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PAPER

Investigation of the electrophilic reactivity of the cytotoxic marine alkaloid discorhabdin B†

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The mechanisms of action of the cytotoxic marine pyrroloiminoquinone alkaloids the discorhabdins are unknown. We have determined that discorhabdin B acts as an electrophile towards biomimetic thiol nucleophiles leading to debrominated adducts. In contrast, less potent cytotoxins discorhabdins D and Q failed to react, supporting an SAR model of cytotoxicity requiring an orchestrated combination of an electrophilic Δ^1 carbon centre and a nucleophilic N-18 amine for potent activity. The stereospecific nature of nucleophile trapping exhibited by both enantiomers of discorhabdin B implies the biogenesis of ovothiol A substituted discorhabdins H, H_2, K and K_2 need not be mediated by enzymatic processes.

Introduction

Natural products that contain electrophilic functional groups have had considerable impact on the treatment of human diseases. For example, the β-lactam-containing penicillin antibiotics form covalent adducts with active site Ser-70 in peptidases associated with bacterial cell-wall biosynthesis, while lipstatin (the tetrahydro analogue of which is marketed as orlistat/xenical) features a β-lactone moiety which irreversibly inhibits pancreatic lipase, leading to patient weight loss.[1] Beyond the pharmaceuticals arena[2] natural product covalent inhibitors have also played valuable roles as molecular probes in the investigation of cellular function of enzymes.[1] In continuation of our interest in exploring the electrophilic reactivity of bioactive marine natural products[3] we now report on the results of our investigation of the reactivity of the cytotoxic alkaloids discorhabdins B, D and Q.[4]

With over 40 analogues reported to date, the discorhabdins (Fig. 1) embody a pyrroloiminoquinone moiety fused to a spiro-substituent at C-6.[4] Some examples contain a sulphur bridge between C-5 and C-8 and bromination at C-2/C-4 (*e.g.* discorhabdins A (**1**) and B (**2**)[5]) while others lack the thioether linkage (*e.g.* discorhabdin C (**3**)[6]). In the specific case of these analogues, natural products have been reported to contain Δ^{16} unsaturation (*e.g.* discorhabdin Q (**4**)[7]), or substitution at C-1 with further ring closure (*e.g.* discorhabdins D (**5**),[8] H (**6**)[9] and L (**7**)[10]) or without ring closure (*e.g.* discorhabdin K (**8**)[11]). Disulfide-linked dimers have been reported (*e.g.* discorhabdin W (**9**)[12,13]) and enantiomeric examples of discorhabdins B (**10**) and Q have been described.[14] Many members of the family exhibit potent nano-molar levels of *in vitro* antiproliferative activity, making them of considerable interest. Despite such potency,

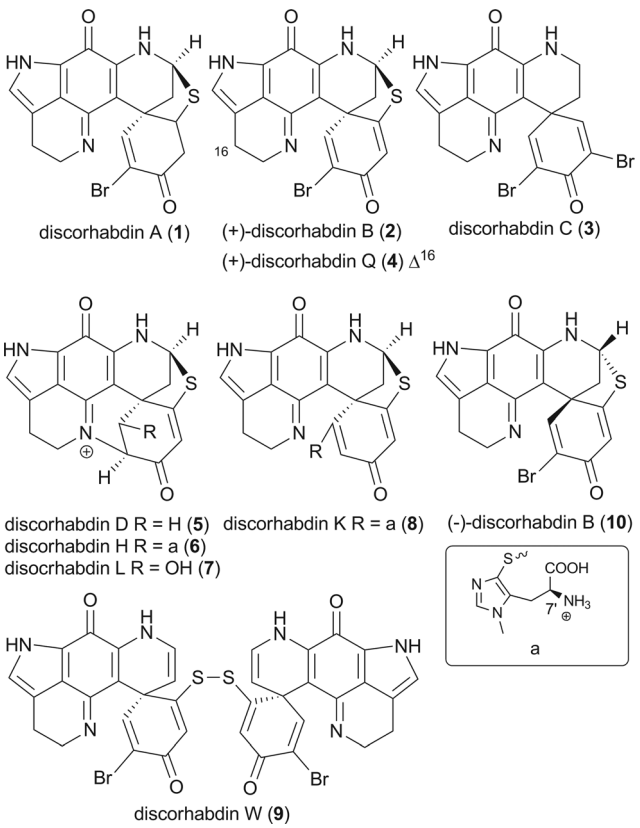

Fig. 1 Structures of selected examples of discorhabdin alkaloids.

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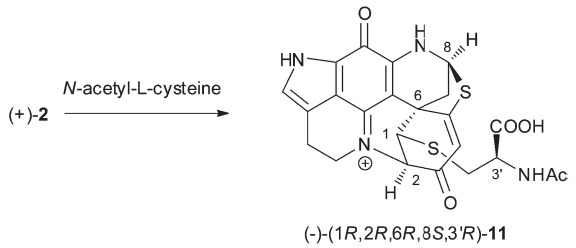


(-)-(1*R*,2*R*,6*R*,8*S*,3′*R*)-**11**

Scheme 1 Reaction of (+)-discorhabdin B (**2**) with *N*-acetyl-L-cysteine yielding **11**.

discorhabdin D (**5**), reported to exhibit mild *in vitro* cytotoxicity, is the only analogue to date to exhibit *in vivo* anti-tumor activity.[8] A structure–activity model of the observed *in vitro* anti-tumor activity of the discorhabdins has been proposed, with a requirement of both the iminoquinone core and the spiro (α-bromo) enone structure being essential, and that C-2–N-18 ring closure (*e.g.* discorhabdins D (**5**), H (**6**) and L (**7**)), substitution at C-1 (*e.g.* discorhabdin K (**8**)), and unsaturation at C-16 (17) (*e.g.* discorhabdin Q (**4**)) are detrimental to activity.[4,15] The critical role played by the spiro α-bromo enone substructure suggests that cytotoxicity of these natural products is potentiated by electrophilic reactivity.[16] No specific cellular target of the discorhabdins has been identified, with Wada *et al.* recently reporting that discorhabdin A failed to inhibit the function of a number of potential targets including kinases, HDAC, FTase, telomerase and the human 20S proteasome.[15] Our previous studies[11,13,14] of the chemistry of New Zealand sponges of the genus *Latrunculia* yielded quantities of both (+)-(6*S*,8*S*)- and (−)-(6*R*,8*R*)-discorhabdin B in addition to discorhabdins D and Q, providing an opportunity to investigate the reactivity of these natural products with biomimetic-type thiol and amine nucleophiles. Herein we describe the results of these studies.

Results and discussion

Reaction of (+)-(6*S*,8*S*)-discorhabdin B (**2**) with *N*-acetyl-L-cysteine (5 equiv.) in DMF-MeOH–H_2O mixture (1 : 1 : 0.1) and triethylamine (6 equiv.) for 30 min yielded a complex mixture[17] from which the major product (**11**, 14%) was purified by C_{18} reversed-phase flash column chromatography (Scheme 1). HRESI mass spectrometry established a molecular formula of $C_{23}H_{21}N_4O_5S_2$ ([M^+] *m/z* 497.0931, calcd 497.0948) indicating the product to be a debrominated mono-*N*-acetylcysteinyl adduct of discorhabdin B. Analysis of ^{1}H, ^{13}C and 2D NMR data established the presence of an *N*-acetylcysteine residue {[δ_H 4.71 (obsc) H-3′; 3.29 (m), 3.04 (m) H_2-2′; 2.03 (3H, s) Ac], [δ_C 35.9 CH_2-2′; 53.5 CH-3′; 173.6 C-5′; 22.5 Ac; C-7′ not observed]}, while the remaining resonances were almost identical to those observed for the 1-(thio)histidine substituted C-2–N-18 ring closed alkaloid (−)-discorhabdin H (**6**) previously reported from *Latrunculia* (*Biannulata*) *wellingtonesis* (Table 1).[11]

Diagnostic NMR evidence for ring closure came in the form of the presence of two alkyl methines [δ_H 3.89 (d, J = 3.2 Hz), δ_C 47.3 (CH-1); δ_H 4.46 (d, J = 3.2 Hz), δ_C 67.6 (CH-2)], and by the observation of an HMBC correlation between H-2 (δ_H 4.46) and C-19 (δ_C 150.3) (Table 1). An HMBC correlation between cysteine methylene resonances H_2-2′ (δ_H 3.29, 3.04) and C-1 located the thiol substituent at C-1. The configuration at positions 2, 6, 8, and 3′ of **11** were defined by the starting materials and, in combination with the observation of a NOESY correlation between H-1 (δ_H 3.89) and the more upfield[11] of the diastereotopic methylene H-7 protons [H-7α (δ_H 2.69)], an absolute configuration of (1*R*,2*R*,6*R*,8*S*,3′*R*) was established. The electronic circular dichroism (ECD) spectrum of **11** was essentially identical to that of (−)-(1*R*,2*R*,6*R*,8*S*,7′*S*)-discorhabdin H[11] (**6**) (Fig. 2) providing further confirmation of the absolute configuration of **11**. Aubart and Heathcock have previously reported a similar ring closure reaction for a dethia analogue of discorhabdin B.[18]

Reaction of enantiomeric (−)-(6*R*,8*R*)-discorhabdin B (**10**)[14] with *N*-acetyl-L-cysteine yielded two products, **12** (36%) and **13** (62%), after C_{18} reversed-phase chromatographic purification (Scheme 2). HRESI mass spectrometric analysis of **12** established a molecular formula of $C_{23}H_{21}N_4O_5S_2$, isomeric with **11**, while the ^{1}H and ^{13}C NMR chemical shifts observed for **12** were similar, but not identical, to those observed for **11**. Differences between the two sets of NMR data were centered upon the resonances assigned to H-1 and H-17a (Table 2).

Extensive analysis of HSQC and HMBC data established that both **11** and **12** shared a common carbon skeleton, while interpretation of NOESY data also established that both alkaloids

Table 1 ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR data (ppm) of compound **11** in CD_3OD[a]

No.	δ_H (mult., J in Hz)	δ_C	HMBC
1	3.89 (d, 3.2)	47.3	3, 5, 6, 2′
2	4.46 (d, 3.2)	67.6	3, 6, 19
3	—	183.4	—
4	6.09 (s)	114.4	2, 6
5	—	172.1	—
6	—	47.6	—
7β	3.24 (m)	39.3	1, 20
7α	2.69 (d, 12.0)	—	5
8	5.62 (d, 2.8)	64.1	5, 6, 10
9	—	—	—
10	—	148.3	—
11	—	167.3	—
12	—	125.5	—
13	—	—	—
14	7.11 (s)	127.4	12, 15, 21
15	—	119.3	—
16A	3.24 (m)	20.7	15
16B	3.12 (m)	—	15
17A	4.07 (m)	52.8	2, 19
17B	3.89 (m)	—	—
18	—	—	—
19	—	150.3	—
20	—	102.0	—
21	—	122.8	—
1′	—	—	—
2′A	3.29 (m)	35.9	—
2′B	3.04 (m)	—	1, 3′
3′	4.71 (obscured)	53.5	—
4′	—	—	—
5′	—	173.6	—
6′	2.03 (s)	22.5	5′
7′	—	N.o.[b]	—

[a] Assigned by analysis of COSY, HSQC, and HMBC experiments. [b] Not observed.

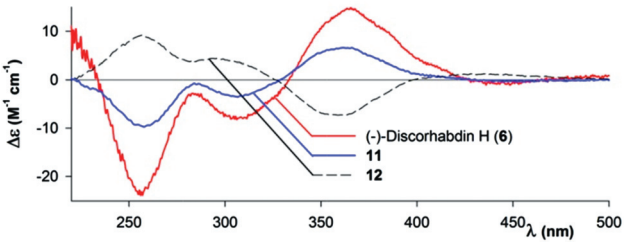
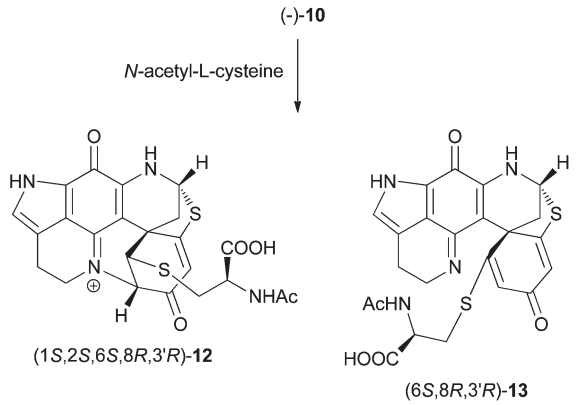


Fig. 2 ECD spectra of **6**, **11**, and **12**.



Scheme 2 Reaction of (−)-discorhabdin B with *N*-acetyl-L-cysteine.

shared the same relative configuration at C-1/C-2/C-6/C-8. Since (−)-(6*R*,8*R*)-discorhabdin B was used as the starting material in this reaction **12** must have an absolute configuration of (1*S*,2*S*,6*S*,8*R*,3′*R*), a conclusion supported by analysis of ECD data (Fig. 2).

The second product (**13**) isolated from the reaction of (−)-discorhabdin B and *N*-acetyl-L-cysteine exhibited a pseudomolecular ion at *m*/*z* 497.0931, also matching $C_{23}H_{21}N_4O_5S_2$. In this case however, ^{1}H and ^{13}C NMR resonances due to sp^3 hybridised carbons at C-1 and C-2 of **12** were absent, replaced by two sp^2 methine resonances (δ_C 162.1, C-1; δ_H 6.59, δ_C 124.1, CH-2) in **13**. Full characterisation of 2D NMR data and comparison with chemical shifts reported for the natural products discorhabdin K (**8**) and K$_2$ (**14**) (Fig. 3)[11] established **13** to be the corresponding 1-*N*-acetyl-L-cysteine analog (Table 3). As expected, the ECD spectrum of **13** closely matched that of (−)-(6*R*,8*R*,7′*S*)-discorhabdin K$_2$ (**14**) (Fig. 4) establishing the absolute configuration of **13** as (6*S*,8*R*,3′*R*).

The stereospecific nature of thiol trapping exhibited by both (+)- and (−)-discorhabdin B (**2**, **10** respectively), and congruence between the observed reaction products **11**–**13** with (thio)methylhistidine-substituted natural products discorhabdins (−)-H (**6**), (+)-H$_2$ (**15**), (+)-K (**8**) and (−)-K$_2$ (**14**) (Fig. 1 and 3)[11] suggests that biogenesis of the latter natural products could arise by adventitious reaction of discorhabdin B with the known free radical scavenging marine metabolite ovothiol A (**16**) (Fig. 3)[19] and may not need to be enzyme mediated.

During the course of these studies it was noticed that dry free base samples of (+)-(6*S*,8*S*)-discorhabdin B stored in a freezer for two weeks degraded to yield a complex mixture from which

Table 2 ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR data (ppm) of compound **12** in CD$_3$OD[a]

No.	δ_H (mult., *J* in Hz)	δ_C	HMBC
1	3.92 (d, 3.0)	46.8	3, 5, 6, 2′
2	4.51 (d, 3.0)	67.4	3, 6, 19
3	—	183.6	—
4	6.09 (s)	114.4	2, 5, 6
5	—	171.8	—
6	—	47.3	—
7*β*	3.07 (m)	39.3	6, 20
7*α*	2.65 (d, 12.1)	—	5, 8, 20
8	5.62 (br m)	64.0	5, 6, 10
9	—	—	—
10	—	148.3	—
11	—	167.3	—
12	—	125.5	—
13	—	—	—
14	7.11 (s)	127.4	12, 15, 21
15	—	119.3	—
16A	3.20 (m)	20.8	15, 17
16B	3.07 (m)	—	15
17A	4.10 (m)	52.9	15
17B	3.89 (m)	—	2, 15, 16, 19
18	—	—	—
19	—	150.3	—
20	—	102.1	—
21	—	122.8	—
1′	—	—	—
2′A	3.36 (dd, 14.1, 4.6)	35.4	1
2′B	2.97 (dd, 14.1, 9.3)	—	1, 3′
3′	4.69 (dd, 9.3, 4.6)	53.3	—
4′	—	—	—
5′	—	173.6	—
6′	2.03 (s)	22.5	5′
7′	—	173.2	—

[a] Assigned by analysis of COSY, HSQC and HMBC experiments.

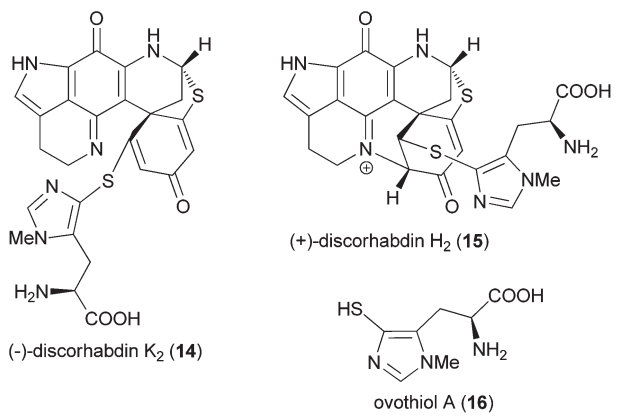


Fig. 3 Structures of discorhabdins K$_2$ (**14**), H$_2$ (**15**) and ovothiol A (**16**).

a major product (−)-**17** was purified in low yield (16%) (Scheme 3).

A molecular formula of $C_{36}H_{24}BrN_6O_4S_2$, determined by (+)-HRESIMS, suggested the product was a mono-debrominated dimer of discorhabdin B. Close inspection of ^{1}H NMR data identified the presence of 1-substituted discorhabdin D-type (*e.g.* **5**) and discorhabdin W-type (**9**, Fig. 1)[12,13] fragments which was further supported by detailed analysis of HSQC and HMBC data

Table 3 ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR data (ppm) of compound **13** in CD_3OD[a]

No.	δ_H (mult., J in Hz)	δ_C	HMBC
1	—	162.1	—
2	6.59 (s)	124.1	4, 6
3	—	181.8	—
4	6.11 (s)	119.4	2, 6
5	—	170.9	—
6	—	52.6	—
7A	2.95 (m)	45.5	5, 6, 20
7B	2.66 (dd, 12.0, 3.9)	—	20
8	5.57 (m)	60.5	6, 10
9	—	—	—
10	—	153.4	—
11	—	166.0	—
12	—	125.5	—
13	—	—	—
14	7.19 (s)	128.0	12, 21
15	—	122.1	—
16	2.89 (m)	19.2	14, 15, 17, 21
17A	4.00 (m)	46.1	15, 16, 19
17B	3.81 (m)	—	15, 16, 19
18	—	—	—
19	—	156.5	—
20	—	99.7	—
21	—	124.1	—
1′	—	—	—
2′A	3.62 (dd, 13.6, 4.9)	33.8	1, 7′
2′B	3.37 (m)	—	1, 3′, 7′
3′	4.73 (dd, 8.4, 4.9)	53.0	—
4′	—	—	—
5′	—	173.5	—
6′	1.96 (s)	22.6	5′
7′	—	172.6	—

[a] Assigned by analysis of COSY, HSQC and HMBC experiments.

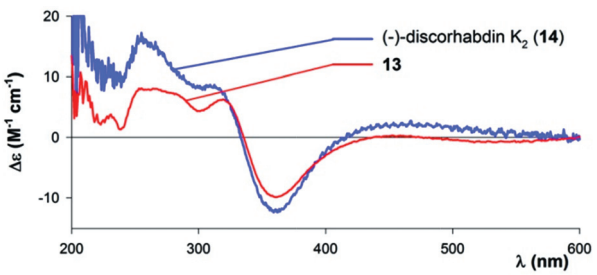
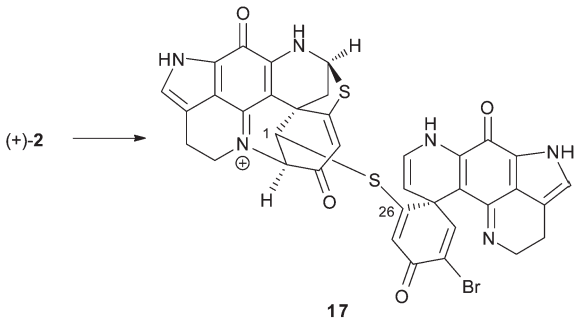


Fig. 4 ECD spectra of compound **13** and (−)-discorhabdin K_2 (**14**).

(ESI Fig. S1 and Table S1†). Interfragment connectivity was established with the observation of an HMBC correlation between H-1 of the discorhabdin D fragment to C-26 of the discorhabdin W fragment (see ESI Fig. S2†). With a (2*R*,6*R*,8*S*,27*S*) configuration of **17** being defined by the (6*S*,8*S*)-**2** starting material, configuration at C-1 was established by *J*-based analysis. A J-HMBC NMR experiment determined a $^{3}J_{H\text{-}1\text{–}C\text{-}5}$ coupling constant of 8.8 Hz requiring an antiperiplanar relationship between H-1 and C-5, defining a 1*R* configuration (Fig. 5). The ECD spectrum of (1*R*,2*R*,6*R*,8*S*,27*S*)-**17** was essentially identical to that of the related natural product (−)-(1*R*,2*R*,6*R*,8*S*,7′*S*)-discorhabdin H (**6**)[11] (ESI Fig. S3†).

Reactivity of (−)-(6*R*,8*R*)- and (+)-(6*S*,8*S*)-discorhabdin B with amine nucleophiles was investigated using *n*-pentylamine



Scheme 3 Semi-synthesis of (+)-discorhabdin B dimer **17**.

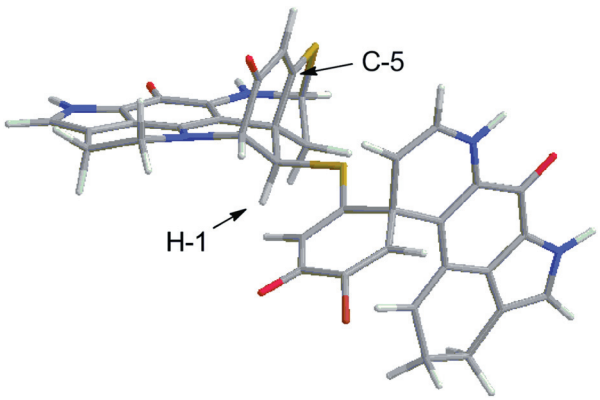


Fig. 5 A $^{3}J_{CH}$ coupling constant of 8.8 Hz defines an antiperiplanar relationship between H-1 and C-5 of **17**.

and *N*α-acetyl-L-lysine. Repeated attempts failed to yield any identifiable amine-linked product, affording either unreacted starting material or complex degradation mixtures. Given the preceding results concerning the reactivity of discorhabdin B towards thiols we speculated that the fact that no Δ^1-containing discorhabdin alkaloids have yet to be reported to exhibit efficacious *in vivo* activity (in any model)[5,15,20] may be due to prompt deactivation and excretion following reaction with glutathione. Reaction of (+)-(6*S*,8*S*)-discorhabdin B with glutathione (5 equiv.) under the standard conditions yielded a complex mixture of products, analysis by (+)-ESIMS identifying the presence of an ion at *m*/*z* 641.1464 attributable to an *S*-glutathionyl adduct (calcd for $C_{28}H_{29}N_6O_8S_2$: 641.1483).

The only discorhabdin alkaloid reported to exhibit *in vivo* antitumor activity, discorhabdin D (**5**), lacks the electrophilic Δ^1 unsaturation. Not surprisingly, (+)-(2*S*,6*R*,8*S*)-discorhabdin D was found to be unreactive towards *N*-acetyl-L-cysteine, *n*-pentylamine, *N*α-acetyl-L-lysine and glutathione under our standard reaction conditions. We conclude that electrophilic reactivity plays no part in the observed biological activity of discorhabdin D.

The original isolation report of discorhabdin Q (**4**) (Δ^{16} discorhabdin B) noted that it exhibited only modest activity against the NCI 60 cell line panel.[7] Our own comparative testing against the P388 murine leukemia cell line has established that discorhabdin Q is approximately 30-fold less potent (IC_{50} 3.0 μM) than discorhabdin B (**2**), and yet both natural products contain a Δ^1 electrophilic dienone moiety. As with discorhabdin D, discorhabdin

Q was found to be unreactive to the suite of biomimetic nucleophiles, either returning unreacted starting material or degrading to complex mixtures. We conclude from these observations that the expected Michael reaction at C-1 of **4** is completely reversible in the absence of a suitably nucleophilic imine (N-18).

Taken together, our results provide a rationalisation of the SAR model of cytotoxicity of the discorhabdins.[4,15] We propose that the nano-molar cytotoxicity of discorhabdin B (and presumably that of the related natural products discorhabdins A (**1**), C (**3**), and W (**9**)) appears to be due to an orchestrated combination of electrophilic reactivity at C-1 and a suitably positioned nucleophilic imine at N-18. Thus future efforts directed towards understanding the mechanism of action of the discorhabdins should also address the identification of proteins that covalently bind to the alkaloids.

Conclusions

In conclusion, we have established that the cytotoxic marine alkaloid discorhabdin B reacts with biomimetic nucelophiles yielding C-1 substituted adducts. A model is proposed whereby an orchestrated combination of Δ^1 electrophilicity and the presence of a nucleophilic imine at N-18 is required for nano-molar levels of antiproliferative activity towards tumour cells. Less potently cytotoxic natural product analogues discorhabdins D (lacking Δ^1) and Q (pyridine N-18) failed to react with nucleophiles, supporting this model. The identification of cellular targets of the discorhabdins is ongoing.

Experimental section

General

Optical rotations were recorded on a Perkin Elmer 341 Polarimeter using a 0.1 dm cell. Ultraviolet-visible spectra were run as MeOH solutions on a UV-2102 PC Shimadzu UV-Vis scanning spectrophotometer. ECD spectra were recorded on an Applied Photophysics Pi star spectropolarimeter. NMR spectra were recorded on either a Bruker Avance DRX-600 spectrometer operating at 600 MHz for ^{1}H nuclei and 150 MHz for ^{13}C nuclei, a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. Proto-deutero solvent signals were used as internal references (CD_3OD: δ_H 3.30, δ_C 49.05). Standard Bruker pulse sequences were utilized. HRMS data were acquired on a Bruker micrOTOF Q II mass spectrometer. Flash column chromatography was performed using reversed-phase Merck Lichroprep RP-8 or RP-18 (40–63 μm). Analytical reversed-phase HPLC was run on a Dionex UltiMate 3000RS using a Grace C_8 column (3 μm platinum, 33 × 7 mm) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 13.5 min at 2 mL min^{-1} and monitoring at 254 nm. The procedures for isolation of (+)- and (−)-discorhabdin B used in this study have been reported elsewhere.[11,14]

Compound 11. *N*-Acetyl-L-cysteine (40 mg, 0.25 mmol) was dissolved in DMF (0.5 mL), MeOH (1 mL) and water (0.1 mL), followed by addition of TEA (40 μL, 0.29 mmol). (+)-(6*S*,8*S*)-Discorhabdin B (**2**) (20 mg, 48.3 μmol) free base was dissolved in DMF (0.5 mL), followed by addition of TEA (9 μL, 65 μmol) and the *N*-acetyl-L-cysteine mixture. The reaction mixture was stirred in air for 30 min before loading the reaction mixture directly onto a reversed-phase C_{18} flash chromatography column and washing with three column volumes of water (0.05% TFA). Elution with 10% MeOH (0.05% TFA) yielded a green fraction which was further purified by C_{18} (25–40 μm) flash column chromatography eluting with a gradient solvent mixture from 0–10% MeOH (0.05% TFA) to yield **11** (4.0 mg, 14%) as a green non-crystalline trifluoroacetate salt; R_T 4.34 min; $[\alpha]_D = 0$ (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 250 (3.94), 274 sh (3.83), 288 sh (3.78), 326 (3.59), 405 (3.55) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 214 (0), 216 (+0.4), 222 (0), 258 (−9.7), 286 (−0.8), 309 (−3.5), 328 (0), 366 (+6.6) nm; ^{1}H and ^{13}C NMR see Table 1; (+)-ESIMS *m*/*z* 497 $[M]^+$; (+)-HRESIMS *m*/*z* $[M]^+$ 497.0931 (calcd for $C_{23}H_{21}N_4O_5S_2$: 497.0948).

Compounds 12 and 13. *N*-Acetyl-L-cysteine (16 mg, 0.10 mmol) was dissolved in DMF (0.5 mL), MeOH (1 mL) and water (0.1 mL), followed by addition of TEA (14 μL, 0.10 mmol). Free base (−)-(6*R*,8*R*)-discorhabdin B (**10**) (10.4 mg, 19.7 μmol) was dissolved in DMF (0.5 mL), followed by addition of TEA (6 μL, 39 μmol) and the *N*-acetyl-L-cysteine mixture. The reaction mixture was stirred in air for 30 min before loading the reaction mixture directly onto a reversed-phase C_{18} flash chromatography column and washed with three column volumes of water (0.05% TFA). Elution with 10% MeOH (0.05% TFA) yielded **13** (7.5 mg, 62%) as a purple–brown non-crystalline trifluoroacetate salt, and further elution with 30% MeOH (0.05% TFA) yielded **12** (4.3 mg, 36%) as a green non-crystalline trifluoroacetate salt.

12: R_T 4.32 min; $[\alpha]_D = 0$ (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.94), 285 (3.79), 323 (3.59), 402 (3.55), 585 (2.48) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 206 (+8.3), 220 (+0.1), 257 (+9.2), 282 (+3.7), 294 (+4.4), 326 (0), 359 (−7.3), 397 (0), 435 (+1.2), 507 (0) nm; ^{1}H and ^{13}C NMR see Table 2; (+)-ESIMS *m*/*z* 497 $[M]^+$; (+)-HRESIMS *m*/*z* $[M]^+$ 497.0910 (calcd for $C_{23}H_{21}N_4O_5S_2$: 497.0948).

13: R_T 4.42 min; $[\alpha]_D = -340$ (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.94), 247 (3.95), 281 (3.80), 336 (3.70), 563 (2.55) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 207 (+10.7), 209 (+5.6), 211 (+9.3), 223 (+2.1), 231 (+3.8), 238 (+1.3), 255 (+8.1), 299 (+4.3), 319 (+6.3), 335 (0), 361 (−9.9), 437(0) nm; ^{1}H NMR and ^{13}C NMR see Table 3; (+)-ESIMS *m*/*z* 497 $[M + H]^+$; (+)-HRESIMS *m*/*z* $[M + H]^+$ 497.0931 (calcd for $C_{23}H_{21}N_4O_5S_2$: 497.0948).

Compound 17. Freshly extracted and purified (+)-(6*S*,8*S*)-discorhabdin B free base (**2**) (15.8 mg, 38.1 μmol) was kept in the freezer for two weeks, after which time analytical HPLC showed one major and several minor non-discorhabdin B peaks. The crude mixture was dissolved in methanol (1 mL) and loaded onto a reversed-phase C_8 flash chromatography column. The major product was eluted with 40% MeOH (0.05% TFA), and further purified by a combination of Sephadex LH-20 (MeOH (0.05% TFA)) and C_8 flash chromatography (MeOH, H_2O–TFA (0.05%)), yielding **17** (3.0 mg, 16%); trifluoroacetate salt dark green oil; $[\alpha]_D = -120$, $[\alpha]_{578} = -140$, $[\alpha]_{546} = -240$, (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.56), 245 (4.48), 293 (4.25), 391 (4.00), 593 (3.06) nm; ECD (MeOH) λ ($\Delta\varepsilon$)

256 (−14.3), 269 (0), 280 (+4.4), 291 (0), 315 (−4.5), 334 (0), 364 (+14.0), 406 (0), 443 (−4.1) nm; ^{1}H and ^{13}C NMR see ESI Table S1;† (+)-HRESIMS *m/z* $[M]^{+}$ 747.0467 (calcd for $C_{36}H_{24}{}^{79}BrN_6O_4S_2$: 747.0478), 749.0453 (calcd for $C_{36}H_{26}{}^{81}BrN_6O_4S_2$: 749.0463).

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