Exploration of the influence of spiro-dienone moiety on biological activity of the cytotoxic marine alkaloid discorhabdin P

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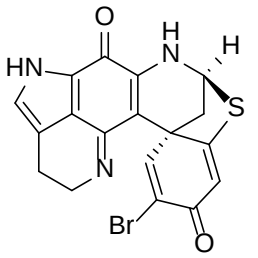
Abstract

In order to clarify the importance of the C-3 carbonyl group to the cytotoxicity observed for discorhabdin marine alkaloids a number of semi-synthetic analogues of discorhabdin P were prepared. C-3 Reduction and acetylation typically resulted in a 4- to 10-fold reduction in cytotoxic potency (P388 cell line) compared to the corresponding keto parent compound. X-ray crystallography of a C-3 dienol derivative of discorhabdin P (**6**) allowed assignment of (3*r*, 6*r*) pseudoasymmetric configuration to the natural product 3-dihydrodiscorhabdin C (**5**). Analogues incorporating increasingly bulky substitution at C-3 only retained cytotoxicity if they bore (3*s*, 6*s*)-configuration at the spiro-dienol moiety. A variety of fluorophore-labelled probes were prepared of which only a dansyl analogue (**14**) exhibited (modest) cytotoxicity.

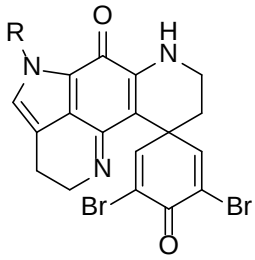
1. Introduction

A diverse array of over forty members of the discorhabdin/prianosin/epinardin family of natural products have been reported from marine sponges.[1,2] These pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline-skeletoned alkaloids, which include discorhabdins B (**1**), C (**2**) and P (**3**), typically exhibit potent cytotoxicity,[3-8] anti-bacterial,[9-11] enzyme inhibitory[11,12] and anti-malarial activities.[10] More recently, several members of the discorhabdin family have been reported to be moderate inhibitors of protein-protein interaction, interrupting the interaction between hypoxia-inducible factor 1α (HIF-1α) and its transcriptional coactivator p300.[13]

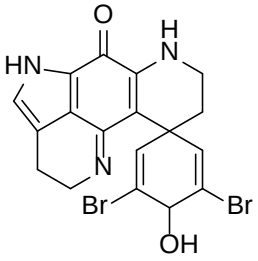
Structure-cytotoxicity relationship studies of discorhabdins have suggested a model for bioactivity with the combination of both the iminoquinone and spiro-enone fragments being essential for cytotoxity.[2,14] In the specific case of discorhabdin B (**1**), we have more recently demonstrated support for this model, showing that the relative electrophilic reactivity of the marine natural product and analogues correlates with cytotoxicity.[15]



1 (+)-discorhabdin B



2 R = H discorhabdin C
3 R = CH_3 discorhabdin P



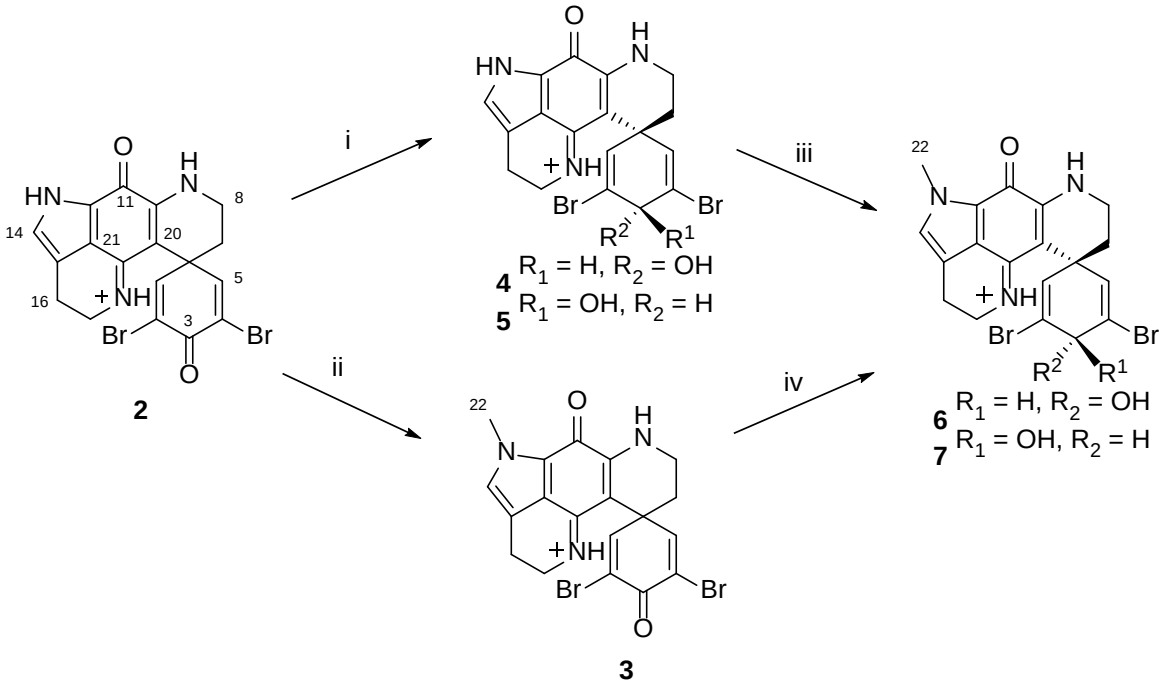
4 3-dihydrodiscorhabdin C

Somewhat contrary to this SAR proposal are the biological activities observed for 3-dihydrodiscorhabdin C (**4**). While the original report of semi-synthetically-derived 3-dihydrodiscorhabdin C noted it to be less cytotoxic towards the murine leukemia cell line P388 (IC_{50} 0.91 μM)[16] compared to spiro-dienone discorhabdin C (IC_{50} 0.09 μM),[16] subsequent reports of naturally occurring **4** [5,10,11] noted that the two alkaloids exhibited similar levels of cytotoxicity towards the K562 human myeloleukemia[11] and Vero monkey kidney non-malignant cell lines.[10]

In an effort to clarify this ambiguity in potency of biological activity, we have prepared a series of C-3 modified analogues of a discorhabdin alkaloid, each bearing variation in substituent steric bulk and stereochemical configuration at C-3. The series of analogues also included fluorescent derivatives that could be utilized in mechanism of action studies or to help define specific cellular targets of the discorhabdins. Herein we report the semi-synthesis and *in vitro* anti-tumour evaluation of a library of C-3 modified analogues of discorhabdin P (*N*-13 methyl discorhabdin C), **3**. During the course of this study, the relative configuration of naturally occurring 3-dihydrodiscorhabdin C was secured by single crystal X-ray analysis of a derivative.

2. Results and discussion

While our original interest was directed towards preparing C-3 derivatives of discorhabdin C (**2**), given the previously noted nucleophilic reactivity of pyrrolic *N*-13,[17] we opted to study the *N*-13 methyl analogue, discorhabdin P (**3**).[12,18] The semi-synthetic preparation of 3-dihydrodiscorhabdin P can be achieved from discorhabdin C via two routes: (i) reduction at C-3, followed by *N*-13-methylation or (ii) the reverse sequence i.e. methylation at N-13 followed by C-3 reduction (**Scheme 1**). Following the first route, reduction of discorhabdin C with $NaBH_4$ in methanol afforded two dienol products **4** and **5** in 2:1 ratio, that were separable by C_{18} reversed-phase flash column chromatography. Direct comparison of ^{1}H NMR data observed for both products with data reported for naturally occurring 3-dihydrodiscorhabdin C [5,10,11,13,16] identified that the lower yielding isomer **5** was identical to the natural product.



Scheme 1. *Reagents and conditions*: (i) $NaBH_4$, MeOH, r.t., 5 min, **4** 46% and **5** 36%; (ii) CH_3I, K_2CO_3, acetone, 70 ^oC, 1 h, 96%; (iii) CH_3I, K_2CO_3, acetone, 70 ^oC, 1 h, 80% for **6** and 90% for **7**; (iv) $NaBH_4$, MeOH, r.t., 5 min, **6** 59% and **7** 32%.

Both dienol isomers were then converted to their respective *N*-13 methyl derivatives **6** and **7** by reaction with methyl iodide in acetone with yields of 80% and 90%, respectively (**Scheme 1**). Using the second route (**Scheme 1**), methylation of discorhabdin C gave discorhabdin P **3**[18] (96% yield), which followed by reduction with $NaBH_4$ in methanol gave **6** (59%) and **7** (32% yield). Spectroscopic data for these products were identical to those observed for samples prepared via the first route. Crystallization of the free base of **6** from CH_3OH – CH_2Cl_2 resulted in red crystals that yielded an X-ray crystal structure, allowing the assignment of the pseudoasymmetric configuration[19] as 3*s*, 6*s* (**Figure 1**). This result allowed indirect assignment of (3*r*, 6*r*) configuration to naturally occurring 3-dihydrodiscorhabdin C (**5**). It is interesting to note that while the major product of hydride reduction at C-3 (**4**) is due to approach of reductant from the 'outside' face of the spiro-dienone, the naturally occurring C-3 dienol derivative of discorhabdin C (**5**) is the result of hydride delivery to the more sterically-crowded 'inner' face of the dienone ring.

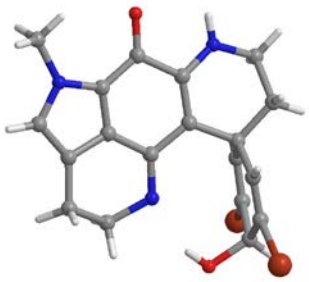
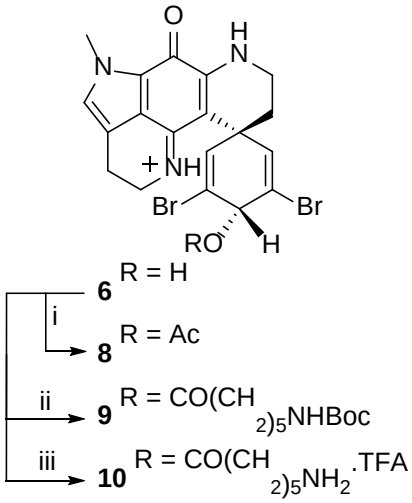
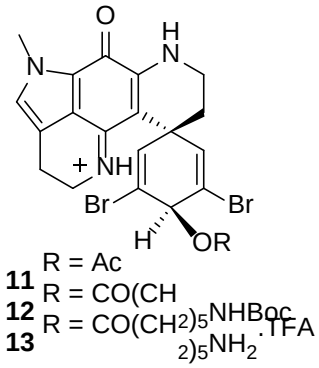


Figure 1. X-Ray crystallographic structure of (3*s*, 6*s*)-3-dihydrodiscorhabdin P (**6**) free base (CCDC 928777).

With stereochemically-defined dienols **6** and **7** in hand, our attention turned to derivatization of the secondary alcohol at C-3. Successful acetylation of dienol **6**, to afford acetate **8** in 79% yield (**Scheme 2**), suggested that attachment of a fluorophore linker at C-3 via esterification was feasible. DCC mediated coupling of 6-(*tert*-butoxycarbonylamino)hexanoic acid to dienol **6** afforded **9** (76% yield), which upon subsequent cleavage with MeOH/H_2O/TFA yielded tethered amine **10** (90% yield). A similar series of transformations were then applied to dienol **7** to afford acetate **11** (93% yield), Boc-protected aminohexanoic acid ester **12** (68% yield) and tethered amine **13** (82% yield).



Scheme 2. *Reagents and conditions:* (i) Ac_2O, pyridine, r.t., 24 h, 79%; (ii) 6-(*tert*-butoxycarbonylamino) hexanoic acid, DCC, DMAP, DMF, r.t., 18 h, 76%; (iii) MeOH/H_2O/TFA, 70 ^oC, 1 h, 90%.



Comparative biological evaluation of the cytotoxicity of **6**–**13** towards the P388 murine leukemia cell line (**Table 1**) identified that 3-dihydro analogues of discorhabdin P exhibit 4- to 10-fold less potent activity than the parent dienone alkaloid, and that acetylated derivatives are essentially equipotent to the corresponding dienol analogue. While incorporation of bulkier substitution at C-3 was tolerated for the (3*s*, 6*s*)-series (**9** and **10**), similar substituted analogues of the (3*r*, 6*r*)-series were noticeably less cytotoxic (**12** and **13**).

Table 1
In vitro anti-tumour activities (μM) of discorhabdin analogues

Compound	**P388 IC_{50}**[a]
2	0.09[b]
3	0.31[c]
6	3.3
7	1.3
8	1.9
9	1.5
10	1.7
11	1.2
12	4.4
13	> 7.6
14	4.5
15	>5.7
16	>6.7

[a] IC_{50} against the P388 D1 murine leukaemia cell line after 72 hours with mitomycin C (IC_{50} 0.5 μM) as the positive control. Values reported are the average of two independent experiments (n=2).
[b] Data taken from Ref. 16.
[c] Data taken from Ref. 18.

Discorhabdin P (**3**) and analogues **7**, **8**, **9** and **11** were also evaluated for cytotoxicity towards a panel of human tumour cell lines as part of the Developmental Therapeutics Program of the National Cancer Institute and in most cases were found to exhibit panel average GI_{50}, TGI and LC_{50} values similar to those observed for discorhabdin P (**3**). Combined with the P388 cytotoxicity data (**Table 1**), these results indicate that N-methylation of discorhabdin C (**2**) [to give discorhabdin P (**3**)] results in a 4-fold decrease in cytotoxicity, with C-3 reduction and acetylation leading to an additional decrease in potency of growth inhibition (GI_{50} data, **Table 2**), but with only modest or no change in cytostatic or anti-proliferative activity (TGI and LC_{50} data, **Table 1**). The exception to this trend was the Boc-protected analogue **9**, which while it demonstrated similar levels of cytotoxicity towards the P388 cell line compared to the other analogues, exhibited less potent activity towards the panel of human tumour cell lines.

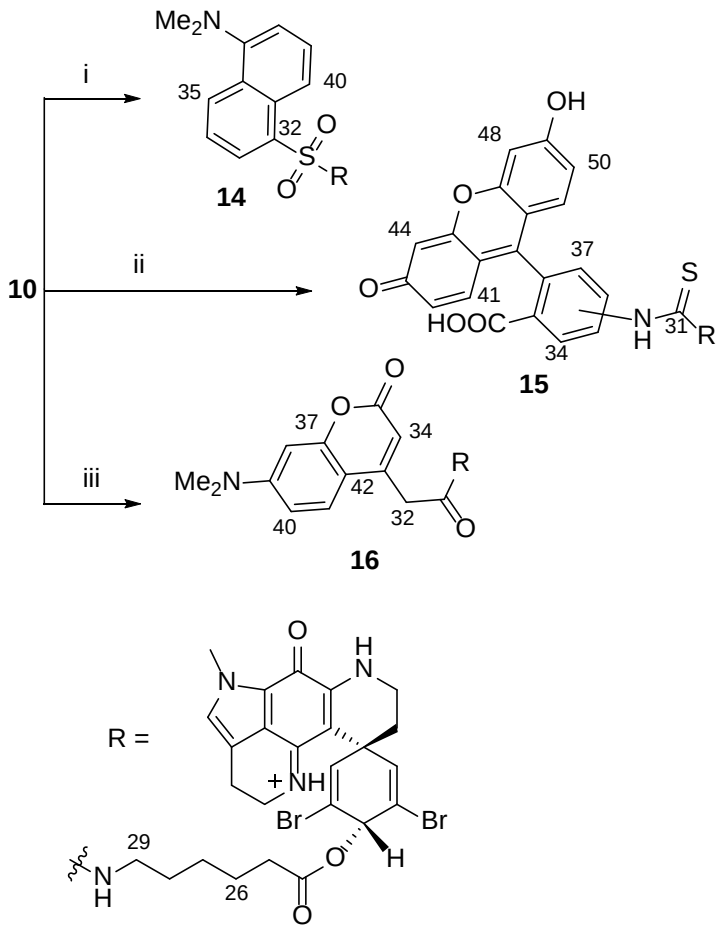
Table 2
Summary of activities of selected discorhabdin analogues against human tumour cell lines

Compound (NSC)[a]	**GI_{50}**[b]	**TGI**	**LC_{50}**
2 (626162)	0.13	0.36	2.3
3 (740265)	0.47	2.1	12.5
7 (747516)	1.2	4.4	14.5
8 (747518)	1.4	5.0	13.5
9 (747519)	4.5	14.8	42.7
11 (747517)	0.81	2.5	8.7

[a] NSC number is the NCI reference number for each compound. Search for this number at http://dtp.cancer.gov to view complete information of all *in vitro* assay profiles.
[b] GI_{50} (50% growth inhibition), TGI (total growth inhibition) and LC_{50} (50% cell kill) data are averaged calculated mean micro-molar values obtained from two experiments at the NCI.

The superior P388 cytotoxicity of amine **10** (**Table 1**) directed our further study of fluorescent analogues. An interesting observation was the lack of cytotoxicity detected for free amine **13**, highlighting what appears to be a structure-activity relationship that is sensitive to stereochemistry and polarity of attached functionality.

Dansyl, 5(6)-fluorescein and 7-*N*,*N*-dimethylaminocoumarin-4-acetic acid (DACA) fluorophores were chosen for attachment to the discorhabdin scaffold. All three fluorophores have been utilized in biological studies related to target identification of small molecules including natural products.[20-22] Reaction of amine **10** with dansyl chloride afforded the fluorescent sulfonamide **14**, with fluorescein-5(6)-isothiocyanate (FITC) gave analogue **15** as a mixture of regioisomers, or with NHS-activated 7-dimethylaminocoumarin-4-acetic acid[17] to afford amide **16** (**Scheme 3**). Biological evaluation of fluorescent analogues **14**–**16** (**Table 1**) revealed that only the dansylated discorhabdin **14** retained activity, albeit modestly (P388, IC_{50} 4.50 μM), suggesting it could play a role in future subcellular localisation and mechanism of action studies.



Scheme 3. *Reagents and conditions*: i) dansyl sulfonyl chloride, K_2CO_3, acetone, r.t., 3.5 h, 40%; ii) Fluorescein-5(6)-isothiocyanate (FITC), K_2CO_3, acetone, r.t., 3.5 h, 38%; (iii) NHS-activated 7-dimethylaminocoumarin-4-acetic acid, K_2CO_3, acetone, r.t. 3.5 h, 37%.

3. Conclusions

In conclusion, we have established that semi-synthetic derivatives of discorhabdin P that embody C-3 reduction and acetylation exhibit 4- to 10-fold reduction in cytotoxic potency towards the P388 cell line compared to the corresponding keto parent compound. A 4-fold decrease in potency of growth inhibition (GI_{50}) towards human tumour cell lines was observed, but with only modest or no change in cytostatic or anti-proliferative activity (TGI and LC_{50}). These results provide some support to the proposed SAR model of cytotoxicity of the discorhabdins, which proposes that nano-molar cytotoxicity requires the presence of an electrophilic spiro-(di)enone ring in the structure.[2] The current results however, also determine that the spiro-dienone moiety is not the sole determinant of cytotoxicity for this class of marine natural products. One of the

derivatives prepared, dienol **6**, was analysed by X-ray crystallography, leading to the first definitive structural assignment of the natural product (3*r*, 6*r*)-3-dihydrodiscorhabdin C (**5**). Analogues incorporating bulky substitution at C-3 only retained cytotoxicity if they bore (3*s*, 6*s*)-configuration at the spiro-dienol moiety.

4. Experimental

4.1 General

Infrared spectra were run as dry films on an ATR crystal and acquired using a Perkin-Elmer Spectrum One Fourier Transform infrared spectrometer with a Universal ATR Sampling Accessory. NMR spectra were recorded on either a Bruker Avance DRX- 400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei or a Bruker Avance DRX-300 spectrometer operating at 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei. Proto-deutero solvent signals were used as internal references (DMSO-d_6: δ_H 2.50, δ_C 39.43; CD_3OD: δ_H 3.30, δ_C 49.05; $CDCl_3$: δ_H 7.25, δ_C 77.0). Standard Bruker pulse sequences were utilized. MS data were acquired on a Bruker micrOTOF Q II mass spectrometer. Analytical reversed-phase HPLC was run on a Waters 600 HPLC photodiode array system using either an Alltech C_{18} or C_8 column (3μ Econosphere Rocket, 7 x 33 mm) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 13.5 min at 2 mL/min. Reversed-phase flash column chromatography was carried out on C_{18}, and CN Merck LiChroprep solid supports. Gel filtration flash chromatography was carried out using Pharmacia Biotech Sephadex LH-20. NHS-activated 7-*N*,*N*-dimethylaminocoumarin-4-acetic acid was prepared by published routes and data obtained were in agreement with those previously reported.[23,24]

*4.1.1. Discorhabdin C (**2**).* Discorhabdin C was isolated from specimens of *Latrunculia* (*Latrunculia*) *trivetricillata* (MNP 6116) collected from the Three Kings Islands, New Zealand using protocols previously reported.[8] Spectroscopic data recorded for the alkaloid were identical to those reported previously and sample purity used for subsequent semi-synthetic preparations was judged >98% by analytical HPLC-DAD and NMR.

*4.1.2. Discorhabdin P (**3**).* Discorhabdin C trifluoroacetate salt (**2**) (10.2 mg, 17.7 mmol) was dissolved in dry acetone (1 mL) along with excess K_2CO_3 (30.0 mg, 0.217 mmol) and CH_3I (30 mL, 92.7 mmol), turning the solution from red/purple to orange. The mixture was heated to 70 ^oC and left to stir for 1 h. The crude mixture was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture of 0–30% MeOH (0.05% TFA) afforded **3** (10.0 mg, 96%) as a red/purple non-crystalline trifluoroacetate salt. t_R 6.41 min; ^1H and ^{13}C NMR and HRMS data matched those previously reported.[18]

*4.1.3. (3s, 6s)-3-Dihydrodiscorhabdin C (**4**) and (3r, 6r)-3-Dihydrodiscorhabdin C (**5**).* Discorhabdin C trifluoroacetate salt (**2**) (8.0 mg, 13.9 µmol) was dissolved in dry MeOH (1 mL) and excess $NaBH_4$ was added, before purging the mixture with N_2. The red/purple solution turned red with addition of $NaBH_4$ and became yellow within 10 seconds, at which the mixture was left to stir for 5 min. The reaction was then opened to air turning the solution back to red. The crude reaction mixture was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–30% MeOH (0.05% TFA) afforded **4** (3.7 mg, 46%) and **5** (2.9 mg, 36%) as dark purple non-crystalline trifluoroacetate salts.

4 (3*s*, 6*s*)-3-Dihydrodiscorhabdin C: t_R 4.89 min; ν_{max} (ATR) 3253, 1675, 1589, 1541, 1328, 1200 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 13.13 (1H, br s, *N*H-13), 9.98 (1H, br s, *N*H-9), 7.82 (1H, br s, *N*H-18), 7.37 (1H, s, H-14), 6.38 (2H, s, H-1, H-5), 4.67 (1H, s, H-3), 3.76 (2H, td, *J* = 7.4, 2.9 Hz, H_2-17), 3.52–3.49 (2H, br m, H_2-8), 2.82 (2H, t, *J* = 7.4 Hz, H_2-16), 1.88 (2H, t, *J* = 5.5 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.7 (C-11), 153.1 (C-19), 151.4 (C-10), 134.1 (C-1, C-5), 127.4 (C-14), 124.4 (C-2, C-4), 123.5 (C-21), 123.1 (C-12), 119.7 (C-15), 96.1 (C-20), 70.8 (C-3), 43.9 (C-17), 41.9 (C-6), 38.1 (C-8), 34.8 (C-7), 18.1 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 463.9589 (calcd. for $C_{18}H_{16}{}^{79}Br_2N_3O_2$, 463.9604), 465.9575 (calcd. for $C_{18}H_{16}{}^{79}Br^{81}BrN_3O_2$, 465.9584), 467.9572 (calcd. for $C_{18}H_{16}{}^{81}Br_2N_3O_2$, 467.9564).

5 (3*r*, 6*r*)-3-Dihydrodiscorhabdin C: t_R 5.68 min; ν_{max} (ATR) 3278, 1675, 1589, 1545, 1328, 1199 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 13.15 (1H, br s, *N*H-13), 10.16 (1H, br s, *N*H-9), 7.79 (1H, br s, *N*H-18), 7.52 (1H, br s, OH-3), 7.37 (1H, s, H-14), 6.48 (2H, s, H-1, H-5), 4.60 (1H, s, H-3), 3.72 (2H, td, *J* = 7.5, 2.7 Hz, H_2-17), 3.60 (2H, obscured by water, H_2-8), 2.83 (2H,

t, J = 7.5 Hz, H_2-16), 1.86 (2H, t, J = 5.7 Hz, H_2-7); NMR data consistent with those in the literature;[16] (+)-HRESIMS *m/z* $[M+H]^+$ 463.9591 (calcd. for $C_{18}H_{16}{}^{79}Br_2N_3O_2$, 463.9604), 465.9573 (calcd. for $C_{18}H_{16}{}^{79}Br^{81}BrN_3O_2$, 465.9584), 467.9563 (calcd. for $C_{18}H_{16}{}^{81}Br_2N_3O_2$, 467.9564).

*4.1.4. (3s, 6s)-3-Dihydrodiscorhabdin P (**6**).* (3*s*, 6*s*)-3-Dihydrodiscorhabdin C trifluoroacetate salt (**4**) (4.7 mg, 8.1 µmol) dissolved in dry acetone (1 mL) along with excess K_2CO_3 (15 mg) and CH_3I (15 µL), turning the solution from red/purple to orange. The mixture was heated to reflux and left to stir for 1 h. The crude mixture was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture of 0–30% MeOH (0.05% TFA) afforded **6** (3.8 mg, 80%) as a dark purple non-crystalline trifluoroacetate salt. t_R 5.98 min; ν_{max} (ATR) 3303, 1672, 1584, 1536, 1322, 1199 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.94 (1H, br s, *N*H-9), 7.79 (1H, br s, *N*H-18), 7.37 (1H, s, H-14), 6.36 (2H, s, H-1, H-5), 4.67 (1H, s, H-3), 3.90 (3H, s, H_3-22), 3.74 (2H, td, J = 7.5, 0.7 Hz, H_2-17), 3.51 (2H, br s, H_2-8), 2.80 (2H, t, J = 7.5 Hz, H_2-16), 1.87 (2H, t, J = 5.7 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.0 (C-11), 152.6 (C-19), 151.3 (C-10), 134.1 (C-1, C-5), 132.0 (C-14), 124.4 (C-2, C-4), 123.3 (C-21), 122.4 (C-12), 119.1 (C-15), 96.2 (C-20), 70.1 (C-3), 43.7 (C-17), 42.0 (C-6), 38.1 (C-8), 35.9 (C-22), 34.8 (C-7), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 477.97500 (calcd. for $C_{19}H_{18}{}^{79}Br_2N_3O_2$, 477.97657), 479.97492 (calcd. for $C_{19}H_{18}{}^{79}Br^{81}BrN_3O_2$, 479.97453), 481.97408 (calcd. for $C_{19}H_{18}{}^{81}Br_2N_3O_2$, 481.97248).

*4.1.5. (3r, 6r)-3-Dihydrodiscorhabdin P (**7**).* (3*r*, 6*r*)-3-Dihydrodiscorhabdin C trifluoroacetate salt (**5**) (3.5 mg, 6.0 µmol) dissolved in dry acetone (1 mL) along with excess K_2CO_3 (15 mg) and CH_3I (15 µL), turning the solution from red/purple to orange. The mixture was heated to reflux and left to stir for 1 h. The crude mixture was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture of 0–30% MeOH (0.05% TFA) afforded **7** (3.2 mg, 90%) as a dark purple non-crystalline trifluoroacetate salt. t_R 6.25 min; ν_{max} (ATR) 3239, 1676, 1585,

1540, 1324, 1198 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.14 (1H, br s, *N*H-9), 7.79 (1H, br s, *N*H-18), 7.41 (1H, s, H-14), 6.50 (2H, s, H-1, H-5), 4.63 (1H, s, H-3), 3.92 (3H, s, H_3-22), 3.72 (2H, obscured by water, H_2-17), 3.61 (2H, obscured by water, H_2-8), 2.84 (2H, t, J = 7.2 Hz, H_2-16), 1.90–1.87 (2H, m, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.7 (C-11), 152.5 (C-19), 152.2 (C-10), 134.1 (C-1, C-5), 132.0 (C-14), 123.1 (C-21), 122.5 (C-2, C-4, C-12), 118.9 (C-15), 92.9 (C-20), 69.5 (C-3), 42.7 (C-17), 42.1 (C-6), 37.5 (C-8), 36.0 (C-22), 33.7 (C-7), 17.9 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 477.97637 (calcd. for $C_{19}H_{18}{}^{79}Br_2N_3O_2$, 477.97657), 479.97300 (calcd. for $C_{19}H_{18}{}^{79}Br^{81}BrN_3O_2$, 479.97453), 481.97351 (calcd. for $C_{19}H_{18}{}^{81}Br_2N_3O_2$, 481.97248).

*4.1.6. Alternative route to the synthesis of (3s, 6s)-3-dihydrodiscorhabdin P (**6**) and (3r, 6r)-3-dihydrodiscorhabdin P (**7**).* Discorhabdin P trifluoroacetate salt (**3**) (5.2 mg, 8.8 µmol) was dissolved in dry MeOH (1 mL) and excess $NaBH_4$ was added, before purging the mixture with N_2. The red/purple solution turned red with addition of $NaBH_4$ and became yellow within 10 seconds, at which the mixture was left to stir for 5 min. The reaction was then opened to air turning the solution back to red. The crude reaction product was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–30% MeOH (0.05% TFA) afforded **6** (3.1 mg, 59%) and **7** (1.7 mg, 32%) as dark purple non-crystalline trifluoroacetate salts. Spectroscopic and spectrometric data observed for these reaction products were identical for those observed for products obtained by the first route.

*4.1.7. (3s, 6s)-3-Acetoxy-3-dihydrodiscorhabdin P (**8**).* (3*s*, 6*s*)-3-Dihydrodiscorhabdin P trifluoroacetate salt (**4**) (3.7 mg, 6.2 µmol) was dissolved in anhydrous acetic anhydride (0.5 mL) and dry pyridine (0.5 mL), the mixture was purged with N_2 and left to stir for 24 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–30% MeOH (0.05% TFA) afforded **8** (3.1 mg, 79%) as a dark purple non-crystalline trifluoroacetate salt. t_R 6.67 min; ν_{max} (ATR) 3247, 1746, 1677, 1537, 1322, 1202 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.99 (1H, br s, *N*H-9), 7.86 (1H, br s, *N*H-18), 7.39 (1H, s, H-14), 6.63

(2H, s, H-1, H-5), 6.46 (1H, s, H-3), 3.96 (3H, obscured by water, H_3-22), 3.72 (2H, obscured by water, H_2-17), 3.55–3.52 (2H, m, H_2-8), 2.81 (2H, t, J = 7.6 Hz, H_2-16), 2.16 (3H, s, H_3-25), 1.83 (2H, t, J = 5.5 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.6 (C-24), 165.8 (C-11), 152.6 (C-19), 151.4 (C-10), 137.5 (C-1, C-5), 131.8 (C-14), 123.2 (C-21), 122.3 (C-12), 119.2 (C-15), 117.9 (C-2, C-4), 95.3 (C-20), 71.0 (C-3), 43.7 (C-17), 42.1 (C-6), 38.1 (C-8), 36.0 (C-22), 35.1 (C-7), 20.3 (C-25), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 519.9865 (calcd. for $C_{21}H_{20}{}^{79}Br_2N_3O_3$, 519.9866), 521.9847 (calcd. for $C_{21}H_{20}{}^{79}Br^{81}BrN_3O_3$, 521.9847), 523.9831 (calcd. for $C_{21}H_{20}{}^{81}Br_2N_3O_3$, 523.9826).

*4.1.8. (3s, 6s)-(3-Dihydrodiscorhabdin P) 6-((tert-butoxycarbonyl)amino)hexanoate (**9**).* (3*s*, 6*s*)-3-Dihydrodiscorhabdin P trifluoroacetate salt (**4**) (21 mg, 35.7 µmol) was dissolved in dry DMF (2 mL) along with DCC (11 mg, 53.3 µmol) and catalytic amount of DMAP. 6-(*tert*-Butoxycarbonylamino) hexanoic acid (17 mg, 74.1 µmol) was added and the mixture was stirred at r.t. for 18 h under N_2. The crude reaction product was purified with a combination of reversed-phase C_{18} and CN flash chromatography eluting with MeOH in H_2O (0.05% TFA) to afford **9** (22 mg, 76%) as a dark purple non-crystalline trifluoroacetate salt. t_R 7.16 min; ν_{max} (ATR) 3399, 3052, 1668, 1182, 1128 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.99 (1H, br s, *N*H-9), 7.87 (1H, br s, *N*H-18), 7.39 (1H, s, H-14), 6.76 (1H, t, J = 5.4 Hz, *N*H-30), 6.63 (2H, s, H-1, H-5), 6.47 (1H, s, H-3), 3.91 (3H, s, H_3-22), 3.73 (2H, td, J = 7.4, 2.9 Hz, H_2-17), 3.55–3.52 (2H, br m, H_2-8), 2.88 (2H, dt, J = 6.5, 5.4 Hz, H_2-29), 2.81 (2H, t, J = 7.4 Hz, H_2-16), 2.40 (2H, t, J = 7.3 Hz, H_2-25), 1.83 (2H, t, J = 5.5 Hz, H_2-7), 1.59 (2H, p, J = 7.3 Hz, H_2-26), 1.40–1.29 (13H, m, H_2-27, H_2-28, $3H_3$-34); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.1 (C-24), 165.8 (C-11), 155.5 (C-31), 152.6 (C-19), 151.4 (C-10), 137.4 (C-1, C-5), 131.8 (C-14), 123.2 (C-21), 122.3 (C-12), 119.2 (C-15), 117.9 (C-2, C-4), 95.4 (C-20), 77.2 (C-33), 70.9 (C-3), 43.7 (C-17), 42.1 (C-6), 39.4 (C-29), 38.1 (C-8), 36.0 (C-22), 35.1 (C-7), 33.2 (C-25), 29.0 (C-28), 28.2 (C-34), 25.6 (C-27), 24.2 (C-26), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 691.11346 (calcd. for $C_{30}H_{37}{}^{79}Br_2N_4O_5$, 691.11307), 693.11144 (calcd. for $C_{30}H_{37}{}^{79}Br^{81}BrN_4O_5$, 693.11102), 695.10851 (calcd. for $C_{30}H_{37}{}^{81}Br_2N_4O_5$, 695.10898).

*4.1.9. (3s, 6s)-(3-Dihydrodiscorhabdin P) 6-aminohexanoate (**10**).* (3*s*, 6*s*)-(3-Dihydrodiscorhabdin P) 6-((*tert*-butoxycarbonyl)amino)hexanoate trifluoroacetate salt (**9**) (10.8 mg, 13.4 µmol) was dissolved in acidified water (2 mL, 0.5% TFA) and minimal MeOH to allow complete dissolution. The mixture was heated to 70 °C and left to stir for 1 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with three column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–30% MeOH (0.05% TFA) afforded **10** (9.9 mg, 90%) as a dark purple non-crystalline trifluoroacetate salt. t_R 5.60 min; ν_{max} (ATR) 3253, 2952, 1676, 1539, 1200, 1133 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.00 (1H, br s, *N*H-9), 7.88 (1H, br s, *N*H-18), 7.73 (3H, br s, *N*H_3-30), 7.39 (1H, s, H-14), 6.64 (2H, s, H-1, H-5), 6.48 (1H, s, H-3), 3.91 (3H, s, H_3-22), 3.73 (2H, td, *J* = 7.2, 2.7 Hz, H_2-17), 3.55–3.50 (2H, m, H_2-8), 2.82–2.73 (4H, m, H_2-16, H_2-29), 2.43 (2H, t, *J* = 7.2 Hz, H_2-25), 1.83–1.79 (2H, m, H_2-7), 1.66–1.50 (4H, m, H_2-26, H_2-28), 1.37 (2H, p, *J* = 7.2 Hz, H_2-27); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.9 (C-24), 165.8 (C-11), 152.6 (C-19), 151.3 (C-10), 137.4 (C-1, C-5), 131.8 (C-14), 123.2 (C-21), 122.3 (C-12), 119.1 (C-15), 117.8 (C-2, C-4), 95.3 (C-20), 70.9 (C-3), 43.6 (C-17), 42.0 (C-6), 38.9 (C-29), 38.0 (C-8), 35.9 (C-22), 35.1 (C-7), 32.9 (C-25), 26.5 (C-28), 25.0 (C-27), 23.9 (C-26), 17.9 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 591.06010 (calcd. for $C_{25}H_{29}{}^{79}Br_2N_4O_3$, 591.06064), 593.05999 (calcd. for $C_{25}H_{29}{}^{79}Br^{81}BrN_4O_3$, 593.05859), 595.05863 (calcd. for $C_{25}H_{29}{}^{81}Br_2N_4O_3$, 595.05655).

*4.1.10. (3r, 6r)-3-Acetoxy-3-dihydrodiscorhabdin P (**11**).* (3*r*, 6*r*)-3-Dihydrodiscorhabdin P trifluoroacetate salt (**5**) (3.5 mg, 5.9 µmol) was dissolved in anhydrous acetic anhydride (0.5 mL) and dry pyridine (0.5 mL), the mixture was purged with N_2 and left to stir for 24 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–30% MeOH (0.05% TFA) afforded **11** (3.5 mg, 93%) as a dark purple non-crystalline trifluoroacetate salt. t_R 6.80 min; ν_{max} (ATR) 3292, 1729, 1679, 1588, 1542, 1324, 1199 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.09 (1H, br s, *N*H-9), 7.77 (1H, br s, *N*H-18), 7.39 (1H, s, H-14), 6.62 (2H, s, H-1, H-5), 5.72 (1H, s, H-3), 3.91 (3H, obscured by water, H_3-22), 3.79 (2H, td, *J* = 7.4, 2.8 Hz, H_2-17), 3.59–3.56 (2H, br m, H_2-8), 2.84 (2H, t, *J* = 7.4 Hz, H_2-16), 2.24 (3H, s, H_3-25), 1.90 (2H, t, *J* = 5.6 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.7 (C-24), 165.9 (C-11), 152.7 (C-19), 151.7 (C-10), 134.9 (C-1, C-5), 132.0 (C-14), 122.9 (C-21), 122.4 (C-12),

118.9 (C-15), 117.2 (C-2, C-4), 95.3 (C-20), 71.8 (C-3), 42.8 (C-17), 42.1 (C-6), 37.5 (C-8), 36.0 (C-22), 34.0 (C-7), 20.7 (C-25), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 519.9857 (calcd. for $C_{21}H_{20}{}^{79}Br_2N_3O_3$, 519.9866), 521.9844 (calcd. for $C_{21}H_{20}{}^{79}Br^{81}BrN_3O_3$, 521.9847), 523.9811 (calcd. for $C_{21}H_{20}{}^{81}Br_2N_3O_3$, 523.9826).

*4.1.11. (3r, 6r)-(3-Dihydrodiscorhabdin P) 6-((tert-butoxycarbonyl)amino)hexanoate (**12**).* (3*r*, 6*r*)-3-Dihydrodiscorhabdin P trifluoroacetate salt (**5**) (6.2 mg, 10.8 µmol) was dissolved in dry DMF (4 mL) along with DCC (2.8 mg, 13.5 µmol) and catalytic amount of DMAP. 6-(*tert*-butoxycarbonylamino) hexanoic acid (5.3 mg, 23.1 µmol) was added and the mixture was stirred at r.t. for 18 h under N_2. The crude reaction product was purified with a combination of reversed-phase C_{18} and CN flash chromatography eluting with MeOH in H_2O (0.05% TFA) to afford **12** (5.9 mg, 68%) as a dark purple non-crystalline trifluoroacetate salt. t_R 7.87 min; ν_{max} (ATR) 3294, 2928, 1679, 1625, 1542, 1200, 1175, 1130 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.08 (1H, br s, *N*H-9), 7.86 (1H, br s, *N*H-18), 7.39 (1H, s, H-14), 6.77 (1H, t, *J* = 5.6 Hz, *N*H-30), 6.63 (2H, s, H-1, H-5), 5.71 (1H, s, H-3), 3.91 (3H, s, H_3-22), 3.79 (2H, td, *J* = 7.4, 2.8 Hz, H_2-17), 3.59–3.56 (2H, m, H_2-8), 2.90 (2H, dt, *J* = 6.4, 5.6 Hz, H_2-29), 2.85 (2H, t, *J* = 7.4 Hz, H_2-16), 2.50 (2H, obscured by water, H_2-25), 1.90 (2H, t, *J* = 5.2 Hz, H_2-7), 1.63 (2H, p, *J* = 7.6 Hz, H_2-26), 1.42–1.34 (13H, m, H_2-27, H_2-28, $3H_3$-34); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.1 (C-24), 165.9 (C-11), 156.5 (C-31), 152.7 (C-19), 151.6 (C-10), 134.9 (C-1, C-5), 132.0 (C-14), 122.9 (C-21), 122.4 (C-12), 118.8 (C-15), 117.3 (C-2, C-4), 94.6 (C-20), 77.2 (C-33), 71.7 (C-3), 42.8 (C-17), 42.1 (C-6), 39.4 (C-29), 37.4 (C-8), 35.9 (C-22), 34.0 (C-25), 33.2 (C-7), 29.0 (C-28), 28.2 (C-34), 25.6 (C-27), 23.7 (C-26), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 691.11174 (calcd. for $C_{30}H_{37}{}^{79}Br_2N_4O_5$, 691.11307), 693.11289 (calcd. for $C_{30}H_{37}{}^{79}Br^{81}BrN_4O_5$, 693.11102), 695.11034 (calcd. for $C_{30}H_{37}{}^{81}Br_2N_4O_5$, 695.10898).

*4.1.12. (3r, 6r)-(3-Dihydrodiscorhabdin P) 6-aminohexanoate (**13**).* (3*r*, 6*r*)-(3-Dihydrodiscorhabdin P) 6-((*tert*-butoxycarbonyl)amino)hexanoate trifluoroacetate salt (**12**) (5.9 mg, 7.3 µmol) was dissolved in acidified water (2 mL, 0.5% TFA) and minimal MeOH to allow complete dissolution. The mixture was heated to 70 °C and left to stir for 1 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with three column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–

30% MeOH (0.05% TFA) afforded **13** (4.9 mg, 82%) as a dark purple non-crystalline trifluoroacetate salt. t_R 6.18 min; ν_{max} (ATR) 3398, 3289, 1678, 1202, 1134 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.10 (1H, br s, *N*H-9), 7.82–7.71 (4H, m, *N*H-18, *N*H_3-30), 7.40 (1H, s, H-14), 6.62 (2H, s, H-1, H-5), 5.72 (1H, s, H-3), 3.91 (3H, s, H_3-22), 3.79 (2H, td, *J* = 7.5, 2.7 Hz, H_2-17), 3.61–3.56 (2H, m, H_2-8), 2.89–2.73 (4H, m, H_2-16, H_2-29), 2.50 (2H, obscured by water, H_2-25), 1.91 (2H, t, *J* = 5.4 Hz, H_2-7), 1.65 (2H, p, *J* = 8.2 Hz, H_2-26/H_2-28), 1.60 (2H, p, *J* = 8.2 Hz, H_2-26/H_2-28), 1.40 (2H, p, *J* = 8.2 Hz, H_2-27); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.0 (C-24), 165.9 (C-11), 152.6 (C-19), 151.6 (C-10), 134.9 (C-1, C-5), 132.0 (C-14), 122.8 (C-21), 122.4 (C-12), 118.8 (C-15), 117.2 (C-2, C-4), 95.3 (C-20), 71.8 (C-3), 42.8 (C-17), 42.1 (C-6), 38.5 (C-29), 37.4 (C-8), 35.9 (C-22), 34.0 (C-7), 33.1 (C-25), 26.5 (C-28), 25.0 (C-27), 23.7 (C-26), 17.9 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 591.05834 (calcd. for $C_{25}H_{29}{}^{79}Br_2N_4O_3$, 591.06064), 593.06029 (calcd. for $C_{25}H_{29}{}^{79}Br^{81}BrN_4O_3$, 593.05859), 595.05689 (calcd. for $C_{25}H_{29}{}^{81}Br_2N_4O_3$, 595.05655).

*4.1.13. (3s, 6s)-C-3-Dansyl amino hexanoate discorhabdin P analogue (**14**).* (3s, 6s)-(3-Dihydrodiscorhabdin P) 6-aminohexanoate trifluoroacetate salt (**10**) (6 mg, 7.3 μmol) was dissolved in dry acetone along with excess K_2CO_3 and 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) (10 mg, 37.1 μmol) and left to stir at r.t. for 3.5 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with three column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–30% MeOH (0.05% TFA) afforded **14** (2.7 mg, 40%) as a purple/brown non-crystalline trifluoroacetate salt. t_R 7.82 min; ν_{max} (ATR) 1742, 1675, 1584, 1538, 1321, 1142 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.99 (1H, br s, *N*H-9), 8.45 (1H, d, *J* = 7.8 Hz, H-35), 8.29 (1H, d, *J* = 8.1 Hz, H-40), 8.09 (1H, d, *J* = 7.8 Hz, H-33), 7.89–7.85 (2H, m, *N*H-18, *N*H-30), 7.62 (1H, t, *J* = 7.8 Hz, H-34), 7.59 (1H, t, *J* = 8.1 Hz, H-39), 7.39 (1H, s, H-14), 7.26 (1H, d, *J* = 8.1 Hz, H-38), 6.61 (2H, s, H-1, H-5), 6.43 (1H, s, H-3), 3.91 (3H, s, H_3-22), 3.72 (2H, td, *J* = 7.6, 2.5 Hz, H_2-17), 3.55–3.51 (2H, m, H_2-8), 2.83 (6H, s, $2H_3$-43), 2.82–2.75 (4H, m, H_2-16, H_2-29), 2.22 (2H, t, *J* = 7.2 Hz, H_2-25), 1.83–1.80 (2H, m, H_2-7), 1.40 (2H, p, *J* = 7.2 Hz, H_2-26), 1.30 (2H, p, *J* = 7.2 Hz, H_2-28), 1.17 (2H, p, *J* = 7.2 Hz, H_2-27); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.9 (C-24), 165.8 (C-11), 152.6 (C-19), 151.4 (C-10), 151.2 (C-37), 137.4 (C-1, C-5), 136.1 (C-32), 131.8 (C-14), 129.2 (C-35, C-41), 129.0 (C-36), 128.1 (C-33), 127.7 (C-39), 123.5 (C-34), 123.2

(C-21), 122.3 (C-12), 119.2 (C-15, C-40), 117.9 (C-2, C-4), 115.1 (C-38), 95.3 (C-20), 70.8 (C-3), 45.0 (C-43), 43.7 (C-17), 42.1 (C-6, C-29), 38.1 (C-8), 36.0 (C-22), 35.1 (C-7), 33.0 (C-25), 28.6 (C-28), 25.2 (C-27), 23.9 (C-26), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 824.1117 (calcd. for $C_{37}H_{40}{}^{79}Br_2N_5O_5S$, 824.1111), 826.1093 (calcd. for $C_{37}H_{40}{}^{79}Br^{81}BrN_5O_5S$, 826.1094), 828.1090 (calcd. for $C_{37}H_{40}{}^{81}Br_2N_5O_5S$, 828.1073).

*4.1.14. (3s, 6s)-C-3-Fluorescein amino hexanoate discorhabdin P analogue (**15**).* (3s, 6s)-(3-Dihydrodiscorhabdin P) 6-aminohexanoate trifluoroacetate salt (**10**) (9.9 mg, 12.0 µmol) was dissolved in dry acetone along with excess K_2CO_3 and fluorescein 5(6)-isothiocyanate (19 mg, (10 mg, 48.8 µmol) and left to stir at r.t. for 3.5 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with three column volumes of water (0.05% TFA). A series of C_{18} (MeOH, 0.05% TFA) and Sephadex LH-20 (MeOH, 0.05% TFA) flash chromatography steps were used to afford **15** (5.0 mg, 38%) as a brown/yellow non-crystalline trifluoroacetate salt. t_R 6.91 min; ν_{max} (ATR) 3286, 1743, 1675, 1611, 1586, 1539, 1322 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.99 (1H, br s, *N*H-9), 7.86 (2H, br s, *N*H-18, *N*H-30), 7.38 (1H, s, H-14), 6.63 (2H, s, H-1, H-5), 6.48 (1H, s, H-3), 3.91 (3H, s, H_3-22), 3.72 (2H, obscured by water, H_2-17), 3.54 (4H, obscured by water, H_2-8, H_2-29), 2.80 (2H, t, J = 6.0 Hz, H_2-16), 2.48–2.37 (2H, m, H_2-25), 1.85–1.80 (2H, m, H_2-7), 1.41–1.68 (6H, m, H_2-26, H_2-27, H_2-28); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.1 (C-24), 165.8 (C-11), 152.6 (C-19), 151.4 (C-10), 137.4 (C-1, C-5), 131.8 (C-14), 122.4 (C-12, C-21), 119.2 (C-15), 117.9 (C-2, C-4), 95.4 (C-20), 70.9 (C-3), 43.7 (C-17, C-29), 42.1 (C-6), 38.1 (C-8), 36.0 (C-22), 35.1 (C-7), 33.2 (C-25), 27.9 (C-28), 25.7 (C-27), 24.2 (C-26), 18.0 (C-16); ^1H and ^{13}C NMR data only assigned up to NH-30 as presence of fluorescein isomers hindered full assignment of fluorescein moiety; (+)-HRESIMS *m/z* $[M+H]^+$ 980.0934 (calcd. for $C_{46}H_{40}{}^{79}Br_2N_5O_8S$, 980.0959), 982.0946 (calcd. for $C_{46}H_{40}{}^{79}Br^{81}BrN_5O_8S$, 982.0944), 984.0949 (calcd. for $C_{46}H_{40}{}^{81}Br_2N_5O_8S$, 984.0921).

*4.1.15. (3s, 6s)-C-3-Coumarin amino hexanoate discorhabdin P analogue (**16**).* (3s, 6s)-(3-Dihydrodiscorhabdin P) 6-aminohexanoate trifluoroacetate salt (**10**) (8.3 mg, 10.1 µmol) was dissolved in dry acetone along with excess K_2CO_3 and NHS-activated 7-dimethylaminocoumarin-4-acetic acid[23,24] (13.9 mg, 40.4 µmol). The reaction mixture was left to

stir at r.t. for 3.5 h. The crude solution was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with three column volumes of water (0.05% TFA). Elution with a gradient solvent mixture from 0–50% MeOH (0.05% TFA) afforded **16** (3.5 mg, 37%) as a purple non-crystalline trifluoroacetate salt. t_R 7.21 min; ν_{max} (ATR) 3337, 1680, 1617, 1205, 1137 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.99 (1H, br s, *N*H-9), 8.17 (1H, t, *J* = 5.5 Hz, *N*H-30), 7.86 (1H, br s, *N*H-18), 7.54 (1H, d, *J* = 9.0 Hz, H-41), 7.38 (1H, s, H-14), 6.72 (1H, dd, *J* = 9.0, 2.4 Hz, H-40), 6.62 (2H, s, H-1, H-5), 6.55 (1H, d, *J* = 2.4 Hz, H-38), 6.47 (1H, s, H-3), 5.99 (1H, s, H-34), 3.90 (3H, s, H_3-22), 3.73 (2H, td, *J* = 7.4, 2.6 Hz, H_2-17), 3.58 (2H, s, H_2-32), 3.55–3.51 (2H, m, H_2-8), 3.05 (2H, dt, *J* = 7.2, 5.5 Hz, H_2-29), 3.01 (6H, s, $2H_3$-44), 2.81(2H, t, *J* = 7.4 Hz, H_2-16), 2.39 (2H, t, *J* = 7.2 Hz, H_2-25), 1.84–1.80 (2H, m, H_2-7), 1.59 (2H, p, *J* = 7.2 Hz, H_2-26), 1.42 (2H, p, *J* = 7.2 Hz, H_2-28), 1.35–1.29 (2H, m, H_2-27); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.1 (C-24), 167.5 (C-31), 165.8 (C-11), 160.6 (C-35), 155.3 (C-37), 152.7 (C-39), 152.6 (C-19), 151.4 (C-33), 151.3 (C-10), 137.4 (C-1, C-5), 131.8 (C-14), 125.9 (C-41), 123.2 (C-21), 122.3 (C-12), 119.2 (C-15), 117.9 (C-2, C-4), 109.3 (C-34), 108.9 (C-40), 108.1 (C-42), 97.4 (C-38), 95.4 (C-20), 70.9 (C-3), 43.7 (C-17), 42.1 (C-6), 39.4 (C-44), 38.5 (C-29, C-32), 38.1 (C-8), 36.0 (C-22), 35.1 (C-7), 33.2 (C-25), 28.5 (C-28), 25.6 (C-27), 24.2 (C-26), 18.0 (C-16); (+)-HRESIMS *m/z* $[M+H]^+$ 820.1345 (calcd. for $C_{38}H_{40}{}^{79}Br_2N_5O_6$, 820.1340), 822.1329 (calcd. for $C_{38}H_{40}{}^{79}Br^{81}BrN_5O_6$, 822.1323), 824.1322 (calcd. for $C_{38}H_{40}{}^{81}Br_2N_5O_6$, 824.1302).

4.2. Cytotoxicity assays

The P388 assay used a two-fold series dilution of the sample incubated for 72 h with murine leukaemia cells (ATCC CCL 46 P388D1). The concentration of the sample required to inhibit cell growth to 50% of the growth of the solvent control was determined using the absorbance obtained upon staining with MTT tetrazolium.[25] Mitomycin C was used as a positive control, with IC_{50} 0.5 μM. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve from which the IC_{50} was determined. Cytotoxic activity was expressed as the average IC_{50} values of two independent experiments

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Supplementary data

Supplementary data related to this article can be found in the online version at

References

1. Antunes, E. M.; Copp, B. R.; Davies-Coleman, M. T.; Samaai, T. *Nat. Prod. Rep.* **2005**, *22*, 62–72.
2. Hu, J-F.; Fan, H.; Xiong, J.; Wu, S-B. *Chem. Rev.* **2011**, *111*, 5465–5491.
3. Perry, N. B.; Blunt, J. W.; McCombs, J. D.; Munro, M. H. G. *J. Org. Chem.* **1986**, *51*, 5476–5478.
4. Reyes, F.; Martin, R.; Rueda, A.; Fernandez, R.; Montalvo, D.; Gomez, C.; Sanchez-Puelles, J. *J. Nat. Prod.* **2004**, *67*, 463–465.
5. Antunes, E. M.; Beukes, D. R.; Kelly, M.; Samaai, T.; Barrows, L. R.; Marshall, K. M.; Sincich, C.; Davies-Coleman, M. T. *J. Nat. Prod.* **2004**, *67*, 1268–1276.
6. Lang, G.; Pinkert, A.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod*. **2005**, *68*, 1796–1798.
7. El-Naggar, M.; Capon, R. J. *J. Nat. Prod.* **2009**, *72*, 460–464.
8. Grkovic, T.; Pearce, A. N.; Munro, M. H. G.; Blunt, J. W.; Davies-Coleman, M. T.; Copp, B. R. *J. Nat. Prod.* **2010**, *73*, 1686–1693.
9. Ford, J.; Capon, R. J. *J. Nat. Prod.* **2000**, *63*, 1527–1528.
10. Na, M.; Ding, Y.; Wang, B.; Tekwani, B. L.; Schinazi, R. F.; Franzblau, S.; Kelly, M.; Stone, R.; Li, X-C.; Ferreira, D.; Hamann, M. T. *J. Nat. Prod.* **2010**, *73*, 383–378.
11. Jeon, J-E.; Na, Z.; Jung, M.; Lee, H-S.; Sim, C. J.; Nahm, K.; Oh, K-B.; Shin, J. *J. Nat. Prod.* **2010**, *73*, 258–262.
12. Gunasekera, S. P.; McCarthy, P. J.; Longley, R. E.; Pomponi, S. A.; Wright, A. E.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **1999**, *62*, 173–175.

13. Goey, A.; Chau, C. H.; Sissung, T. M.; Cook, K. M.; Venzon, D. J.; Castro, A.; Ransom, T. R.; Henrich, C. J.; McKee, T. C.; McMahon, J. B.; Grkovic, T.; Cadelis, M. M.; Copp, B. R.; Gustafson, K. R.; Figg, W. D. *J. Nat. Prod.* **2016**, *79*, 1267–1275.
14. Wada, Y.; Harayama, Y.; Kamimura, D.; Yoshida, M.; Shibata, T.; Fujiwara, K.; Morimoto, K.; Fujioka, H.; Kita, Y. *Org. Biomol. Chem.* **2011**, *9*, 4959–4976.
15. Lam, C. F. C.; Grkovic, T.; Pearce, A. N.; Copp, B. R. *Org. Biomol. Chem.* **2012**, *10*, 3092–3097.
16. Copp, B. R.; Fulton, K. F.; Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *J. Org. Chem.* **1994**, *59*, 8233–8238.
17. Lam, C. F. C.; Giddens, A. C.; Chand, N.; Webb, V. L.; Copp, B. R. *Tetrahedron* **2012**, *68*, 3187–3194.
18. Grkovic, T.; Kaur, B.; Webb, V. L.; Copp, B. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1944–1946.
19. http://goldbook.iupac.org/P04921.html (accessed 6-March-2017).
20. Fuchs, S.; Otto, H.; Jehle, S.; Henklein, P.; Schluter, A. D. *Chem. Commun.* **2005**, 1830–1832.
21. Shukla, N. M.; Mutz, C. A.; Ukani, R.; Warshakoon, H. J.; Moore, D. S.; David, S. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6384–6386.
22. Alexander, M. D.; Burkart, M. D.; Leonard, M. S.; Portonovo, P.; Liang, B.; Ding, X.; Joullie, M. M.; Gulledge, B. M.; Aggen, J. B.; Chamberlin, A. R.; Sandler, J.; Fenical, W.; Cui, J.; Gharpure, S. J.; Polosukhin, A.; Zhang, H-R.; Evans, P. A.; Richardson, A. D.; Harper, M. K.; Ireland, C. M.; Vong, B. G.; Brady, T. P.; Theodorakis, E. A.; La Clair, J. J. *ChemBioChem* **2006**, *7*, 409–416.
23. Ma, Y.; Luo, W.; Quinn, P. J.; Liu, Z.; Hider, R. C. *J. Med. Chem.* **2004**, *47*, 6349–6362.
24. Angelides, K. J.; Nutter, T. J. *J. Biol. Chem.* **1983**, *258*, 11948–11957.
25. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.