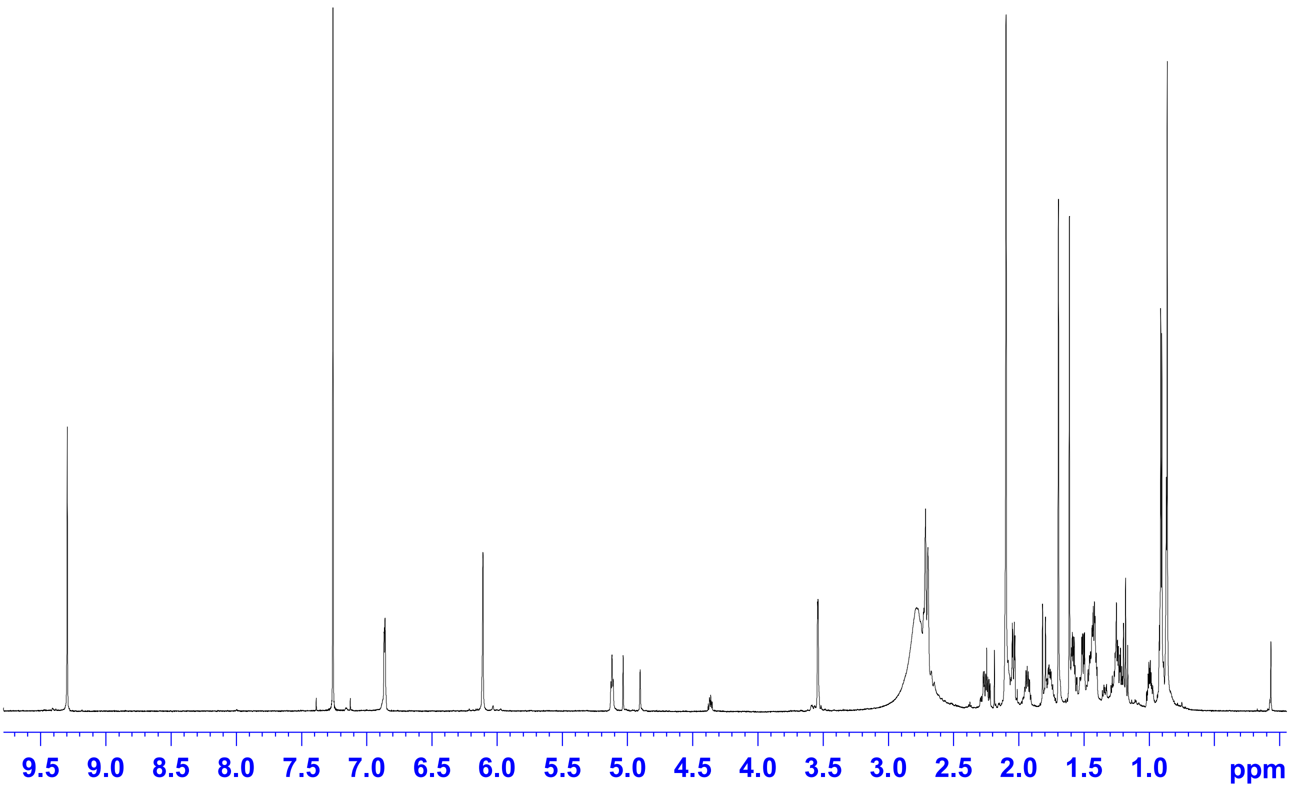



**Figure S10.** $^{13}$C-NMR spectrum of 6-epiophiobolin N in $CDCl_3$ at 200 MHz.

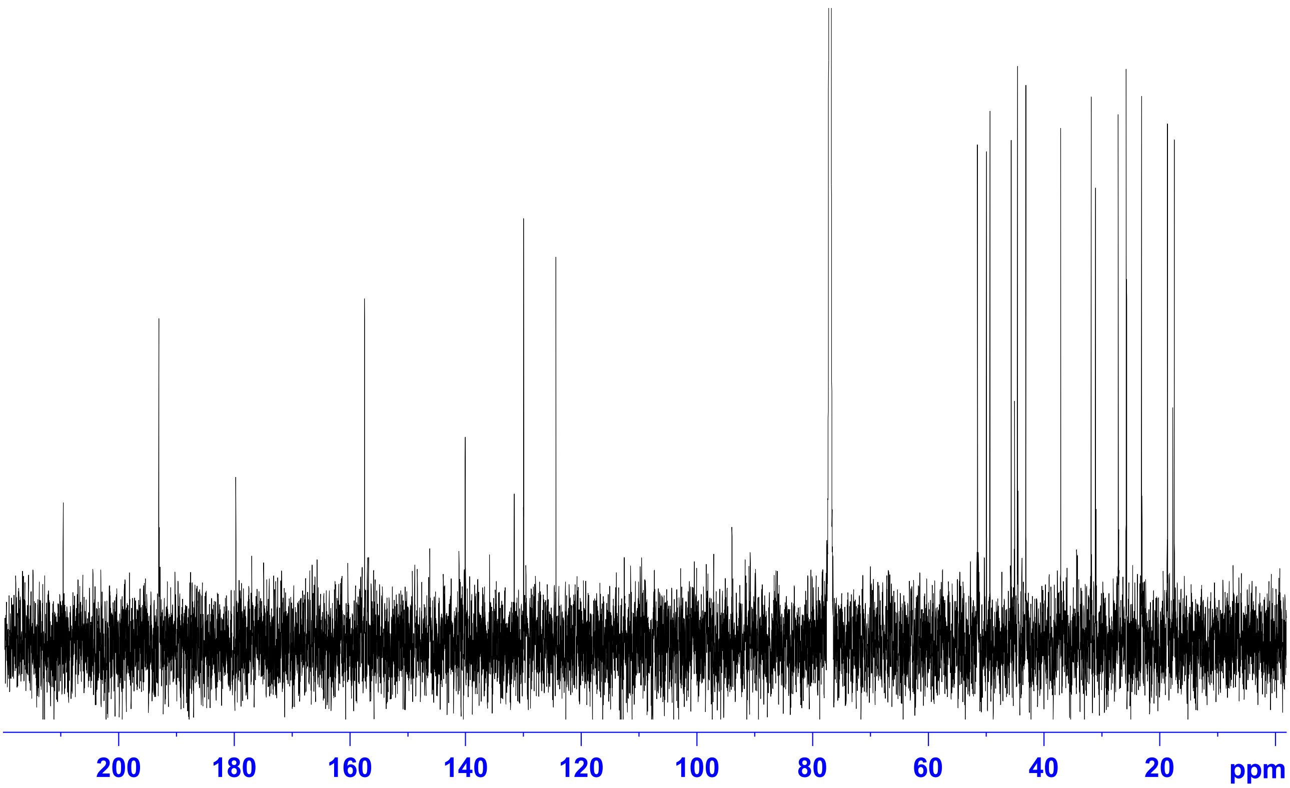


**Table S12.** [1]H and [13]C NMR for 6-epiophiobolin N in $CDCl_3$.at 800 MHz for [1]H and 200 MHz for [13]C.

| | $\delta_H$ mult. (*J* (Hz)) | $\delta_C$ | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.18 m | 45.6 | 2; 3; 10; 12; 22 | 1b, 6, 10, 12a/15 * |
| 1b | 2.04 dd (3.7; 13.2) | 45.6 | 2, 6, 10, 11 | 1a, 10, 12a/15 *, 12b, 22 |
| 2 | 2.71 m | 49.3 | 8, 11 | 8. 9a, 16b, 20, 22 |
| 3 | | 179.8 | | |
| 4 | 6.11 s | 130.0 | 3; 5; 6; 20 | 20 |
| 5 | | 209.6 | | |
| 6 | 3.54 d (3.7) | 49.9 | 1; 2; 5; 7; 8; 21 | 1a; 2; (8); 9a, 10 |
| 7 | | 140.1 | | |
| 8 | 6.86 dd (2.0; 6.3) | 157.3 | 6; 10; 21 | 2/9b **, (6), 9a, 21, 22 |
| 9a | 2.25 m | 31.0 | 7; 8; 10 | 2/9b **, 6, 8, 12a, 22 |
| 9b | 2.71 m | 31.0 | 8, 11 | 8. 9a, 12a/15 *, 20, 22 |
| 10 | 2.72 m | 43.1 | 1, 7, 13, 14, 22 | 1a, 1b, 6, 14, 16b |
| 11 | | 45.0 | | |
| 12a | 1.42 m | 44.5 | 1, 13, 22 | 1a, 1b, 9b, 12b, 13b, 14, 17a, 23 |
| 12b | 1.51 m | 44.5 | 10; 11; 14; 22 | 1b; 12a/15 *; 13a; 13b; 22 |
| 13a | 1.23 m | 27.1 | 12; 14; 15 | 12b; 13b; 22; 23 |
| 13b | 1.58 m | 27.1 | 10; 11 | 13a; 14, 12a/15 *; 23 |
| 14 | 1.75 m | 51.1 | | 9b, 10, 12a/15 *, 13b |
| 15 | 1.42 m | 31.8 | | 1a, 1b, 9b, 12b, 13b, 14, 17a, 23 |
| 16a | 0.99 m | 37.1 | 15; 17; 18; 23 | 10; 17b; 18 |
| 16b | 1.45 m | 37.1 | 23 | 2/9b **; 10, 16a |
| 17a | 1.94 m | 25.6 | 16; 18; 19 | 12a/15 *; 17b; 23 |
| 17b | 2.07 m | 25.6 | | 16a; 17a; 23 |
| 18 | 5.12 t (7.0) | 124.4 | 24; 25 | 15; 16a; 25 |
| 19 | | 131.6 | | |
| 20 | 2.10 s | 17.4 | 2, 3, 4 | 2/9b **; 4 |
| 21 | 9.30 s | 193.0 | 6, 7 | 8 |
| 22 | 0.86 m | 23.1 | 1; 10; 12 | 1b; 2/9b **; 8; 9a; 12b; 13a |
| 23 | 0.91 d (6.4) | 18.6 | 14; 15; 16 | 10; 13a; 13b; 15; 16a; 17a; 17b |
| 24 | 1.61 s | 17.7 | 18; 19; 25 | 25 |
| 25 | 1.69 s | 25.7 | 18; 19; 24 | 18; 24 |

* Not possible to distinguish between H12a and H15; ** Not possible to distinguish between H2 and H9b.

## 7.6 Ophiobolin K isolated from a new sp. in the *Aspergillus* section *Usti* (IBT 18591)

**Figure S11.** $^1$H-NMR spectrum of ophiobolin K in $CDCl_3$ at 800 MHz.

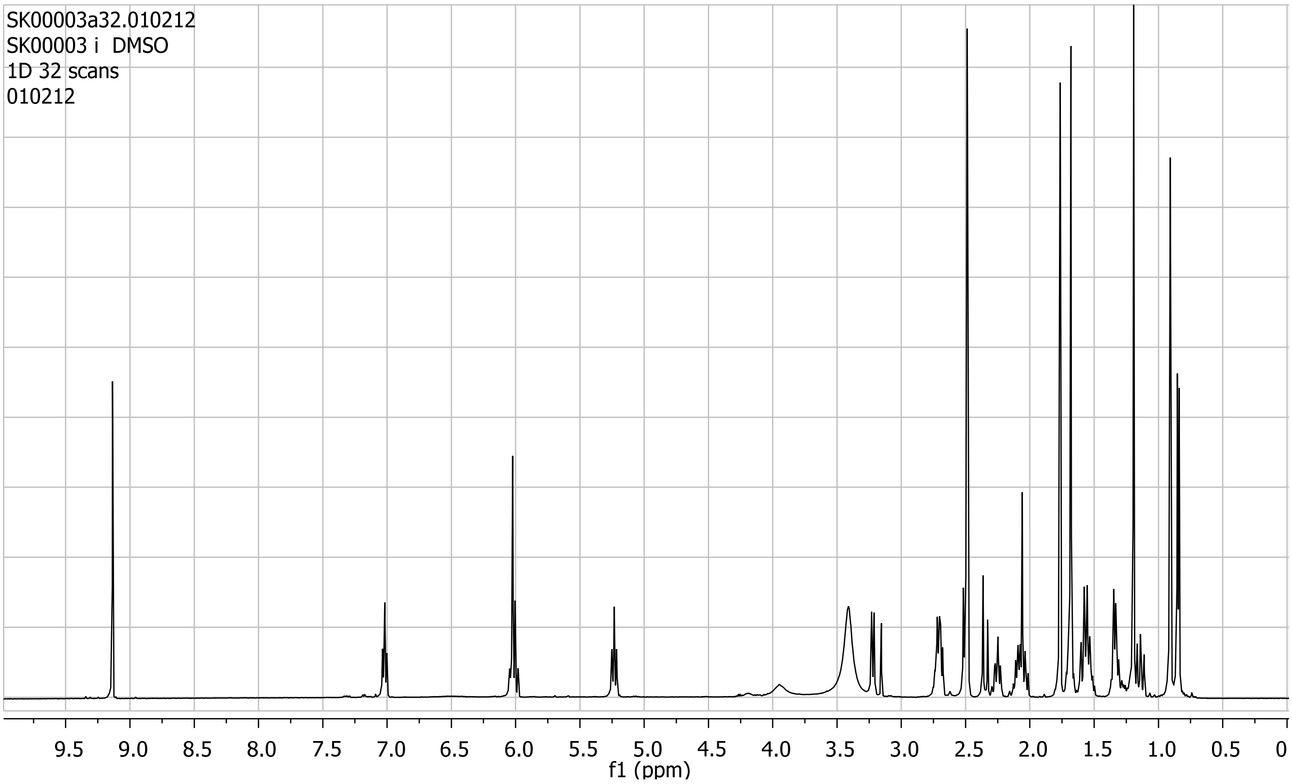


**Table S13.** $^{1}H$ and $^{13}C$-NMR for ophiobolin K in DMSO-$d_6$ at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$.

| | $\delta_H$ mult. ($J$ (Hz)) | $\delta_C$ * | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.14 m | 34.5 | 6, 10, 11, 22 | |
| 1b | 1.58 m | | 6, 10, 11 | 2, 12a, 20 |
| 2 | 2.25 m | 49.2 | 1, 4, 6, 7 | 1b, 4a, 6, 20, 22 |
| 3 | | 76.2 | | |
| 4a | 2.35 m | 53.9 | 3, 5, 20 | 2, 20 |
| 4b | 2.50 m | | 2, 3, 5 | |
| 5 | | 217.0 | | |
| 6 | 3.22 d (9.8) | 48.3 | 2, 3, 5, 7, 8, 21 | 2, 9a, 22 |
| 7 | | 140.6 | | |
| 8 | 7.02 t (8.5) | 157.6 | 6, 9, 21 | 9b, 10, 21 |
| 9a | 2.04 m | 24.3 | 7, 8, 10, 11 | 6, 22 |
| 9b | 2.69 m | | 7, 8, 10, 11, 14 | 8, 10, 16 |
| 10 | 1.56 m | 53.0 | 1, 8, 9, 11, 14, 22 | 8, 9b, 12 |
| 11 | | 43.3 | | |
| 12a | 1.33 m | 42.2 | 10, 11, 13, 14, 22 | 1b, 13b, 22 |
| 12b | 1.35 m | | | |
| 13a | 1.54 m | 25.4 | 11, 15 | 14, 22, 23, 24 |
| 13b | 1.69 m | | 11 | 12a, 14 |
| 14 | 2.09 m | 46.2 | 10, 11, 13, 15, 16, 23 | 13a/b, 15, 16 , 23 |
| 15 | 2.72 m | 34.4 | 16, 17, 23 | 14, 18, 22, 23 |
| 16 | 5.23 t (9.5) | 136.9 | 14, 15, 18, 23 | 9b, 14, 17, 23 |
| 17 | 5.99 m | 121.7 | 15, 18 | 16, 24 |
| 18 | 6.04 m | 119.9 | 16, 19, 24, 25 | 15, 23, 25 |
| 19 | | 134.6 | | |
| 20 | 1.19 s | 25.7 | 2, 3, 4 | 1b, 2, 4a |
| 21 | 9.14 s | 193.1 | 6, 7, 8 | 8 |
| 22 | 0.91 s | 18.2 | 1, 10, 11 | 2, 6, 9a, 12a, 13a,15 |
| 23 | 0.85 d (6.6) | 19.6 | 14, 15, 16 | 13a, 14, 15, 16, 18 |
| 24 | 1.68 s | 18.0 | 18, 19, 25 | 13a, 17, 25 |
| 25 | 1.76 s | 25.9 | 18, 19, 24 | 18, 24 |

* $^{13}C$ NMR chemical shifts determined from HSQC and HMBC experiments.

## 7.7 6-Epiophiobolin K isolated from a new sp. in the *Aspergillus* section *Usti* (IBT 18591)

**Figure S12.** $^{1}$H-NMR spectrum of 6-epiophiobolin K in $CDCl_3$ at 800 MHz.

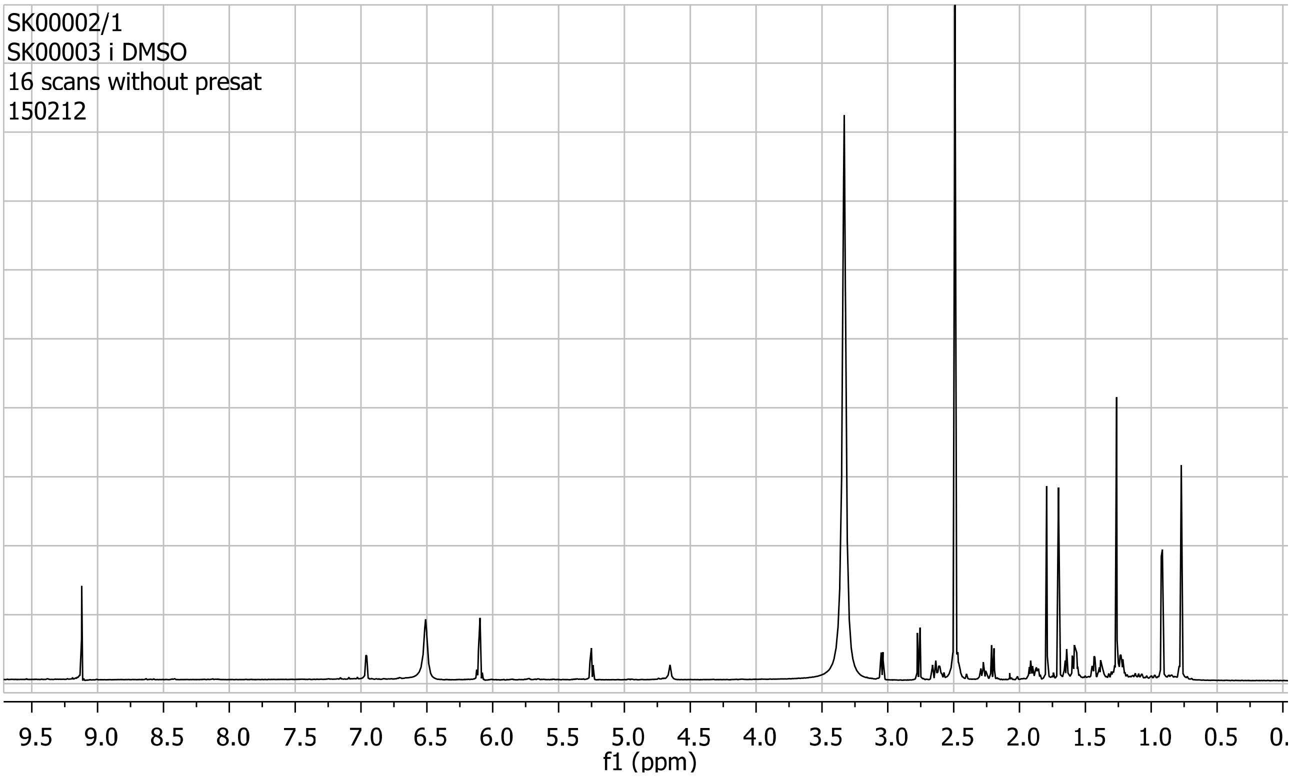



**Figure S13.** $^{13}$C-NMR spectrum of 6-epiophiobolin K in $CDCl_3$ at 200 MHz.

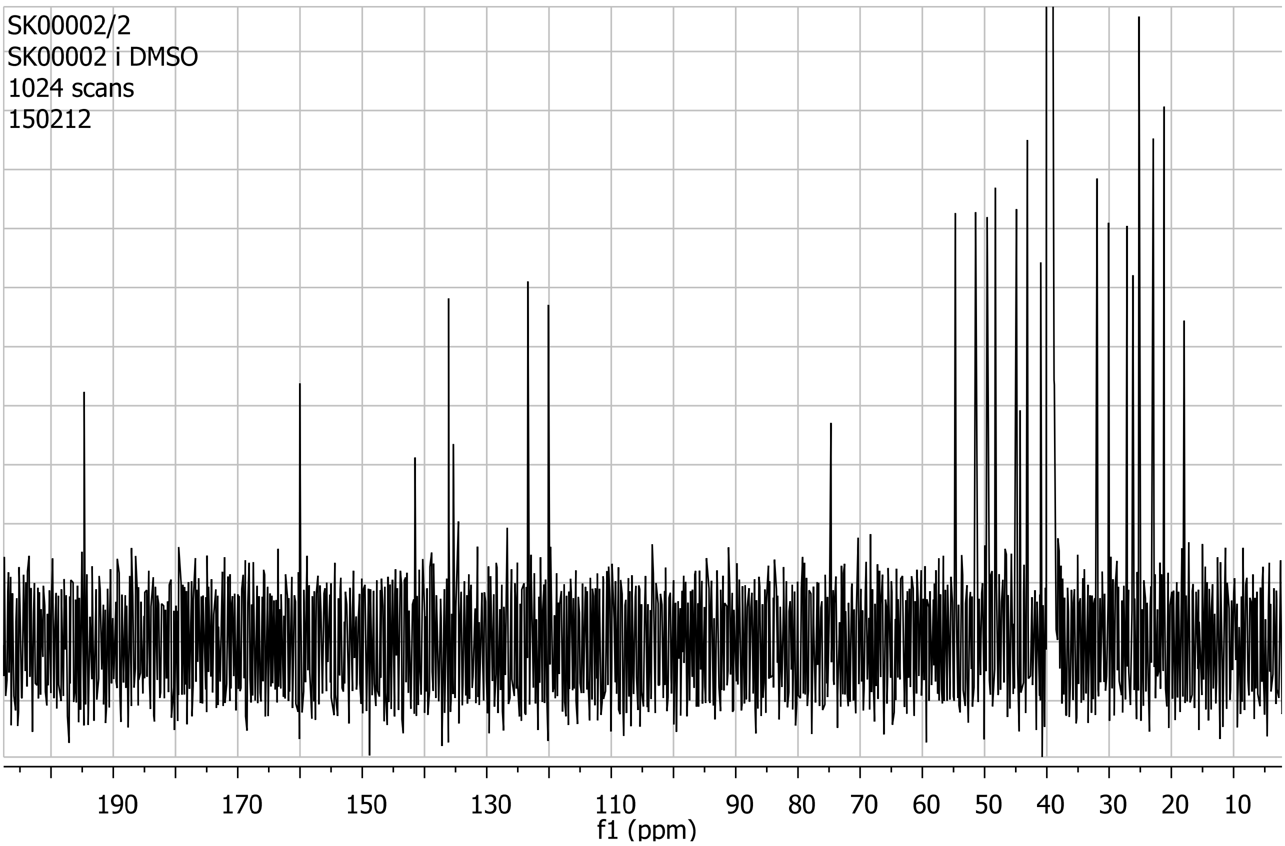


**Table S14.** $^{1}$H and $^{13}$C-NMR for 6-epiophiobolin K in DMSO-$d_6$ at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C.

| | $\delta_H$ mult. ($J$ (Hz)) | $\delta_C$ | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.58 m | 41.0 | 2, 6, 10, 22 | 6, 12a |
| 1b | 1.65 m | | | 2, 20, 22 |
| 2 | 1.91 m | 49.6 | 6, 7 | 1a/b , 4b, 20, 22 |
| 3 | | 74.7 | | |
| 3-OH | 6.51 br.s. | | | |
| 4a | 2.21 m | 54.7 | 2, 3, 5 | 20 (w) |
| 4b | 2.77 d (16.0) | | 3, 5, 20 | 2, 20 |
| 5 | | 216.1 | - | |
| 6 | 3.04 d (10.8) | 48.2 | 1, 2, 5, 7, 21 | 1a, 2 (w), 10 |
| 7 | | 141.5 | | |
| 8 | 6.96 m | 159.9 | 6, 10, 21 | 9a/b, 21, 22 |
| 9a | 2.27 m | 30.0 | 7, 8 | 8, 22 (w) |
| 9b | 2.65 m | | | 8, 10 |
| 10 | 2.46 m | 43.1 | | 6, 9b, 14 |
| 11 | | 44.9 | | |
| 12a | 1.38 m | 44.4 | 10, 22 | 1a |
| 12b | 1.44 m | | 10, 22 | 22 |
| 13a | 1.22 m | 27.1 | 14, 15 | 22 |
| 13b | 1.57 m | | | 14, 23 |
| 14 | 1.87 m | 51.4 | | 10, 13b, 16, 23 |
| 15 | 2.61 m | 31.9 | | 17, 23 |
| 16 | 5.25 t (9.3) | 136.0 | 14, 18 | 14, 17, 23 |
| 17 | 6.11 m | 123.3 | 15, 19 | 16, 24 |
| 18 | 6.09 m | 120.1 | 24, 25 | 15, 23, 25 |
| 19 | | 135.3 | | |
| 20 | 1.26 s | 25.2 | 2, 3, 4 | 1b, 2, 4b |
| 21 | 9.12 s | 194.7 | 6, 7, 8 | 8 |
| 22 | 0.77 s | 22.8 | 1, 10, 11 | 1b, 2, 8, 9a, 12b, 13a |
| 23 | 0.92 d (6.6) | 21.1 | 14, 15, 16 | 13b, 14, 15, 16, 17 |
| 24 | 1.70 s | 17.9 | 18, 19, 25 | 17 |
| 25 | 1.79 s | 26.1 | 18, 19, 24 | 17 |

## 7.8 Ophiobolin C isolated from *A. calidoustus* (IBT 25726)

**Figure S14.** $^{1}$H-NMR spectrum of ophiobolin C in $CDCl_3$ at 800 MHz.

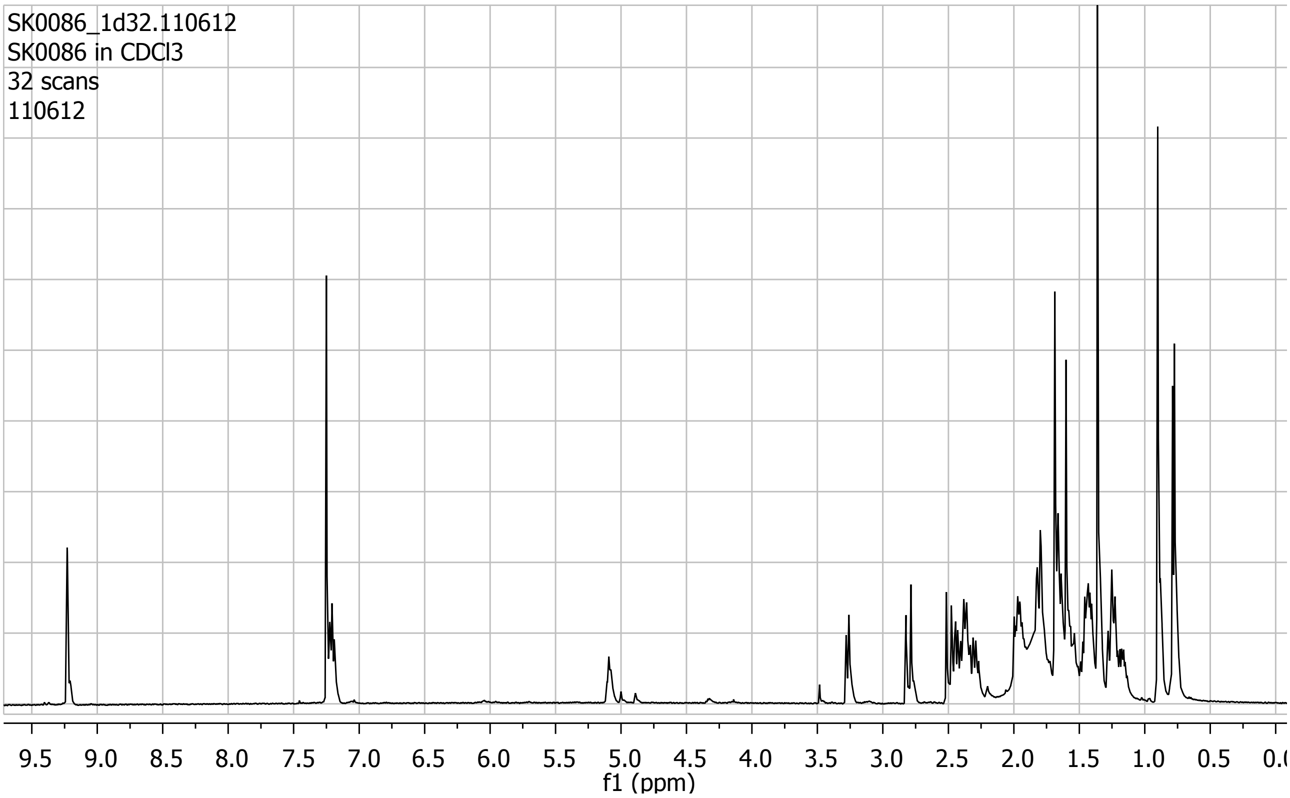


**Table S15.** $^{1}$H and $^{13}$C-NMR for ophiobolin C in $CDCl_3$ at 800 MHz for $^{1}$H and 200 MHz for $^{13}$C.

| | $\delta_H$ mult. (*J* (Hz)) | $\delta_C$ * | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.26 m | 36.0 | 2, 10, 11 | |
| 1b | 1.81 m | | 6, 10 | 2, 12a, 20 |
| 2 | 2.38 m | 50.8 | | 1b, 4a, 6, 20, 22 |
| 3 | | 76.6 | | |
| 4a | 2.49 m | 54.6 | 5, 20 | 2, 20 |
| 4b | 2.80 m | | 2, 3, 5 | 20 |
| 5 | | 217.5 | | |
| 6 | 3.26 m | 48.4 | 2, 3, 5, 7, 8, 21 | 2, 9a, 22 |
| 7 | | 141.5 | | |
| 8 | 7.21 m | 163.8 | 6, 9, 21 | 9b, 10, 21 |
| 9a | 2.31 m | 24.7 | 7, 8, 10, 11 | 6, 22, 23 |
| 9b | 2.45 m | | 7, 8, 10, 11 | 8, 10 |
| 10 | 1.67 m | 53.4 | 8, 14, 15 | 8, 9b, 16b |
| 11 | | 43.6 | | |
| 12a | 1.41 m | 42.6 | | 1b, 22 |
| 12b | 1.44 m | | | |
| 13a | 1.46 m | 22.8 | 11 | 23 |
| 13b | 1.55 m | | 10, 11 | 14 |
| 14 | 2.36 m | 45.2 | | 13b, 15, 16b, 17b |
| 15 | 1.65 m | 32.7 | 16 | 14, 23 |
| 16a | 1.18 m | 36.8 | 14, 15, 17, 18, 23 | 17a, 23 |
| 16b | 1.24 m | | 14, 15, 17, 18, 23 | 1b, 10, 14,17b, 18 |
| 17a | 1.95 m | 26.0 | 15, 16, 18, 19 | 16a,18 |
| 17b | 2.00 m | | 16, 18, 19 | 14, 16b, 23, 24 |
| 18 | 5.09 m | 124.3 | 24, 25 | 16b, 17a, 25 |
| 19 | | 131.0 | | |
| 20 | 1.36 s | 25.4 | 2, 3, 4 | 1b, 2, 4a/b |
| 21 | 9.23 s | 195.9 | 6, 7 | 8 |
| 22 | 0.90 s | 19.0 | 10, 11, 12 | 2, 6, 9a, 12a |
| 23 | 0.78 d (6.8) | 16.4 | 14, 15, 16 | 9a, 13a, 15, 16a, 17b |
| 24 | 1.61 s | 17.6 | 18, 19, 25 | 17b |
| 25 | 1.69 s | 25.6 | 18, 19, 24 | 18 |

* $^{13}$C NMR chemichal shifts determinated from HSQC and HMBC experiments.

## 7.9 6-epiophiobolin G isolated from *A. calidoustus* (IBT 25726)

**Figure S15.** $^{1}$H-NMR spectrum of 6-epiophiobolin G in $CDCl_3$ at 800 MHz.

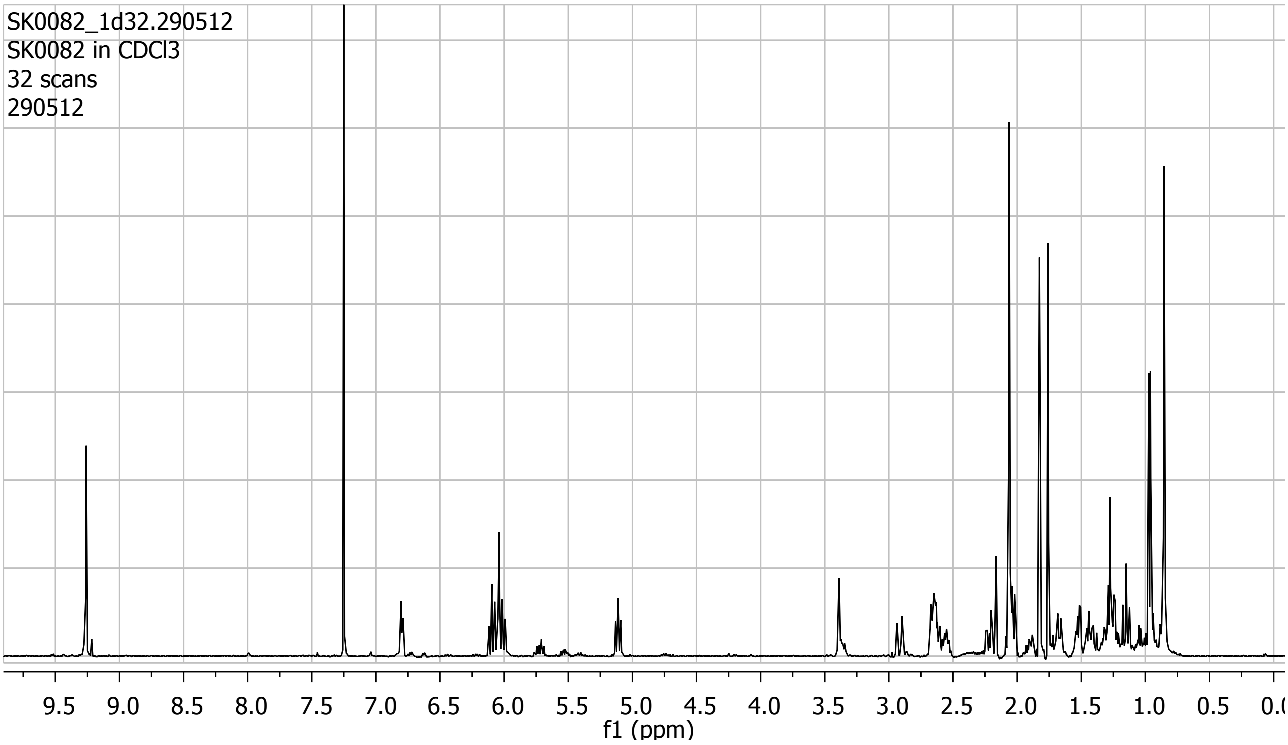



**Figure S16**. $^{13}$C-NMR spectrum of 6-epiophiobolin G in $CDCl_3$ at 200 MHz.

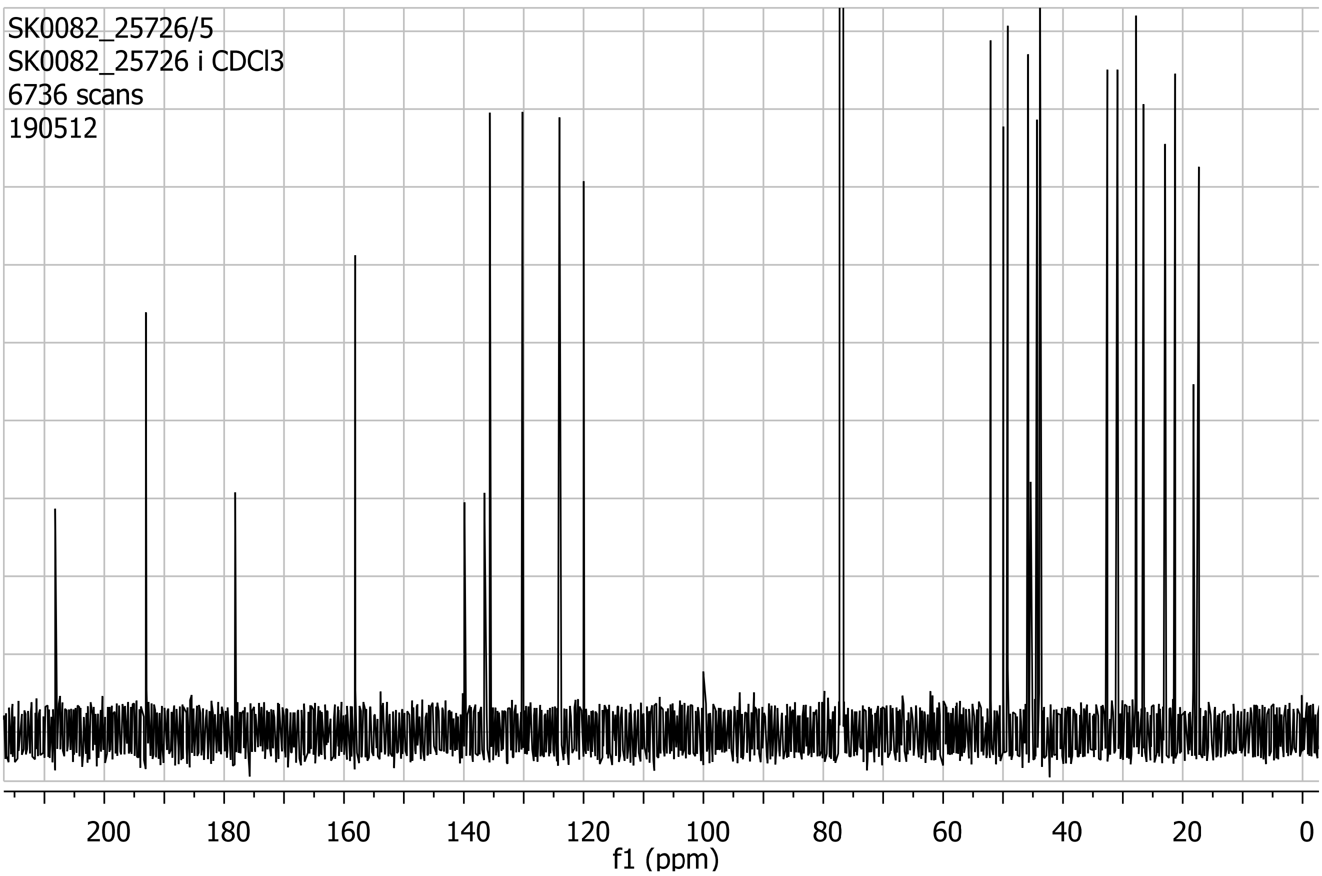
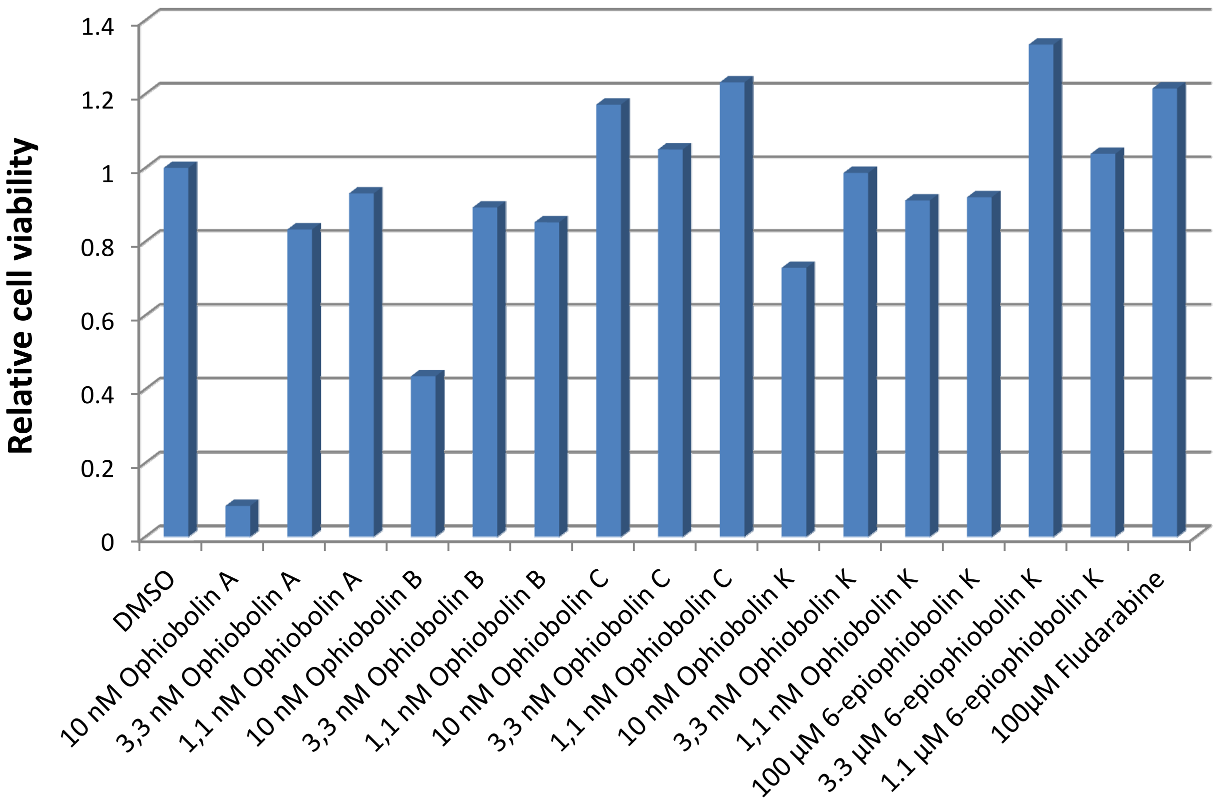


**Table S16.** $^{1}H$ and $^{13}C$-NMR for 6-epiophiobolin G in $CDCl_3$ at 800 MHz for $^{1}H$ and 200 MHz for $^{13}C$.

| | $\delta_H$ mult. (*J* (Hz)) | $\delta_C$ | HMBC connectivities | NOE connectivities |
|---|---|---|---|---|
| 1a | 1.15 m | 45.8 | 2, 3, 10, 11, 22 | 6, 10, 12a |
| 1b | 2.03 m | | 3, 6, 12, 22 | 2, 22 |
| 2 | 2.66 m | 49.2 | 1 | 1b, 22 |
| 3 | | 178.1 | | |
| 4 | 6.04 s | 130.2 | 3, 5, 6, 20 | 20 |
| 5 | | 208.2 | | |
| 6 | 3.40 m | 50.0 | 1, 2, 5, 7, 8, 21 | 1a, 10 |
| 7 | | 139.9 | | |
| 8 | 6.80 m | 158.1 | 6, 9, 10, 21 | 9a/b, 21, 22 |
| 9a | 2.20 m | 30.9 | 7, 8, 10, 14 | 8, 15, 22 |
| 9b | 2.93 m | | 7, 8, 10, 11 | 8, 10 |
| 10 | 2.63 m | 43.8 | 8, 9, 11, 13, 14, 22 | 1a, 6, 9b, 14 |
| 11 | | 45.4 | | |
| 12a | 1.43 m | 44.3 | 1, 13, 14, 22 | 1a, 13b |
| 12b | 1.52 m | | 1, 10, 13, 14, 22 | 22 |
| 13a | 1.25 m | 27.8 | 12, 14, 15 | 22 |
| 13b | 1.67 m | | 10, 11, 14 | 12a, 14, 23 |
| 14 | 1.89 m | 52.1 | 9, 10, 13, 15, 16, 23 | 10, 13b, 16, 23 |
| 15 | 2.55 m | 32.6 | 14, 16, 17, 23 | 9a/b, 18, 23 |
| 16 | 5.11 t (1.3) | 135.7 | 14, 15, 17, 18, 23 | 14, 17, 23 |
| 17 | 6.10 m | 124.0 | 15, 18, 19, 23 | 16, 24 |
| 18 | 6.00 m | 120.0 | 16, 17, 24, 25 | 15, 23, 25 |
| 19 | | 136.6 | | |
| 20 | 2.06 s | 17.3 | 2, 3, 4, 5 | 4 |
| 21 | 9.26 s | 193.0 | 6, 7 | 8 |
| 22 | 0.85 s | 22.9 | 1, 12 | 1b, 2, 8, 9a, 12b, 13a |
| 23 | 0.97 d (6.8) | 21.3 | 14, 15, 16 | 13b, 14, 15, 16,18 |
| 24 | 1.76 s | 18.2 | 18,19, 25 | 17 |
| 25 | 1.83 s | 26.5 | 18,19, 24 | 18 |

**7.10 Activity of ophiobolin A, B, C and K + 6-epiophiobolin K towards healthy fibroblasts (Wi-38) cells**

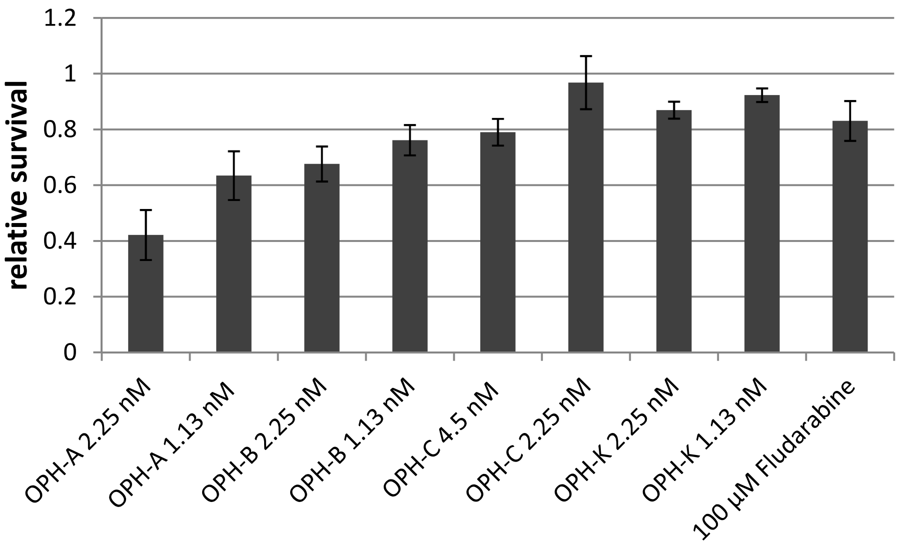
**Figure S17.** Activity of ophiobolin A, B, C and K + 6-epiophioboln K towards healthy fibroblasts (Wi-38).



## 7.11 Apoptosis induction in CLL cells by different ophiobolins

**Figure S18.** CLL cells cultured in DMEM were treated for 24 hours with two different concentrations of ophiobolin A, ophiobolin B, ophiobolin C and ophiobolin K. In order to analyze apoptosis induction, cells of each well were divided into two parts and analyzed by flow cytometry using two different staining strategies. (**a**) cell survival was analyzed by gating on cells that were negative for Annexin V-phycoerythrin (PE) and 7-amino-actinomycin (7-AAD). Relative survival compared to DMSO control (0.1%) is depicted as mean values +SD of 4 independent CLL samples; (**b**) Caspase-3 activity is depicted as mean values +SD of 4 independent CLL samples relative to DMSO control (0.1%).

(**a**)




(**b**)