SUPPLEMENTARY INFORMATION

Biosynthesis of the angiogenesis inhibitor borrelidin: directed biosynthesis of novel analogues

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SUPPLEMENTARY INFORMATION

Fermentation and isolation of compounds

General fermentation method

A seed flask containing NYG medium (30 cm^3 in a 250 cm^3 Erlenmyer flask) was inoculated with strain BIOT-1302 from a mycelial stock culture (0.5 cm^3; generated by 1:1 dilution of a 2 day seed culture with cryo-preservative (20% w/v glycerol and 10% w/v lactose in deionised water)). NYG medium contains, in deionised water: beef extract (0.3% w/v), Bacto peptone (0.5% w/v), glucose (1% w/v) and yeast extract (0.5% w/v). After 2 days on a rotary incubator (5 cm throw; 30°C; 250 rpm) the resulting cream-coloured culture was used to inoculate PYDG production medium (30 cm^3 in a 250 cm^3 Erlenmeyer flask; 10% v/v inoculum). PYDG medium contains, in deionised water: peptonised milk nutrient (1.5% w/v), yeast autolysate (0.15% w/v), dextrin (4.5% w/v) and glucose (0.5% w/v) adjusted to pH 7.0. After 24 hours on a rotary incubator (5 cm throw; 30°C; 250 rpm), the carboxylic acid supplement was added as a 0.05 cm^3 aliquot of a stock solution (0.6 M in methanol). The culture was incubated for a further 5 days and then harvested for analysis.

Chemical analysis of BIOT-1302 cultures

A portion of fermentation broth (1 cm^3) was mixed with an equal volume of methanol and shaken vigorously for 15 min on a vibrax. The resulting mixture was centrifuged for 10 min to remove cell debris and the supernatant was taken for LCMS analysis. Metabolites were separated by chromatography over reverse phase silica (Hypersil C18-BDS, 150 x 4.6 mm column, 3μ particle size) eluted at 1 $cm^3.min^{-1}$ using the following gradient: T=0 min, 25% B; T=15, 100% B. Mobile phase A: 10% acetonitrile:90% water,

containing 10 mM ammonium acetate and 0.1% v/v TFA; Mobile phase B: 90% acetonitrile:10% water, containing 10 mM ammonium acetate and 0.1% v/v TFA. The content of borrelidin(s) was calculated by comparison to a standard calibration curve.

Larger scale fermentation & extraction method

The example is given for the production of **4** but is general to all compounds. 3 x 7 dm^3 Applikon fermentors were run as below and the fermentation broths combined for extraction.

Initial seed inocula were prepared as in the section above. Secondary seeds were also prepared as above (but with 250 cm^3 NYG in 2 dm^3 Erlenmyer flasks). PYDG production medium (4 dm^3) prepared as above but with 0.01% v/v Pluronic L0101 to prevent foaming was inoculated with the secondary seed inocula (10% v/v). This was allowed to ferment in a 7 dm^3 Applikon fermenter for 6 days at 30°C. After 24 h cyclobutane1,2-*trans*-dicarboxylic acid in methanol was added to give a final concentration of 4 mM. Airflow was set at 0.75 v/v/m, with tilted baffles and the impeller speed controlled electronically between 250 and 600 rpm in order to maintain dissolved oxygen tension at or above 30% of air saturation. The fermentation broth of three such fermenters was combined (12 dm^3) and assayed; a total of 339 mg of **4** was produced (at an average titre 28 $mg.dm^{-3}$).

The fermention broth was adjusted to pH~5.5 (conc. HCl) and then clarified by centrifugation. The supernatant was extracted with ethylacetate (2 x 1 volume equivalent), and the cell pellet extracted with methanol (2 x 1 volume equivalent). The combined organic extracts were evaporated to yield a brown aqueous slurry. This was

diluted with water (to 1 dm^3) and extracted with ethylacetate (3 x 1 dm^3). The combined organic extracts were evaporated to yield a brown oil (~4.5 g). The oil was dissolved into acetone, flash silica gel 60 was added (150 g) and the solvent removed by evaporation. This was applied to a flash silica column (5 x 25 cm) pre-prepared in heptane. The column was eluted with a stepped gradient from 100% heptane to 100% ethyl acetate. Fractions containing **4** were identified by TLC, combined, and the solvent removed by evaporation to give a yellow oil (~1 g). This oil was then dissolved in the minimum volume of solvent mixture (heptane:$CHCl_3$:ethanol, 10:10:1) and applied to a column of Sephadex LH-20 (2.5 x 60 cm) pre-prepared in the same solvent mixture. The column was eluted under gravity with approx. 3 dm^3 of the same solvent mixture, collecting 12 cm^3 fractions. Fractions containing **4** were combined and the solvent removed by evaporation. The resulting pale yellow solid (550 mg) was crystallised by dissolving into a minimum amount of $CHCl_3$ and then adding hexane until becoming cloudy. The resulting solution was stored at -20°C overnight and the resulting solid collected by filtration and then washed with cold hexane. The mother liquor was treated similarly again. The two batches were combined to yield **4** (190 mg).

Spectroscopic analysis and structural determination

General methods

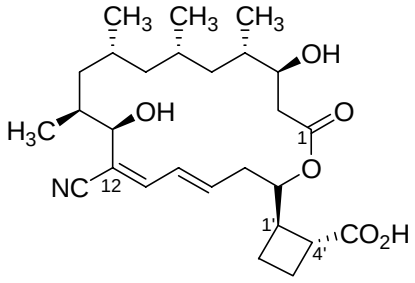
LCMS analysis was performed on an Agilent HP1100 HPLC system in combination with a Bruker Daltonics Esquire 3000+ ion trap mass spectrometer fitted with an electrospray source. The MS was operated in both positive and negative ion modes (continuous switching) and over a mass range of 100-1000 amu; UV analysis was performed at 258

nm on an Agilent DAD detector recording between 190-450 nm. NMR spectra were recorded on a Bruker Advance 500 spectrometer at 298 K operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C respectively. Standard Bruker pulse programs were used to acquire the ^{1}H-^{1}H COSY, APT, HMQC and HMBC spectra; coupling constants are given in hertz. NMR experiments were run in $CDCl_3$ (compounds **4**-**8**) or d_6-DMSO (compound **9**); these were referenced to the residual proton resonating at δ_H 7.26 and carbon at δ_C 77.0 for $CDCl_3$; and δ_H 2.49 and δ_C 39.5 for d_6-DMSO.

Assessment of compound purity

Purified compounds were analysed using the LCMS assay described above. Purity was assessed by MS and at multiple UV wavelengths. All compounds were >95% purity by this method and by inspection of the ^{1}H and ^{13}C NMR spectrum.

*Compound **4***



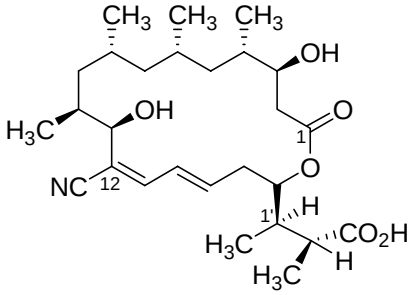
Position		δ_C	δ_H (mult., Hz)	H-H COSY	H-C HMBC
1	O-C=O	172.3	-	-	-
2	CH_2	34.3	2.35 dd (16.7, 2.0) 2.47 dd(16.7, 10.3)	3	1,3, 4
3	CH-OH	70.0	3.89 dt (10.4, 2.2)	2, 4	1,4, 5,18
4	CH	35.7	1.61 m	3, 5a, 18	
5	CH_2	43.1	1.24 ddd (10.4,10.4, 2.2) 0.90 ddd (19.0, 8.3, 2.7)	4, 6	3,4,7,18,19

6	CH	27.4	1.54 m	5, 7, 19	
7	CH_2	47.6	1.12 ddd 0.96 m	6 8	5,8,9,19,20
8	CH	26.2	1.60 m	7, 9, 20	
9	CH_2	37.4	1.04 m 0.71td (14.3,3.1)	8,9b, 10 8,9a	8,10,11,20,21
10	CH	35.2	1.86 m	9a, 11, 21	
11	CH-OH	73.1	4.11 d (9.7)	10	9,10, 12,13,21
12	C=	118.2	-	-	-
13	CH=	144.0	6.80 d (11.3)	14	11, 12, 15, 22
14	CH=	126.9	6.39 dd (14.9, 11.5)	13, 15	13, 22
15	CH=	138.5	6.10 ddd (8.9, 3.4, 3.0)	14, 16	13 17
16	CH_2	34.3	2.46 m	15, 17	15,17, 1’
17	CH-O	75.5	5.11 m	16, 1’	1’,2’,1, 15
18	4-CH_3	17.0	0.84 d (6.7)	4	3, 4,5
19	6-CH_3	18.2	0.79 d (6.3)	6	5
20	8-CH_3	20.2	0.82 d (5.2)	8	7, 8,9
21	10-CH_3	14.9	1.04 d (6.4)	10	9,11
22	-CN	115.9	-	-	-
1’	CH	40.2	2.89 quint (9.0)	17, 2’, 4’	2’, 4’,5’,17
2’	CH	41.9	3.08 qrad (9.0)	1’, 3’	1’,3’, 5’,17
3’	CH_2	21.5	2.15 m	2’, 4’	1’
4’	CH_2	21.1	2.02 m 1.76 quint (9.6)	1’, 3’	1’,3’,17
5’	-CO_2H	177.3	-	-	-

ESI-MS *m/z*: 498.4 $[M+Na]^+$, 476.3 $[M+H]^+$, 458.4 $[M-18]^+$; 474.5 $[M-H]^-$, 456.4 $[M-18]^-$

UV: λ_{max}(DAD) = 258 nm

*Compound **5***



SUPPLEMENTARY INFORMATION

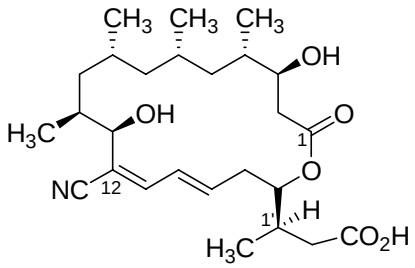
Position		δ_C	δ_H (mult., Hz)	H-H COSY	H-C HMBC
1	O-C=O	172.3	-	-	-
2	CH_2	37.9	2.37 m	3	1
3	CH-OH	71.0	3.89 td (6.2,3.9)	2,4	5,18
4	CH	35.5	1.80 m	3,5,18	-
5	CH_2	43.1	1.18 m 0.97 m	4,6	3,4,6,7,19
6	CH	27.2	1.67 m	5,7	-
7	CH_2	48.0	0.95 m 1.09 m	6,8	8,9,20
8	CH	26.2	1.61 m	7	-
9	CH_2	37.5	0.69 td (13.2, 3.2) 1.06 m	10	10,11,21
10	CH	35.2	1.83 m	9,11,21	-
11	CH-OH	73.0	4.11 d (9.8)	10	9,10,12,13,21
12	C=	118.2	-	-	-
13	CH=	143.7	6.81 d (11.3)	14	11,12,14,15,22
14	CH=	127.2	6.37 dd (13.8, 6.4)	13,15	13,16,22
15	CH=	138.1	6.17 ddd (14.8, 9.7, 5.1)	14,16	13,16
16	CH_2	34.3	2.59 m	15,17	14,15,1’
17	CH-O	74.2	4.9 dt(10.7,3.6)	16, 1’	1, 15, 1’
1’	CH	35.2	2.51 m	17, 4’	17, 5’
2’	CH	39.9	2.56 m	5’	3’, 4’, 5’
3’	$-CO_2H$	180.2	-	-	-
4’	1’-CH_3	12.2	0.96 d (6.9)	1’	1’,2’,17
5’	2’-CH_3	10.7	1.08 d (7.1)	2’	1’,2’,3’
18	4-CH_3	18.1	0.83 d	4	3,5
19	6-CH_3	18.1	0.83 d	6	7
20	8-CH_3	20.2	0.83 d	8	9
21	10-CH_3	14.9	1.04 d (6.4)	10	9,10,11
22	-CN	116.0	-	-	-

ESI-MS *m/z*: 500.5 $[M+Na]^+$, 478.5 $[M+H]^+$, 460.4 $[M-18]^+$; 476.4 $[M-H]^-$, 458.4 $[M-18]^-$

UV: λ_{max}(DAD) = 258 nm

*Compound **6***

SUPPLEMENTARY INFORMATION



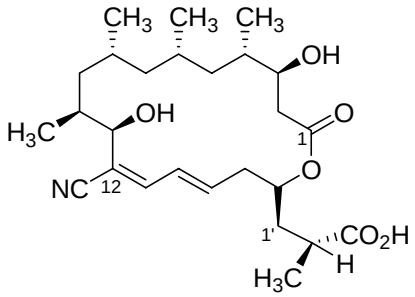
Position		δ_C	δ_H (mult., Hz)	H-H COSY	H-C HMBC
1	O-C=O	172.3	-	-	-
2	CH_2	38.1	2.38 m	3	1
3	CH-OH	70.8	3.88 td (6.2,3.9)	2,4	5,18
4	CH	35.2	1.77 m	3,5,18	-
5	CH_2	43.0	1.18 m 0.96 m	4,6	3,4,6,7,19
6	CH	27.1	1.63 m	5,7	-
7	CH_2	47.9	0.95 m 1.08 m	6,8	8,9,20
8	CH	26.2	1.61 m	7	-
9	CH_2	37.4	0.70 td (13.2, 3.2) 1.10 m	10	10,11,21
10	CH	35.1	1.87 m	9,11,21	-
11	CH-OH	73.0	4.11 d (9.5)	10	9,10,12,13,21
12	C=	118.2	-	-	-
13	CH=	143.8	6.81 d (11.5)	14	11,12,14,15,22
14	CH=	127.1	6.38 dd (13.8, 11.5)	13,15	13,16,22
15	CH=	138.1	6.17 ddd (13.8, 9.7, 5.1)	14,16	13,16
16	CH_2	34.1	2.58 m	15,17	14,15,1’
17	CH-O	76.1	4.85 dt(10.7,3.6)	16, 1’	1, 15, 1’
1’	CH	30.7	2.53 m	17, 4’	17
2’	CH_2	31.7	2.56 m	1’	3’, 4’
3’	$-CO_2H$	176.9	-	-	-
4’	1’-CH_3	16.7	1.09 d (6.9)	1’	1’,2’,17
18	4-CH_3	18.1	0.84 d	4	3,5
19	6-CH_3	17.7	0.83 d	6	7
20	8-CH_3	20.2	0.82 d	8	9
21	10-CH_3	14.8	1.05 d (6.4)	10	9,10,11
22	-CN	115.9	-	-	-

ESI-MS *m/z*: 486.5 $[M+Na]^+$, 464.6 $[M+H]^+$, 446.4 $[M-18]^+$; 463.5 $[M-H]^-$, 444.4 $[M-18]^-$

SUPPLEMENTARY INFORMATION

UV: λ_{max}(DAD) = 258 nm

Compound 7



Position		δ_C	δ_H (mult., Hz)	H-H COSY	H-C HMBC
1	O-C=O	172.4	-	-	-
2	CH_2	38.3	2.28 m	3	1
3	CH-OH	70.9	3.86td (6.2,3.9)	2,4	5,18
4	CH	35.3	1.74 m	3,5,18	-
5	CH_2	43.1	1.15m 0.97 m	4,6	3,4,6,7,19
6	CH	27.3	1.64 m	5,7	-
7	CH_2	47.9	1.08 m 1.18 m	6,8	8,9,20
8	CH	26.2	1.62 m	7	-
9	CH_2	37.5	0.70 td (13.2, 3.2) 1.10 m	10	10,11,21
10	CH	35.1	1.88 m	9,11,21	-
11	CH-OH	72.9	4.11 d (9.5)	10	9,10,12,13,21
12	C=	118.3	-	-	-
13	CH=	143.9	6.80 d (11.5)	14	11,12,14,15,22
14	CH=	127.1	6.38 dd (13.8, 11.5)	13,15	13,16,22
15	CH=	138.4	6.17 ddd (13.8, 9.7, 5.1)	14,16	13,16
16	CH_2	35.9	2.56 m	15,17	14,15,1’
17	CH-O	70.7	5.19 dt(10.7,3.6)	16, 1’	1, 15, 1’
1’	CH	37.0	2.48 m	17	17
2’	CH_2	36.7	2.44 m	1’,4’	1’,3’, 4’
3’	-CO_2H	180.6	-	-	-
4’	2’-CH_3	17.2	1.22 d (6.9)	1’	1’,2’, 3’
18	4-CH_3	18.1	0.85 d	4	3,5
19	6-CH_3	17.8	0.83 d	6	7
20	8-CH_3	20.2	0.81 d	8	9
21	10-CH_3	14.9	1.04d (6.4)	10	9,10,11
22	-CN	116.0	-	-	-

ESI-MS *m/z*: 486.5 $[M+Na]^+$, 464.5 $[M+H]^+$, 446.4 $[M-18]^+$; 463.4 $[M-H]^-$, 444.4 $[M-18]^-$

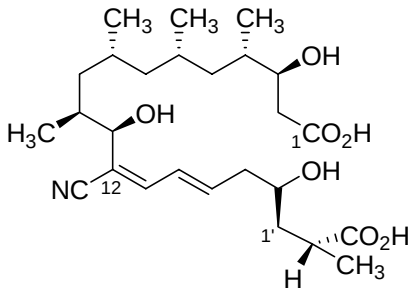
UV: λ_{max}(DAD) = 258 nm

*Compound **8***

ESI-MS *m/z*: 486.5 $[M+Na]^+$, 464.4 $[M+H]^+$, 446.4 $[M-18]^+$; 463.5 $[M-H]^-$, 444.4 $[M-18]^-$

UV: λ_{max}(DAD) = 258 nm

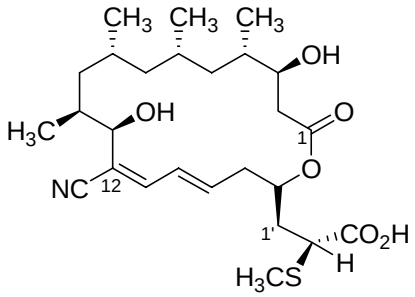
Following the purification of **8** and its submission for cancer cell line screening we observed an issue with the NMR sample; the remaining material had hydrolysed to the ring open *seco* form. LCMS analysis confirmed this, and that the original batch submitted for biological screening was correct. The NMR data below is thus for the *seco* form.



Position		δ_C	δ_H (mult., Hz)	H-H COSY	H-C HMBC
1	O-C=O	179.3	-	-	-
2	CH_2	39.1	2.42 m	3	1
3	CH-OH	70.6	3.94 td (6.2,3.9)	2,4	5,18
4	CH	35.3	1.85 m	3,5,18	-
5	CH_2	41.1	1.16m 0.95 m	4,6	3,4,6,7,19
6	CH	27.3	1.57 m	5,7	-

7	CH_2	45.8	0.95 m 1.18 m	6,8	8,9,20
8	CH	27.2	1.59 m	7	-
9	CH_2	38.5	0.85 td (13.2, 3.2) 1.01 m	10	10,11,21
10	CH	35.7	1.85 m	9,11,21	-
11	CH-OH	72.3	4.30 d (9.5)	10	9,10,12,13,21
12	C=	118.9	-	-	-
13	CH=	143.8	6.78 d (11.5)	14	11,12,14,15,22
14	CH=	127.4	6.6.53 dd (13.8, 11.5)	13,15	13,16,22
15	CH=	138.4	6.08 ddd (13.8, 9.7, 5.1)	14,16	13,16
16	CH_2	36.6	2.70 m	15,17	14,15,1’
17	CH-O	76.9	4.44 dt(10.7,3.6)	16, 1’	15, 1’
1’	CH	36.1	2.58 m	17	17
2’	CH_2	35.8	2.49 m	1’,4’	1’,3’
3’	$-CO_2H$	179.3	-	-	-
4’	2’-CH_3	14.7	1.26 d (6.9)	1’	1’,2’, 3’
18	4-CH_3	20.2	0.86 d	4	3,5
19	6-CH_3	15.0	0.85 d	6	7
20	8-CH_3	20.6	0.84 d	8	9
21	10-CH_3	14.8	1.00d (6.4)	10	9,10,11
22	-CN	116.3	-	-	-

*Compound **9***



Position		**δ_C**	**δ_H (mult., Hz)**	**H-H COSY**	**H-C HMBC**
1	O-C=O	170.8	-	-	-
2	CH_2	39.4	2.13 m 2.34 dd (15.3, 3.0)	3	1,3,4
3	CH-OH	70.4	3.68 dt (10.0, 3.0)	2,4	1,5,18
4	CH	36.1	1.59 m	3,5,18	-
5	CH_2	43.7	0.81 m 1.13 m	3,6	3,4,18
6	CH	27.4	1.66 m	5,19	-
7	CH_2	48.1	0.87 m 1.02 m	6,8	5,8,18,20 5,8,9,18

8	CH	26.4	1.54 m	9, 20	
9	CH_2	37.5	0.56 dd (13.8, 2.9) 1.13 m	8	8,10,20,21
10	CH	34.8	1.59 m	11, 21	-
11	CH-OH	71.1	4.05 d (9.7)	10	10,12,13
12	C=	119.6	-	-	-
13	CH=	143.9	6.94 d (11.1)	14	11,12,14,15,22
14	CH=	127.7	6.46 dd (14.4, 12.0)	13,15	13,15,16,22
15	CH=	139.9	6.28 m	14,16	13,14,16
16	CH_2	36.4	2.48 m	15,17	14,15,17,1’
17	CH-O	70.2	5.12 m	1’, 16	-
1’	CH_2	34.2	1.80 m 2.12 m	2’, 17	2’,3’,16,17
2’	CH	46.8	3.0 t (7.1)	1’	17, 1’, 3’, 4’
3’	$-CO_2H$	173.2	-	-	-
4’	CH_3-S	13.7	2.01 s	-	2’
18	4-CH_3	18.3	0.74 m	4	3,4,5
19	6-CH_3	18.7	0.93 d (6.4)	6	5,6,7
20	8-CH_3	20.8	0.73 m	8	7,8,9
21	10-CH_3	15.3	0.91 d (6.4)	10	9,10,11
22	-CN	116.6	-	-	-

ESI-MS *m/z*: 518.5 $[M+Na]^+$, 478.5 $[M+H]^+$, 460.5 $[M-18]^+$; 476.5 $[M-H]^-$, 458.5 $[M-18]^-$

UV: λ_{max}(DAD) = 258 nm

In vitro bioassay for anticancer activity

In vitro evaluation of compounds for anticancer activity in a panel of 12 human tumour cell lines using a monolayer proliferation assay were carried out at the Oncotest Testing Facility, Institute for Experimental Oncology, Oncotest GmbH, Freiburg.

Origin of cell lines

The 12 cell lines chosen for analysis were MCF-7, MDA-MB-231, MDA-MB-468, NCI-H460, SF-268, OVCAR-3, A498, GXF 251L, MEXF 394NL, UXF 1138L, LNCAP and

DU145. The Oncotest cell lines were established from human tumor xenografts as described previously.[1] The origin of the donor xenografts has been described.[2] Other cell lines were either obtained from the NCI (H460, SF-268, OVCAR-3, DU145, MDA-MB-231, MDA-MB-468) or purchased from DSMZ, Braunschweig, Germany (LNCAP). All cell lines, unless otherwise specified, were grown at 37°C in a humidified atmosphere (95% air, 5% CO_2) in a 'ready-mix' medium containing RPMI 1640 medium, 10% fetal calf serum, and 0.1 $mg.cm^{-3}$ gentamicin (PAA, Cölbe, Germany). A description of cell line characteristics is given in the table below:

Cell line	**Characteristics**
MCF-7	Breast, NCI standard
MDA-MB-231	Breast, PTEN positive, resistant to 17-AAG
MDA-MB-468	Breast, PTEN negative, resistant to 17-AAG
NCI-H460	Lung, NCI standard
SF-268	CNS, NCI standard
OVCAR3	Ovarian, p85 mutated, AKT amplified
A498	Renal, high MDR expression
GXF 251L	Gastric
MEXF 394NL	Melanoma
UXF 1138L	Uterus
LNCAP	Prostate, PTEN negative
DU145	Prostate, PTEN positive

Monolayer assay: brief description of protocol

A modified propidium iodide assay was used to assess the effects of the test compound(s) on the growth of twelve human tumor cell lines.[3] Briefly, cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96 well flat-bottomed

microtitre plates at a cell density dependent on the cell line (5 - 10.000 viable cells/well). After 24 h recovery to allow the cells to resume exponential growth, 0.010 cm^3 of culture medium (6 control wells per plate) or culture medium containing borrelidin are added to the wells. Each concentration is plated in triplicate. Compounds are applied to give two concentrations (1 μM and 10 μM). Following 4 days of continuous exposure, cell culture medium with or without test compound is replaced by 0.2 cm^3 of an aqueous propidium iodide (PI) solution (7 $\mu g.cm^{-3}$). To measure the proportion of living cells, cells are permeabilized by freezing the plates. After thawing the plates, fluorescence is measured using the Cytofluor 4000 microplate reader (excitation 530 nm, emission 620 nm), giving a direct relationship to the total number of viable cells. Growth inhibition is expressed as treated/control x 100 (%T/C). %T/C data is averaged over *n* runs for each cell line vs. compound.

Results

Cell line	**%T/C for borrelidin analogues assayed [at 1μM/10μM(*n*)]**						
	1	**4**	**5**	**6**	**7**	**8**	**9**
SF268	3/3(2)	15/4(2)	12/3(3)	79/13(3)	86/14(3)	86/57(3)	79/54(3)
251L	9/8(2)	24/9(2)	22/12(3)	62/26(3)	68/32(3)	107/81(3)	99/67(3)
H460	5/4(2)	75/7(2)	32/3(3)	94/38(3)	98/49(3)	97/84(3)	102/79(3)
MCF7	15/13(2)	12/9(2)	13/9(3)	23/12(3)	45/16(3)	100/76(3)	97/48(3)
MDA231	14/12(1)	24/8(1)	20/7(3)	81/20(3)	89/26(3)	89/90(3)	100/81(3)
MDA468	7/6(2)	18/6(2)	17/7(3)	93/15(3)	103/25(3)	102/62(3)	91/59(3)
394NL	2/2(2)	16/3(2)	14/4(3)	79/9(3)	90/9(3)	98/67(3)	95/64(3)
OVCAR3	11/11(2)	43/16(2)	22/6(3)	87/32(3)	100/29(3)	101/91(3)	97/102(3)
DU145	7/5(2)	86/16(2)	61/7(3)	89/55(3)	99/68(3)	79/74(3)	99/75(3)
LNCAP	5/7(2)	54/9(2)	22/7(3)	103/24(3)	116/34(3)	108/92(3)	98/59(3)
A498	8/8(2)	22/9(2)	23/9(3)	74/21(3)	77/30(3)	102/78(3)	95/68(3)
1138L	3/2(2)	5/3(2)	3/3(3)	59/5(3)	87/6(3)	83/56(3)	81/42(3)

References

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