Supporting Information

Antimicrobial metabolites against Methicillin-Resistant *Staphylococcus aureus* from the endophytic fungus *Neofusicoccum australe*

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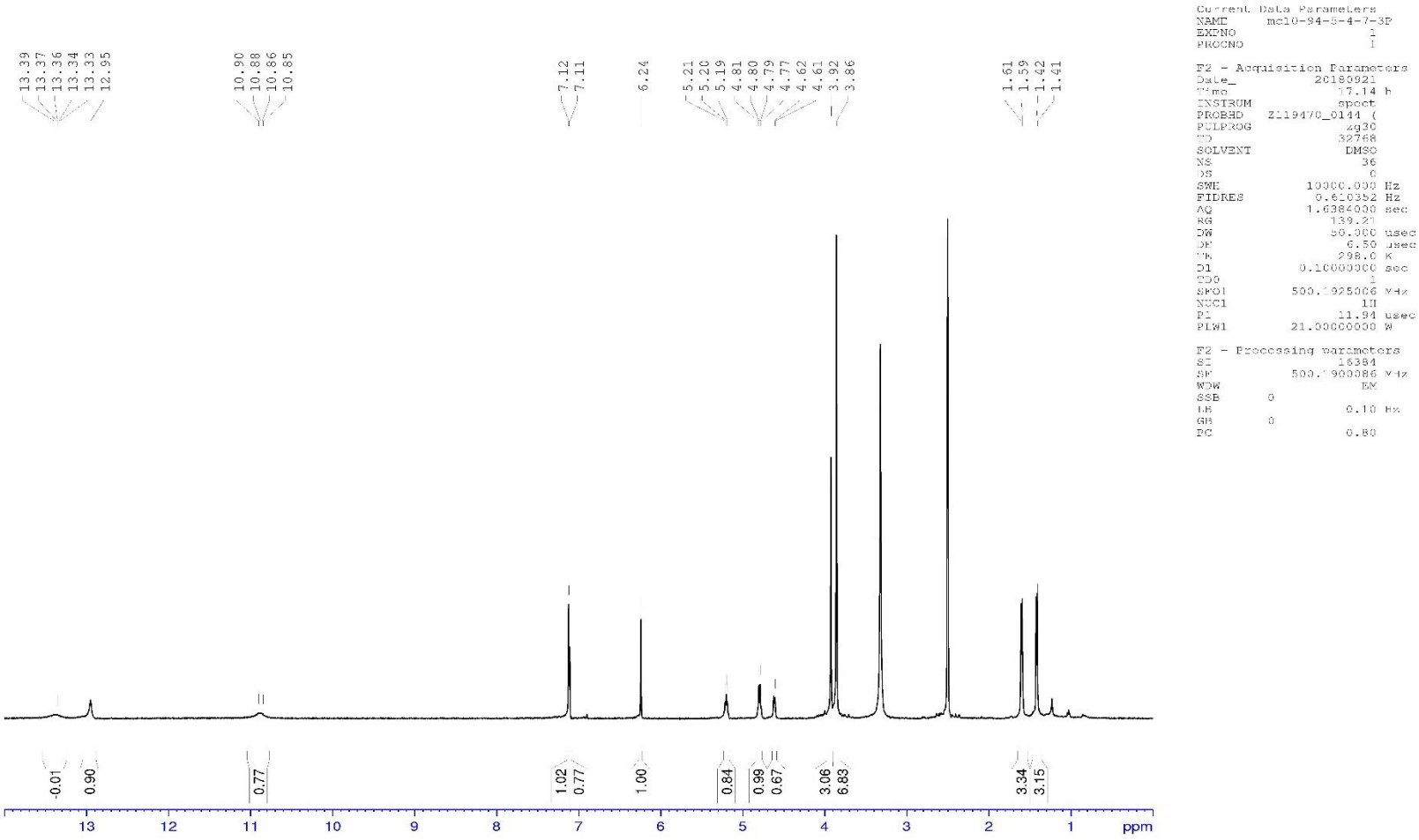
Content

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TE                 298.0 K
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NUC1                  1H
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PLW1          21.00000000 W

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Figure S1 ^{1}H NMR spectrum (500 MHz, DMSO) of neofusnaphthoquinone B (**1**)

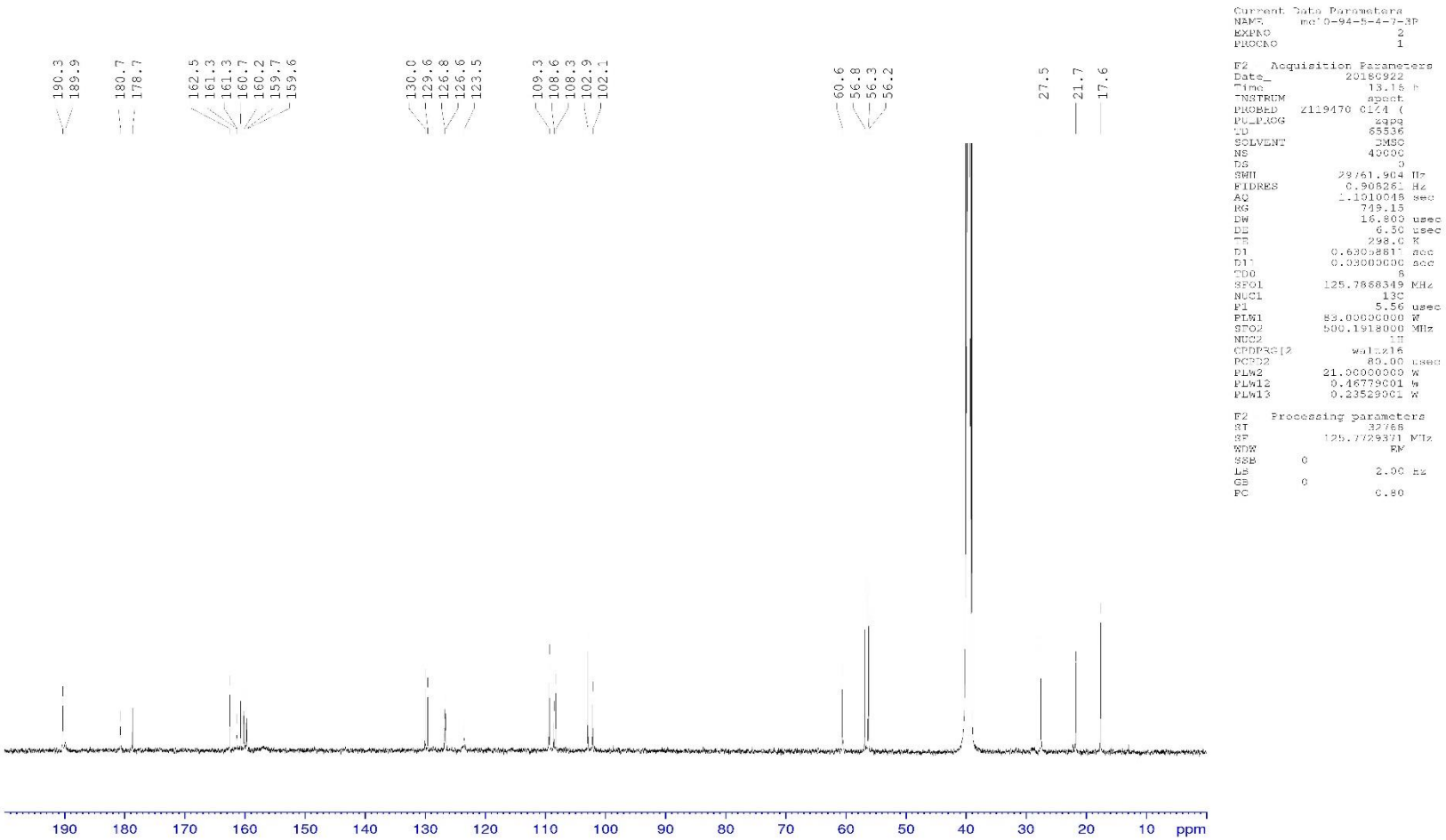
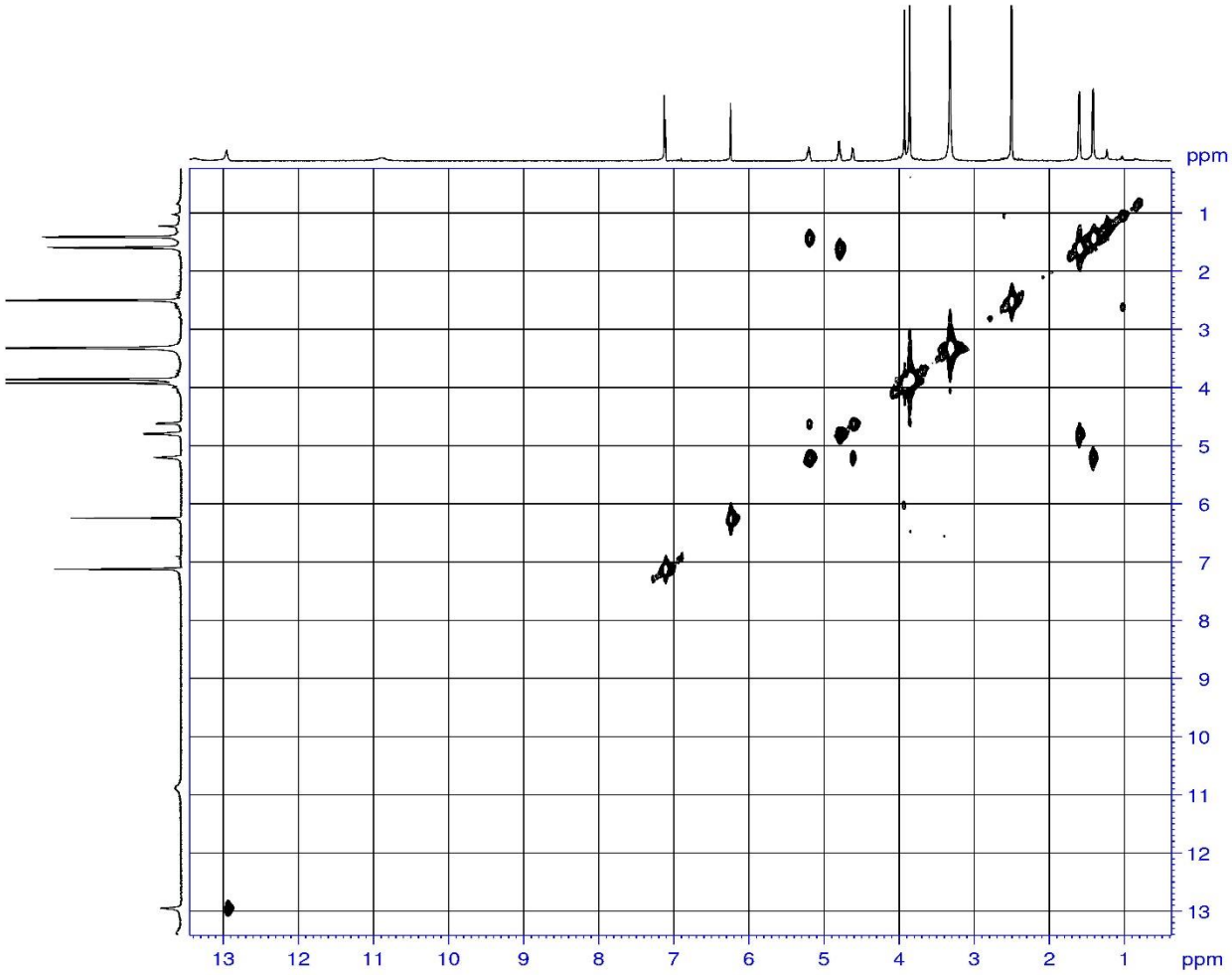


Figure S2 ^{13}C NMR spectrum (125 MHz, DMSO) of neofusnaphthoquinone B (**1**)

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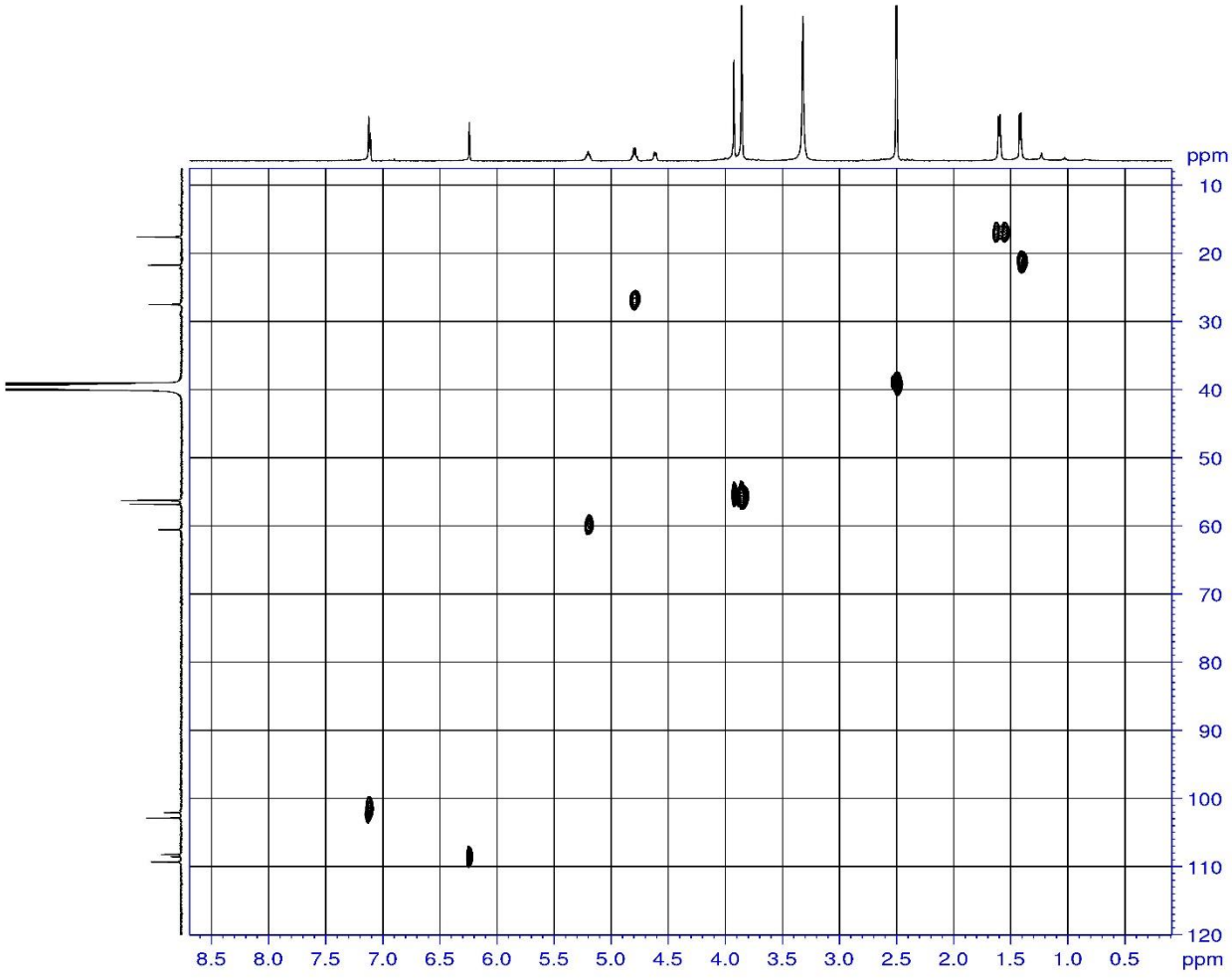
PULPROG       cosygpppqf
TD                  2048
SOLVENT             DMSO
NS                    10
DS                     8
SWH           10000.000 Hz
FIDRES            9.765625 Hz
AQ              0.1024000 sec
RG                  277.87
DW                  50.000 usec
DE                   6.50 usec
TE                 298.0 K
D0            0.00000300 sec
D1            1.39999998 sec
D11           0.03000000 sec
D12           0.00002000 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00010000 sec
TDav                    1
SFO1          500.1925010 MHz
NUC1                   1H
P0                  11.94 usec
P1                  11.94 usec
P17               2500.00 usec
PLW1         21.00000000 W
PLW10         3.32649994 W
GPNAM[1]        SINE.100
GPZ1              10.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1          500.1925 MHz
FIDRES         78.125000 Hz
SW                19.992 ppm
FnMODE                QF

F2 - Processing parameters
SI                    1024
SF          500.1900296 MHz
WDW                   SINE
SSB       0
LB        0 Hz
GB        0
PC                    0.80

F1 - Processing parameters
SI                     256
MC2                     QF
SF          500.1900119 MHz
WDW                   SINE
SSB       0
LB        0 Hz
GB        0
```

Figure S3 COSY spectrum of neofusnaphthoquinone B (**1**)

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PROCNO                   1

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Time                 14.23 h
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PROBHD   X11970_0144 (
PULPROG  hsqcedetgpsisp.2
TD                     2048
SOLVENT                DMSO
NS                       40
DS                       16
SWH              10000.000 Hz
FIDRES               9.765625 Hz
AQ                0.1024000 sec
RG                   645.04
DW                   50.000 usec
DE                    6.50 usec
TE                  298.0 K
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CNST17            0.5000000
D0                0.00003300 sec
D1                1.00000000 sec
D7                0.00178571 sec
D11               0.03000000 sec
D16               0.00020000 sec
D21               0.00357143 sec
D24               0.00089286 sec
LN0               0.00001670 sec
TD4v                     1
SFO1            500.1925013 MHz
NUC1                    1H
P1                   11.90 usec
P2                   23.80 usec
P28               1000.00 usec
PLW1          21.00000000 W
SFO2            125.7868347 MHz
NUC2                   13C
CPDPRG[2              garp
P3                   10.00 usec
P14                 500.00 usec
P24                2000.00 usec
PCPD2                70.90 usec
PLW0       0 W
PLW2          83.00000000 W
PLW12          1.69389999 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3                0.500
SPOFFS3    0 Hz
SPW3          12.68099976 W
SPNAM[7]     Crp60comp.4
SPOAL7                0.500
SPOFFS7    0 Hz
SPW7          12.68099976 W
GPNAM[1]          SINE.100
GPZ1                 80.00 %
GPNAM[2]          SINE.100
GPZ2                 20.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      256
SFO1             125.7868 MHz
FIDRES           233.907181 Hz
SW               238.023 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                      2024
SF            500.1900200 MHz
WDW                    QSINE
SSB                        2
LB         0 Hz
GB         0
PC                      0.80

F1 - Processing parameters
SI                       256
MC2        echo-antiecho
SF           125.7729757 MHz
WDW                    QSINE
SSB                        2
LB         0 Hz
GB         0
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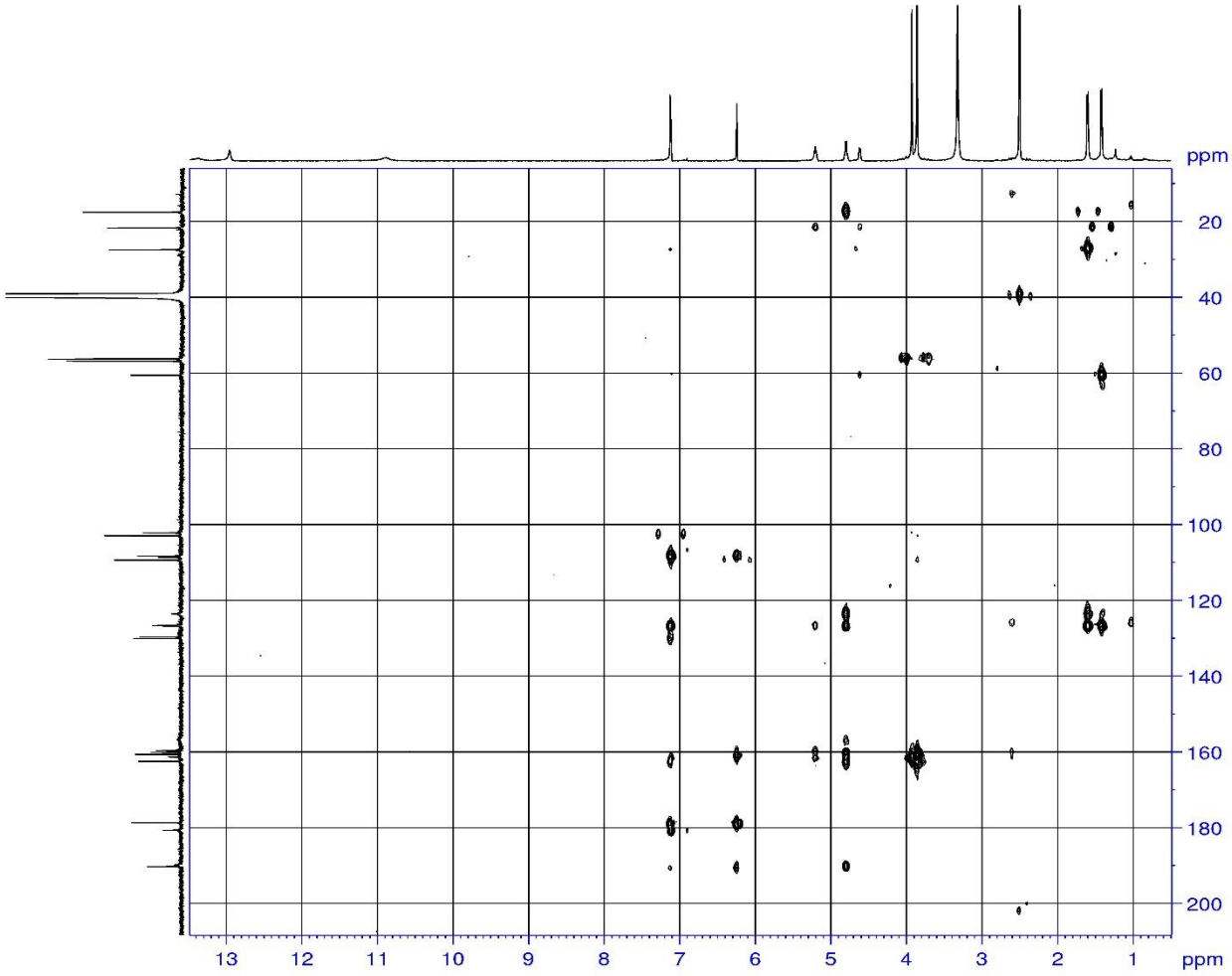
Figure S4 HSQC spectrum of neofusnaphthoquinone B (**1**)

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EXPNO                     5
PROCNO                    1

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Time                 17.46 h
INSTRUM                spect
PROBHD   Z119470_0144 (
PULPROG    hmbcgp'prdq'
TD                     2048
SOLVENT                DMSO
NS                      320
DS                       16
SWH              10000.000 Hz
FIDRES               9.765625 Hz
AQ                   0.1024000 sec
RG                      635.04
DW                      50.000 usec
DE                       6.50 usec
TE                      298.0 K
CNST2            140.0000000
CNST13             8.3299999
D0                   0.00000300 sec
D1                   1.00000000 sec
D2                   0.00357143 sec
D6                   0.06002401 sec
D16                  0.00020000 sec
IN0                  0.00001670 sec
TDav                          1
SFO1             500.1925010 MHz
NUC1                         1H
P1                       11.94 usec
P2                       23.88 usec
PLW1             21.00000000 W
SFO2             125.7868347 MHz
NUC2                        13C
P3                       10.00 usec
PLW2             83.00000000 W
GPNAM[1]            SINE.100
GPZ1                    50.00 %
GPNAM[2]            SINE.100
GPZ2                    30.00 %
GPNAM[3]            SINE.100
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                         256
SFO1             125.7868 MHz
FIDRES           233.907181 Hz
SW                   238.023 ppm
FnMODE                    QF

F2 - Processing parameters
SI                        1024
SF               500.1900288 MHz
WDW                       SINE
SSB                          2
LB          0 Hz
GB          0
PC                        0.80

F1 - Processing parameters
SI                         256
MC2                         QF
SF               125.7729345 MHz
WDW                       SINE
SSB          0
LB           0 Hz
GB           0
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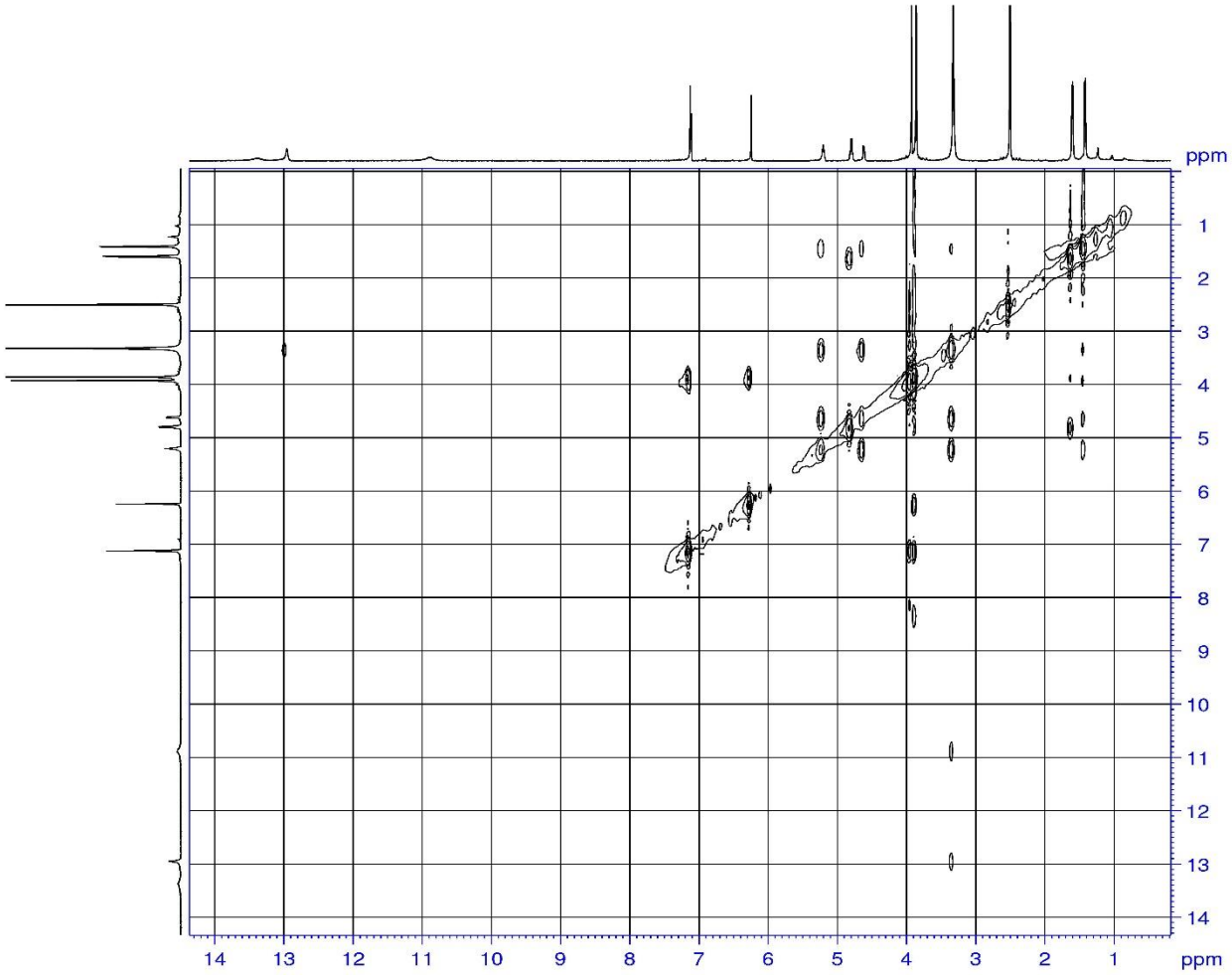
Figure S5 HMBC spectrum of neofusnaphthoquinone B (**1**)

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Current Data Parameters
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EXPNO                    6
PROCNO                   1

F2 - Acquisition Parameters
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Time              20.32 h
INSTRUM             spect
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PULPROG        roesyphpp
TD                  2048
SOLVENT             DMSO
NS                   200
DS                    16
SWH           10000.000 Hz
FIDRES            9.765625 Hz
AQ              0.10240000 sec
RG                 635.04
DW                 50.000 usec
DE                  6.50 usec
TE                298.0 K
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D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
IN0           0.00010000 sec
TDav                    1
SFO1         500.1925010 MHz
NUC1                   1H
P1                 11.94 usec
P15            230000.00 usec
P17              2500.00 usec
PLW1          21.00000000 W
PLW10          3.32649994 W
PLW11          0.24742000 W

F1 - Acquisition parameters
TD                    162
SFO1          500.1925 MHz
FIDRES        123.456787 Hz
SW              19.992 ppm
FnMODE             TPPI

F2 - Processing parameters
SI                  1024
SF           500.1900000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                 0.80

F1 - Processing parameters
SI                   256
MC2                 TPPI
SF           500.1900000 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```

Figure S6 ROESY spectrum of neofusnaphthoquinone B (**1**)

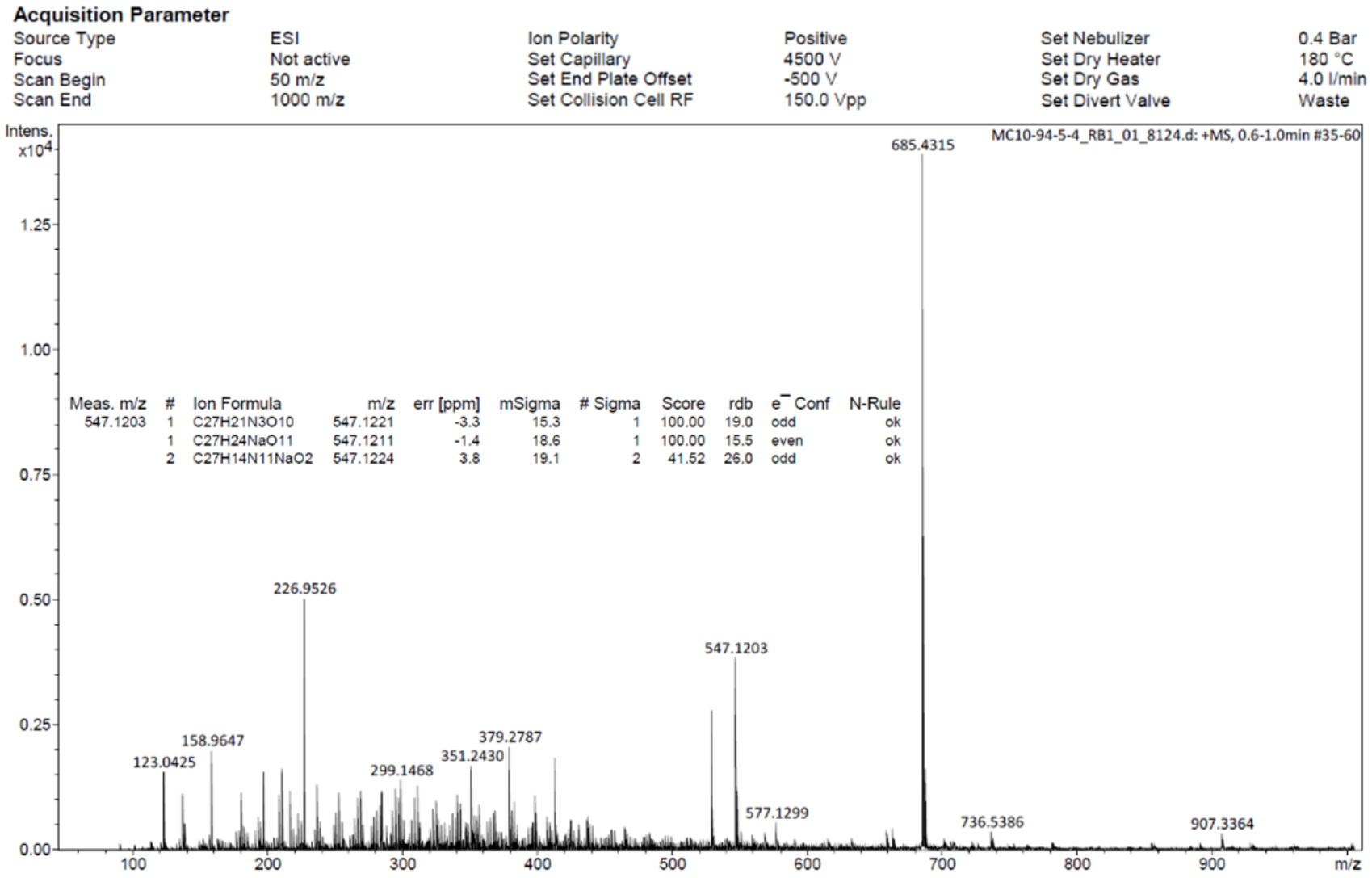


Figure S7 HRESIMS of neofusnaphthoquinone B (**1**)

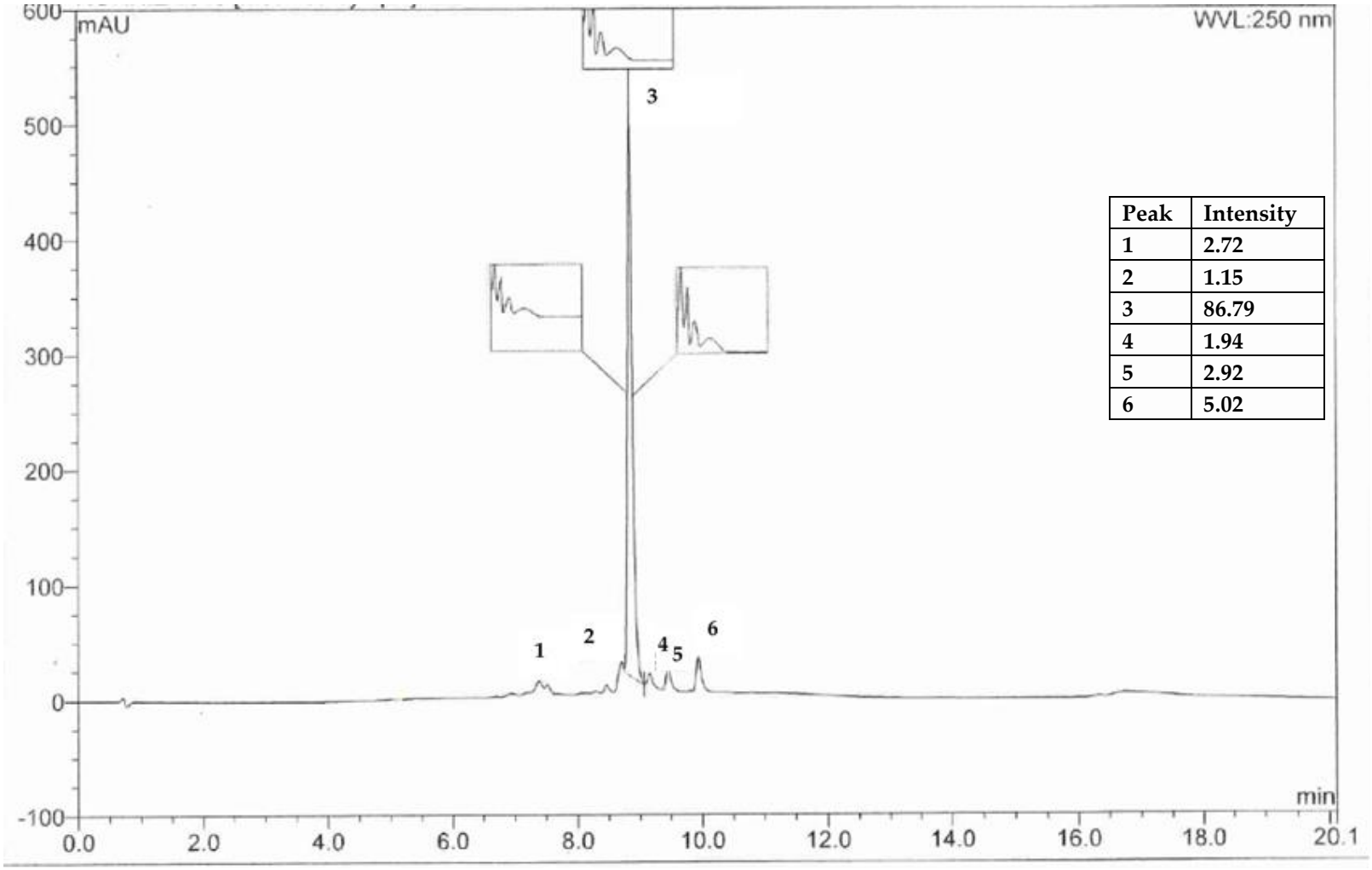
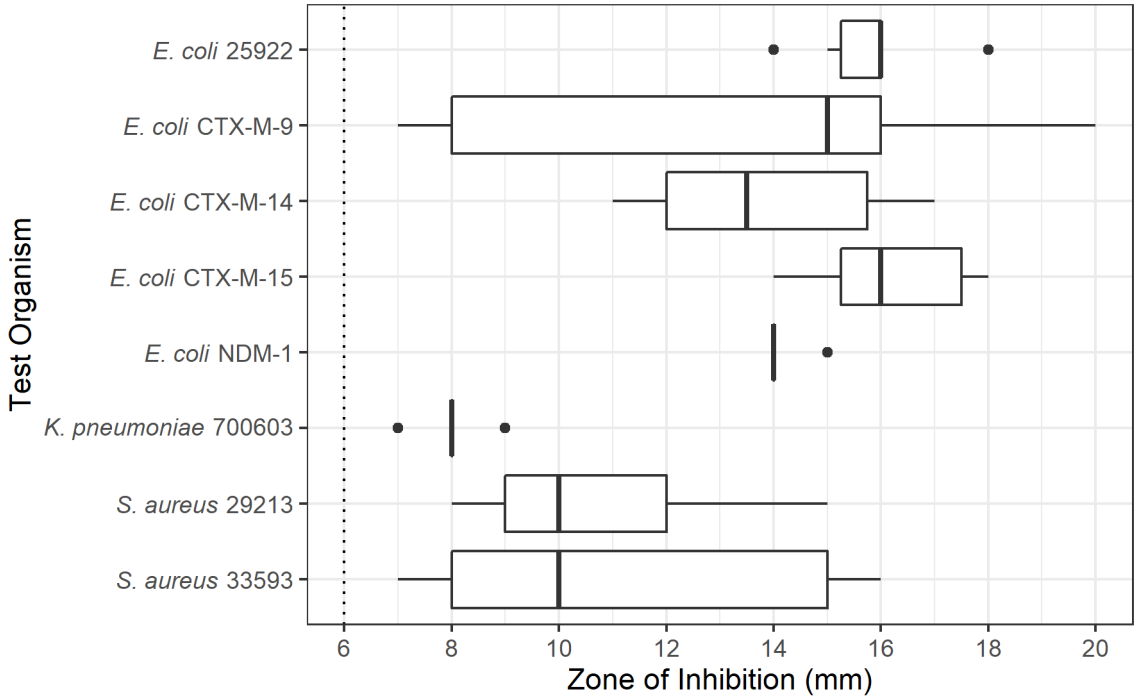


Figure S8 HPLC trace of neofusnaphthoquinone B (**1**) (Analytical reversed-phase HPLC on a Dionex UltiMate 3000RS using a C_8 column (3 μm Platinum, 33 × 7 mm) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 20 mins at 2 mL/min and monitoring at 216 nm).

Figure S9 Antibacterial activity testing using the zone of inhibition (ZOI) assay

To perform zone of inhibition (ZOI) testing, potato dextrose agar (PDA) plates were inoculated with a lawn of either antibiotic-sensitive *Escherichia coli* (ATCC 25922) or resistant clinical isolates (CTX-M-9, CTX-M-14, CTX-M-15, NDM-1) or antibiotic-resistant *Klebsiella pneumoniae* (ATCC 700603). Similarly, Mueller-Hinton agar plates were inoculated with a lawn of either antibiotic-sensitive *Staphylococcus aureus* (ATCC 29213) or antibiotic-resistant *S. aureus* (ATCC 33593). *Neofusicoccum australe* was grown on PDA plates and fungal plugs removed using a 6 mm punch biopsy tool (Catalogue number: SH241, Amtech Medical, New Zealand). Fungal plugs were placed onto the bacterial lawns, alongside PDA plugs containing no fungus. Plates were incubated inverted at 37°C for 24 h before measuring any zones of inhibition (in mm) produced.



Antibacterial activity of *Neofusicoccum australe* (ICMP 21498) against *E. coli*, *K. pneumoniae*, and *S. aureus*.

The dotted line represents the diameter of the fungal plugs with no activity. Data is presented as box-whisker plots. Raw data is available at DOI: 10.17608/k6.auckland.11888184.