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New natural products in the discorhabdin A- and B-series from New Zealand-sourced *Latrunculia* spp. sponges

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ABSTRACT

A survey of the secondary metabolite chemistry profiles of New Zealand sponges of the genus *Latrunculia* has yielded new members of the discorhabdin A- and B-type families. The structure elucidation of (+)-(6*R*,8*S*)-1-thiomethyldiscorhabdin G*/I (**5**) and both enantiomers of 16a,17a-dehydrodiscorhabdin W (**6**) are reported. Absolute configurations were assigned by comparison with a dataset of recently reported electronic circular dichroism spectra of discorhabdin alkaloids. A stereochemical correction of the recently reported natural product (+)-3-dihydrodiscorhabdin A from (3*S*,5*R*,6*S*,8*S*)-(**7**) to the C3-epimeric (+)-(3*R*,5*R*,6*S*,8*S*)-(**8**) and assignment of absolute configuration is also presented. Semi-synthesis of (+)-(3*S*,5*R*,6*S*,8*S*)-(**7**) from (+)-discorhabdin A provided further evidence for this structure revision. Cytotoxicity data is reported for **5**–**8**.

1. Introduction

Since the first reports of discorhabdin C in 1986[1] and prianosin A/discorhabdin A (+)-**1** in 1987,[2,3] close to forty members of the discorhabdin/prianosin/epinardin family of natural products have been reported from marine sponges.[4] These alkaloids, which contain a core pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline skeleton bearing a spirocyclic moiety at C-6, and the possible presence of sulfur linkage between C-5 and C-8 and/or ring closure between C-2 and N-18, exhibit potent biological activities including cytotoxic, antibiotic, and more recently, antimalarial properties. The first dimer, discorhabdin W, reported in 2005, was isolated from a New Zealand collection of *Latrunculia* sp.[5]

We recently disclosed the first examples of enantiomeric pairs of discorhabdins B (**2**), G*/I (**3**), L and W (**4**), isolated from distinct sponge populations collected from geographically distant locales in New Zealand.[6] Comparison of experimental electronic circular dichroism (ECD) spectra with those calculated using time dependent density functional theory (TD-DFT) yielded a dataset affording the ability to assign the core skeleton absolute configuration of discorhabdin A-, B-, G*/I-, D- and W-type alkaloids. As part of an ongoing investigation of the structure–activity relationship and mechanism of action of the discorhabdins[7] three new examples were isolated from New Zealand *Latrunculia* sponges, namely (+)-(6*R*,8*S*)-1-thiomethyldiscorhabdin G*/I (**5**) and both enantiomers of 16a,17a-dehydrodiscorhabdin W (**6**). Herein we report their isolation, structure elucidation, determination of absolute configuration and results of a preliminary evaluation of cytotoxic properties. In addition, a stereochemical correction of the recently reported natural product (+)-3-dihydrodiscorhabdin A from (3*S*,5*R*,6*S*,8*S*)-(**7**)[8] to the C3-epimeric (+)-(3*R*,5*R*,6*S*,8*S*)-(**8**) and assignment of absolute configuration for the compound is presented.

2. Results and discussion

Chemical screening of Wellington Harbour specimens of *Latrunculia* (*Biannulata*) *wellingtonesis* sponge led to the isolation of twelve pigments. Dereplication by NMR, MS, LC–PDA, [α] and ECD analysis identified ten of these compounds as the previously reported (+)-discorhabdin/prianosin A (**1**),[2,3] (+)-discorhabdin B (**2**),[3] $(-)_{546}$-discorhabdin D,[9] (−)-discorhabdin H,[10] (+)-discorhabdin G*/I (**3**),[10,11] $(-)_{578}$-discorhabdin L,[11] (−)-discorhabdin N,[10] (+)-discorhabdin Q,[12] (−)-discorhabdin W (**4**)[5] and (+)-3-dihydrodiscorhabdin A.[8] Recently El-Naggar and Capon reported a number of new discorhabdin alkaloids from southern Australian sponges of the genera *Higginsia* and *Spongosorites*, including (+)-3-dihydrodiscorhabdin A (**7**).[8] Based upon their observation of carbinol H-3 as being a triplet (CD_3OD, δ_H 4.32, t, J=2.8 Hz), combined with a CHEM3D minimised conformation (as shown in Fig. 1a), the authors concluded the C-3 configuration to be 3S*. A biogenetic reasoning of co-occurrence with

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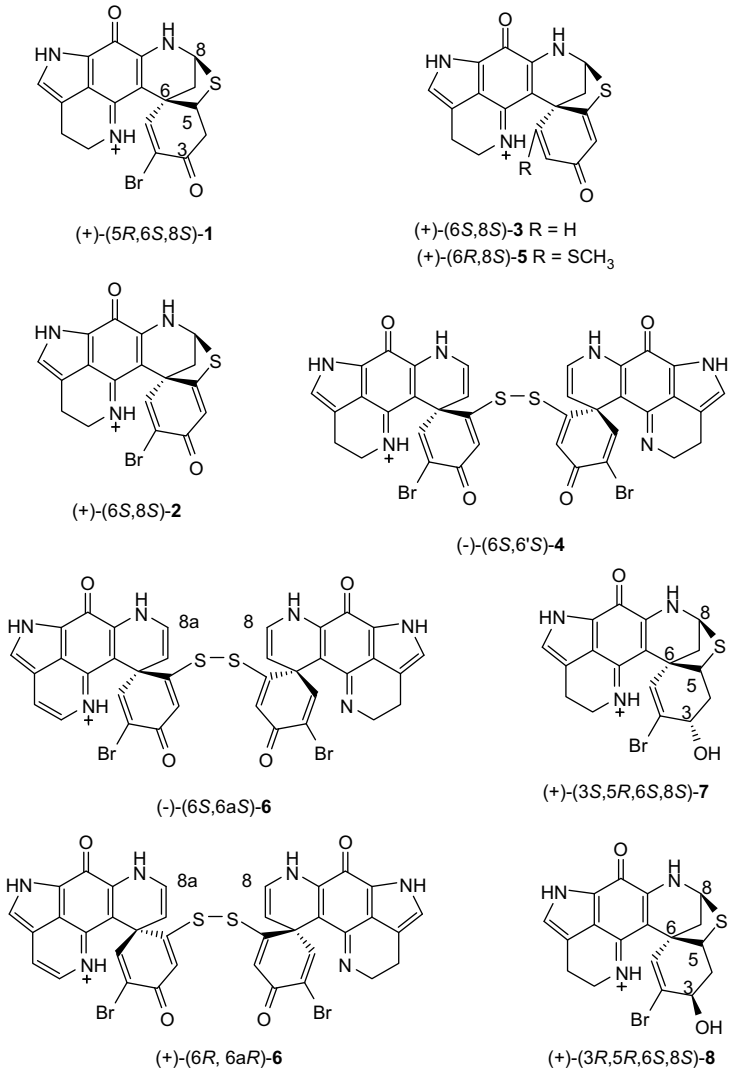

(+)-(5*R*,6*S*,8*S*)-discorhabdin A (**1**), which has defined absolute configuration,[2,6] led them to conclude a *putative* absolute configuration of (3*S**,5*R**,6*S**,8*S**)-(**7**).[8]

Analysis of the spectroscopic data observed for our alkaloid isolated from a Wellington Harbour collection of *L. (Biannulata) wellingtonesis* also led us to conclude the natural product was the 3-dihydro derivative of discorhabdin A. Like El-Naggar and Capon, we also observed carbinol H-3 as a triplet (D_2O, δ_H 4.37, t, J=3.0 Hz) and as both compounds exhibited the same sign and similar magnitude $[\alpha]_D$ values we were convinced the two isolated natural products were in fact the same. Where we differed in our structural analysis however, was in the determination of the preferred solution conformation adopted by the alkaloid. This conformation is critical, as the geometry of the H-3–2H-4 spin system and resultant predicted vicinal coupling constants are used to define the relative configuration at C-3. Force-field conformational analysis and subsequent DFT B3LYP/6-31G* geometry optimisation (Spartan 06) of the El-Naggar and Capon conformation (Fig. 1a) identified it as being the higher energy (13.1 kJ/mol) of two accessible conformers (Table 1). The ene-ol-containing ring geometry of the dominant conformer

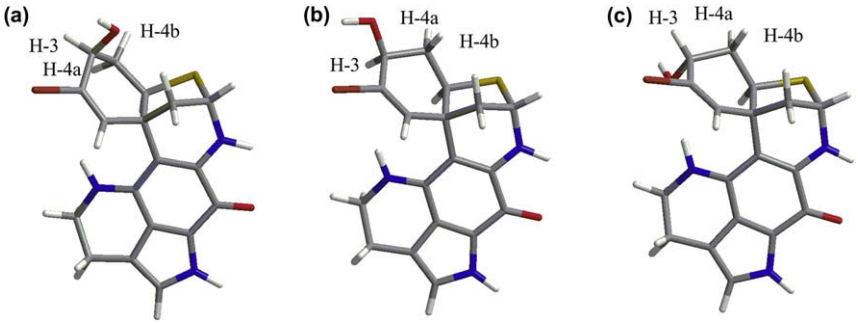

Figure 1. (a) CHEM3D minimised conformation of (+)-(3*S**,5*R**,6*S**,8*S**)-3-dihydrodiscorhabdin A (**7**) as proposed by El-Naggar and Capon[8]; (b) dominant conformer of (3*S*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A (**7**) identified by conformational analysis and DFT/B3LYP/6-31G* geometry optimisation calculations; (c) dominant conformer of (3*R*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A (**8**) identified by conformational analysis and DFT/B3LYP/6-31G* geometry optimisation calculations.

(conformer 1, 99.5%) of (3*S*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A (Fig. 1b) would lead to carbinol H-3 being expected to be observed as a doublet of doublets (ϕ 52.1°, Karplus equation predicted $J_{H\text{-}3/H\text{-}4a}$ 5.3 Hz; ϕ 167.3°, $J_{H\text{-}3/H\text{-}4b}$ 10.5 Hz), which is markedly different from the triplet (J=3.0 Hz) observed for H-3 in the natural product.

Similar conformational analysis of the C-3 epimer of **7**, i.e., (3*R*,5*R*,6*S*,8*S*)-(**8**), identified only one accessible conformer (Fig. 1c) with predicted coupling constants (ϕ 55.7°, $J_{H\text{-}3/H\text{-}4a}$ 4.7 Hz; ϕ 61.2°, $J_{H\text{-}3/H\text{-}4b}$ 4.0 Hz) in close agreement with those observed for the natural product (t, J=3.0 Hz). *Thus we believe the 3S* configuration recently reported for (+)-3-dihydrodiscorhabdin A (**7**)[8] is in error, and the relative configuration should be reassigned as 3R* (**8**).*

Table 1
Conformer energy analysis of (3*S*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A (**7**)

Conformer	Free energy (au)	Free energy difference (kJ/mol)	Boltzmann population (%)
1	−3982.69874	0	99.5
2	−3982.69375	13.0915	0.5

The absolute stereochemistry of (+)-**8** was determined upon comparison of the ECD spectrum with that observed for Wellington-sourced (+)-discorhabdin A [(+)-**1**] (Fig. 2). The stereochemistry of (+)-**1** has previously been established as (5*R*,6*S*,8*S*) for the free base form with a single crystal X-ray study[2] and more recently, via TD-DFT calculations of the ECD for the trifluoroacetate salt.[6] The ECD spectrum of (+)-**8** was consistent in both sign and magnitude to that of the (+)-(5*R*,6*S*,8*S*)-discorhabdin A (**1**), thereby establishing the absolute stereochemistry of the C-5, C-6 and C-8 chiral centers as (5*R*,6*S*,8*S*). The absolute stereochemistry of the natural product (+)-3-dihydrodiscorhabdin A (**8**) was therefore concluded to be (3*R*,5*R*,6*S*,8*S*).

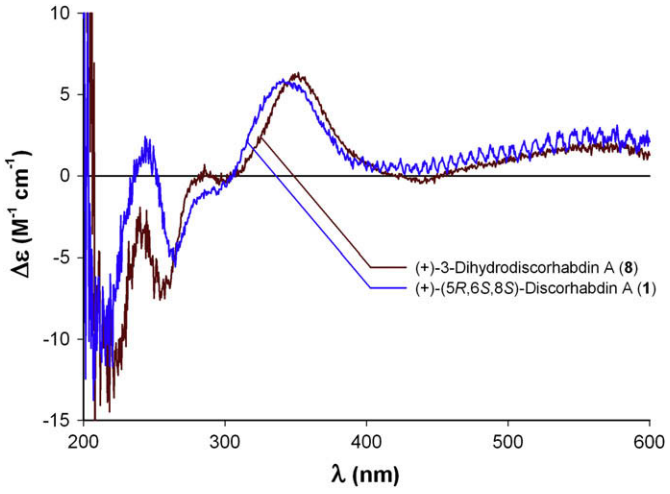

Figure 2. Experimental ECD spectra of (+)-(3*R*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A [(+)-**8**] (red) and (+)-(5*R*,6*S*,8*S*)-discorhabdin A [(+)-**1**] (blue) as trifluoroacetate salts in methanol.

In order to experimentally establish a biosynthetic link between **1** and **8**,(+)-(5*R*,6*S*,8*S*)-discorhabdin A (**1**) was reduced with $NaBH_4$ in methanol. Colour change from dark red to yellow, followed by aerial oxidation back to dark red was observed, as previously noted for the reduction of discorhabdin C.[13] Spectroscopic characterization of purified product revealed chemical shift differences between the semi-synthetic derivative and both starting material **1** and natural product **8**, centred mainly on resonances associated with the spiro-ring. While the chemical shifts and coupling constants observed for H-5 (D_2O, δ_H 4.21, dd, J=13.3, 4.6 Hz) and H-4a (δ_H 2.27, m) and H-4b (δ_H 2.03, m) in the reaction product were similar to those observed for (+)-(3*R*,5*R*,6*S*,8*S*)-**8**, the resonances observed for H-1 (δ_H 6.52, d, J=1.9 Hz) and critically, H-3 (δ_H 4.54, ddd, J=10.3, 5.7, 1.9 Hz) were quite different. Significantly, the coupling constants observed for H-3 (D_2O, $J_{H\text{-}3/H\text{-}4a}$ 5.7 Hz, $J_{H\text{-}3/H\text{-}4b}$ 10.3 Hz) were those expected for the dominant conformer of (3*S*,5*R*,6*S*,8*S*)-dihydrodiscorhabdin A (**7**) discussed earlier (Fig. 1b). Thus we conclude that the semi-synthetic product resulting from hydride reduction of naturally-occurring (+)-(5*R*,6*S*,8*S*)-discorhabdin A (**1**) is (3*S*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A (**7**). It is interesting to note that the small size of the reducing agent ($NaBH_4$) yields a product resulting from *Re* face attack, whilst the naturally occurring stereoisomer (+)-(3*R*,5*R*,6*S*,8*S*)-3-dihydrodiscorhabdin A (**8**) is the result of *Si* face attack.

The second new pigment (**5**) was isolated as a dark purple/brown oil. High resolution FABMS established a molecular formula of $C_{19}H_{16}N_3O_2S_2$, which required fourteen double bond equivalents. Compound **5** showed three exchangeable proton signals in the DMSO-d_6 ^{1}H NMR spectrum, at 13.28, 10.83 and 8.02 ppm, corresponding to *N*H-13, *N*H-9 and *N*H-18 positions in a discorhabdin B-type alkaloid.[3] Close comparison of ^{1}H and ^{13}C NMR spectroscopic data observed for **5** with those reported for discorhabdin G*/I (**3**)[10,11] suggested **5** to be a thiomethyl (CD_3OD; δ_H 2.56 3H, s; δ_C 14.6)[14] substituted analogue. Chemical shift differences centered upon the spiro-enone ring were noted for both compounds. In the case of **5** these resonances comprised a carbonyl (δ_C 181.6), two quaternary sp^2 (δ_C 170.7 and 164.8), one quaternary sp^3 (δ_C 52.9) and two olefinic methines (δ_C 123.3 and 119.2) in the ^{13}C NMR spectrum, and two olefinic doublets (δ_H 6.40, J=1.1 Hz; 6.12, J=0.6 Hz) observed in the ^{1}H NMR spectrum. The two methine resonances at 6.40 and 6.12 ppm were assigned to the spirodienone ring positions H-2 and H-4 respectively, based on their long-range ^{1}H–^{1}H COSY correlations to each other and ^{1}H–^{13}C HMBC correlations from H-2 to C-4 (δ_C 119.2) and C-6 (δ_C 52.9), and from H-4 to C-2 (δ_C 123.3) and C-6. The relative assignments of protons at positions C-2 and C-4 were confirmed by observation of a long-range planar zig-zag COSY correlation between H-4 and H-7α.[9] The *S*-methyl resonance (δ_H 2.65) was placed at C-1 (δ_C 164.8) based on ^{1}H–^{13}C HMBC correlations from the methyl protons to C-1 and C-2 on the spirodienone ring and by virtue of observed long-range COSY and NOESY correlations between the methyl singlet and H-2. This established **5** as 1-thiomethyldiscorhabdin G*/I. Near equivalence of the ECD spectrum observed for (+)-**5** with that previously reported for (+)-(6*S*,8*S*)-discorhabdin G*/I (**3**)[6] (Fig. 3) established the (6*R*,8*S*) absolute configuration of **5** as shown.

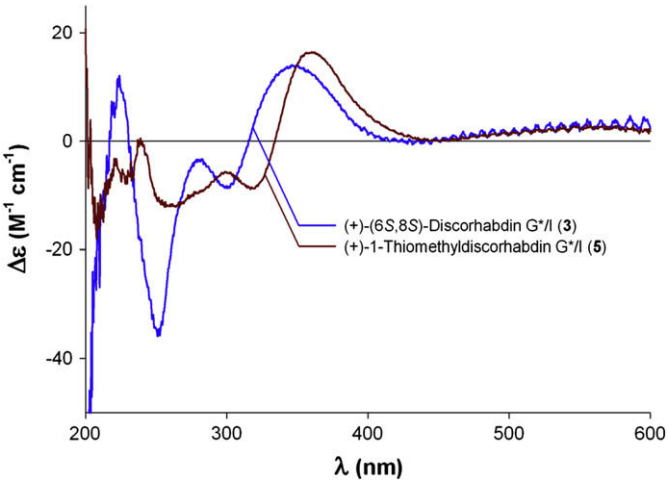

Figure 3. Experimental ECD spectra of (+)-1-thiomethyldiscorhabdin G*/I [(+)-**5**] (red) and (+)-(6*S*,8*S*)-discorhabdin G*/I [(+)-**3**] (blue) as trifluoroacetate salts in methanol.

ESI mass spectrometric analysis of the third pigment (**6**) showed a pseudomolecular ion $[M+H]^+$ at *m*/*z* 824, with the doubly protonated ion $[M+2H]^{2+}$ also present at *m*/*z* 413, suggestive of a metabolite of similar molecular weight to the dimeric discorhabdin W (**4**).[5] As with discorhabdin W, the isotope pattern of the molecular ion of **6** also showed peaks in the 1:2:1 ratio indicating the presence of two bromine atoms in the molecule. The ^{13}C NMR spectrum of **6** in CD_3OD revealed the presence of 40 resonances, suggesting that the structure of this large molecule was not symmetrical as in the case of discorhabdin W. The ^1H NMR spectrum (CD_3OD) of **6** showed 16 resonances. Interpretation of ^1H–^1H COSY and ^1H–^{13}C HMBC spectra suggested that nine proton resonances were consistent with a discorhabdin W-type subunit, and the remaining 7 showed similarities with a discorhabdin T-type[14] molecule. With the structure of the two subunits identified, a connection through the C-5 disulfide was made to give the irregular dimer, 16a,17a-dehydrodiscorhabdin W (**6**). The ECD spectrum of **6** was essentially identical to that observed for (−)-(6*S*,6′*S*)-discorhabdin W[6] (**4**) (Fig. 4), thereby establishing the absolute configuration of (−)-**6** as (6*S*,6a*S*).

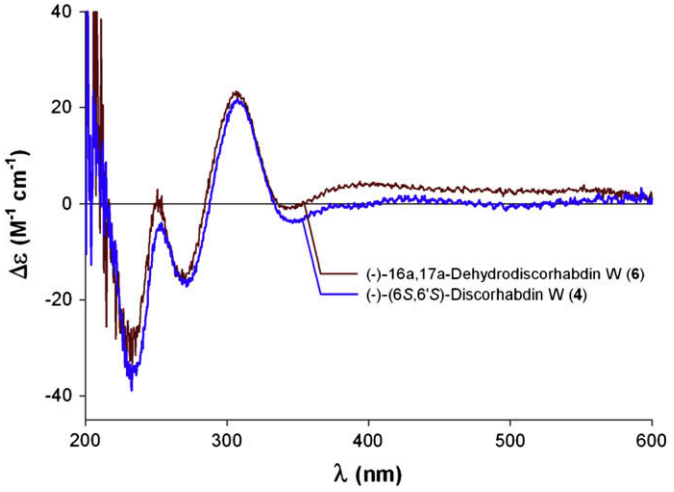

Figure 4. Experimental ECD spectra of (−)-16a,17a-dehydrodiscorhabdin W [(−)-**6**] (red) and (−)-(6*S*,6′*S*)-discorhabdin W [(−)-**4**] (blue) as free bases in methanol.

We have previously noted that while most collections of *Latrunculia* in New Zealand yield discorhabdin alkaloids with absolute configuration related to (6*S*,8*S*)-discorhabdin B, sponges collected in the remote Doubtful Sound region biosynthesise the enantiomeric series of alkaloids.[6] Further close inspection of chromatographic fractions derived from a Doubtful Sound collection of *Latrunculia* sp. revealed the presence of **6** in very low quantities. Subsequent purification yielded a product that exhibited identical mass spectrometric and NMR properties to those observed for Wellington-sourced **6** but for which an equal and opposite ECD spectrum was recorded (Fig. 5). Thus the Doubtful Sound-sourced compound was concluded to be (+)-(6*R*,6a*R*)-**6**.

Biological activity against the murine leukaemia P388 cell line was determined for **5**–**8**. Potent antiproliferative activity was observed for **5** (IC_{50} 0.28 μM) and both enantiomers of **6** (IC_{50} 0.45 μM), which are of similar magnitude to the respective 'parent' compounds (+)-discorhabdin G*/I (**3**, IC_{50} 0.6 μM) and discorhabdin W (IC_{50} 0.13 μM). The central role played by the C-3 keto group in the potency of antiproliferative activity observed for discorhabdin A (**1**) (IC_{50} 0.11 μM) is demonstrated by the modest activity observed for naturally occurring **8** (IC_{50} 1.8 μM) and semi-synthetic analogue **7** (IC_{50} 2.1 μM). A similar structure–activity relationship has been reported for discorhabdin C.[13]

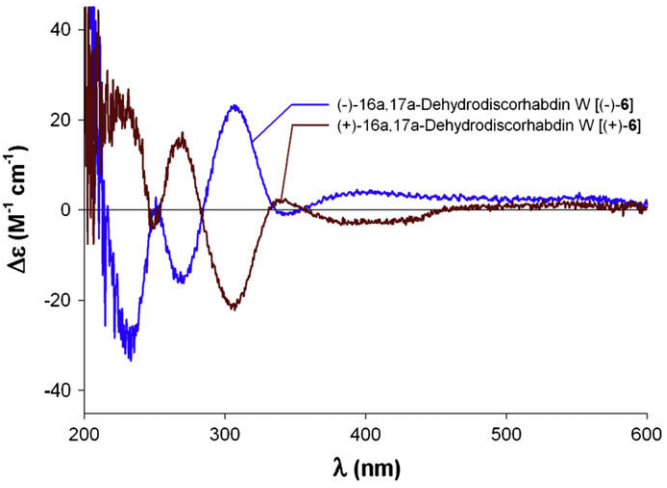

Figure 5. Experimental ECD spectra of (−)- and (+)-enantiomers of 16a,17a-dehydrodiscorhabdin W (**6**) as free bases in methanol.

3. Conclusions

New Zealand specimens of sponges of the genus *Latrunculia* continue to provide new examples of biologically active pyrroloiminoquinone alkaloids. Amongst the growing number of examples reported in the literature, it is apparent that the potency of cytotoxic activity towards tumour cell lines is dependent upon the presence of a 3-keto group.[4] This trend is further supported by our current results, whereby 3-keto-containing alkaloids **5** and **6** all exhibit sub-micromolar activity, while ene-ol analogues **7** and **8** were markedly less cytotoxic. In addition, the current study has provided another example of the location specific biosynthesis of enantiomers in the discorhabdin-series.[6] We have shown that with a dataset of electronic circular dichroism spectra available for the 'parent' structures of the different sub-families of discorhabdin alkaloids (i.e., discorhabdin A, B, G*/I, L and W)[6] assignment of absolute configuration to new members of this alkaloid class can be confidently made.

4. Experimental

4.1. General experiment procedures

General experimental procedures have been reported elsewhere.[6,15] All conformational analysis and geometry optimisation molecular modelling calculations used Spartan '06.[16] Predicted coupling constants based upon dihedral angles were calculated using the modified Karplus equation.[17]

4.2. Extraction and isolation

A single specimen of wet *L.* (*Biannulata*) *wellingtonesis* (Barret's Reef) was extracted with MeOH. The solvent was filtered and then removed in vacuo to give a dark brown crude extract (4.43 g), which was subjected to combinations of C_{18}, C_8 and CN flash (MeOH, H_2O–TFA (0.05%)) and Sephadex LH-20 (MeOH–TFA (0.05%)) flash column chromatography. The following discorhabdin alkaloids were isolated: (+)-discorhabdin A (**1**) trifluoroacetate salt (6.6 mg, 0.15% wet weight), (+)-discorhabdin B (**2**) trifluoroacetate salt (80.6 mg, 1.82% wet weight), $(-)_{546}$-discorhabdin D trifluoroacetate salt (12.7 mg, 0.29% wet weight), (+)-discorhabdin G*/I (**3**) trifluoroacetate salt (24.6 mg, 0.56% wet weight), (−)-discorhabdin H trifluoroacetate salt (52.9 mg, 1.19%

wet weight), $(-)_{578}$-discorhabdin L trifluoroacetate salt (39.0 mg, 0.88% wet weight), (−)-discorhabdin N trifluoroacetate salt (20.0 mg, 0.45% wet weight), (+)-discorhabdin Q trifluoroacetate salt (4.8 mg, 0.11% wet weight) and (+)-1-thiomethyldiscorhabdin G*/I (**5**) trifluoroacetate salt (3.2 mg, 0.072% wet weight).

A single specimen of freeze dried *L.* (*Biannulata*) *wellingtonesis* (SEAS-LAT-BR-15-1) (12.472 g) was extracted with MeOH. The solvent was filtered and then removed in vacuo to give a dark brown crude extract (3.84 g). A portion of the crude extract (582 mg) was subjected to combinations of Sephadex LH-20 (MeOH), C_{18}, and C_8 flash (MeOH, H_2O–TFA (0.05%)) chromatography, yielding: (+)-discorhabdin B (**2**) as a free base (15.8 mg, 0.83% dry weight), (+)-discorhabdin G*/I (**3**) trifluoroacetate salt (11.8 mg, 0.62% dry weight), $(-)_{578}$-discorhabdin L trifluoroacetate salt (20.4 mg, 1.08% dry weight), (+)-discorhabdin Q as a free base (3.0 mg, 0.16% dry weight), (−)-discorhabdin W (**4**) as a free base (9.1 mg, 0.48% dry weight), (+)-3-dihydrodiscorhabdin A (**8**) trifluoroacetate salt (3.7 mg, 0.20% dry weight), and (−)-16a,17a-dehydrodiscorhabdin W (**6**) as a free base (2.4 mg, 0.12% dry weight).

A single specimen of freeze dried Doubtful Sound-sourced *Latrunculia* sp. sponge material (4.782 g) was extracted with MeOH (4×200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (1.290 g). A portion of the crude extract (420 mg) was subjected to combinations of Sephadex LH-20 and C_{18} flash (MeOH, H_2O–TFA (0.05%)) column chromatography, yielding (−)-discorhabdin B (**2**) (2.49 mg, 0.16% dry weight), discorhabdin C (0.1 mg, 0.006% dry weight), (−)-discorhabdin G*/I (**3**) (1.50 mg, 0.096% dry weight), $(+)_{578}$-discorhabdin L (1.80 mg, 0.12% dry weight), (−)-discorhabdin K (2.14 mg, 0.14% dry weight), (+)-discorhabdin W (**4**) (2.13 mg as free base, 0.13% dry weight; and 5.53 mg converted to a TFA salt, 0.36% dry weight) and (+)-16a,17a-dehydrodiscorhabdin W (**6**) (converted to a TFA salt 0.6 mg, 0.04% dry weight).

*4.2.1. (+)-(3R,5R,6S,8S)-3-Dihydrodiscorhabdin A trifluoroacetate salt, [(+)-**8**]*

$[\alpha]_D$ +40, $[\alpha]_{578}$ −160, $[\alpha]_{546}$ −480 (*c* 0.05, MeOH); IR (smear) ν_{max} 3232, 3102, 1667, 1532, 1179, 1123, 1024 cm^{-1}; UV (MeOH) λ_{max} 204 (20,700), 249 (18,910), 347 (8130), 402 (6990), 586 (1130) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 219 (−14.5), 240 (−1.9), 258 (−7.5), 281 (0), 352 (+6.4), 419 (0) nm; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.18 (1H, br s, *N*H-13), 10.51 (1H, br s, *N*H-9), 7.94 (1H, br s, *N*H-18), 7.37 (1H, s, H-14), 6.42 (1H, s, H-1), 5.21 (1H, d, *J*=2.1 Hz, H-8), 4.28 (1H, dd, *J*=13.2, 4.9 Hz, H-5), 4.16 (1H, br s, H-3), 3.89 (1H, m, H-17a), 3.83 (1H, m, H-17b), 2.88 (2H, m, H-16), 2.50 (under solvent peak, H-7a), 2.40 (1H, d, *J*=12.0 Hz, H-7b), 2.11 (1H, td, *J*=13.6, 3.0 Hz, H-4a), 1.90 (1H, ddd, *J*=13.6, 4.8, 3.1 Hz, H-4b); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.9 (C-11), 153.7 (C-19), 150.9 (C-10), 132.6 (C-1), 127.3 (C-14), 126.5 (C-2), 123.2 (C-12), 123.0 (C-21), 119.6 (C-15), 103.3 (C-20), 69.4 (C-3), 58.8 (C-8), 50.0 (C-6), 48.9 (C-5), 43.7 (C-17), 40.7 (C-7), 38.3 (C-4), 18.1 (C-16); ^{1}H NMR (CD_3OD-d_4, 400 MHz) δ 7.16 (1H, s, H-14), 6.46 (1H, s, H-1), 5.20 (1H, dd, *J*=3.5, 1.1 Hz, H-8), 4.33 (2H, m, H-3/H-5), 3.91 (2H, m, H-17), 2.95 (2H, m, H-16), 2.59 (1H, dd, *J*=12.2, 3.7 Hz, H-7a), 2.46 (1H, d, *J*=12.0 Hz, H-7b), 2.17 (1H, td, *J*=13.7, 3.0 Hz, H-4a), 2.00 (1H, ddd, *J*=13.9, 5.0, 3.1, H-4b); ^{1}H NMR (D_2O, 400 MHz) δ 7.16 (1H, s, H-14), 6.55 (1H, s, H-1), 5.27 (1H, dd, *J*=3.6, 1.1 Hz, H-8), 4.37 (1H, t, *J*=3.0 Hz, H-3), 4.16 (1H, dd, *J*=13.3, 5.0 Hz, H-5), 3.95 (1H, m, H-17a), 3.83 (1H, m, H-17b), 2.88 (2H, m, 2H-16), 2.58 (1H, dd, *J*=12.5, 3.7 Hz, H-7a), 2.42 (1H, d, *J*=12.5 Hz, H-7b), 2.23 (1H, ddd, *J*=14.4, 13.5, 3.7 Hz, H-4a), 2.05 (1H, ddd, *J*=13.3, 4.9, 2.6 Hz, H-4b); ^{13}C NMR (D_2O, 100 MHz) δ (C-11 not observed), 157.3 (C-19), 153.9 (C-10), 136.2 (C-1), 130.0 (C-14), 128.6 (C-21), 126.4 (C-2), 125.5 (C-12), 123.0 (C-15), 105.6 (C-20), 72.1 (C-3), 61.5 (C-8), 51.9 (C-5 or C-6), 51.8 (C-6 or C-5), 46.6 (C-17), 42.7 (C-7), 40.7 (C-4), 20.5 (C-16); HRFABMS *m*/*z* $[M+H]^+$ 418.02314 (calcd for $C_{18}H_{17}{}^{79}BrN_3SO_2$, 418.02248), 420.02051 (calcd for $C_{18}H_{17}{}^{81}BrN_3SO_2$, 420.02044).

*4.2.2. Semi-synthetic 3-dihydrodiscorhabdin A,(+)-(3S,5R,6S,8S)-3-dihydrodiscorhabdin A trifluoroacetate salt, [(+)-**7**]*

(+)-(5*R*,6*S*,8*S*)-Discorhabdin A (**1**) TFA salt (1.9 mg, 3.6 μmol) was dissolved in dry methanol (2 mL) to which an excess of $NaBH_4$ was added. Solution was stirred for 10 min under N_2, during which the color changed from dark red to bright yellow. After stirring in air for 5 min, the solution had reverted to red. The solvent was removed in vacuo and the solid purified by C_8 flash (MeOH, H_2O–TFA (0.05%)) chromatography, yielding semi-synthetic 3-dihydrodiscorhabdin A (**7**) TFA salt (1.7 mg, 3.2 μmol, 89% yield). Blue-green powder; $[\alpha]_D$ +200, $[\alpha]_{578}$ −40, $[\alpha]_{546}$ −400 (*c* 0.05, MeOH); IR (smear) ν_{max} 3102, 2926, 1676, 1525, 1184, 1123, 1024 cm^{-1}; UV (MeOH) λ_{max} 204 (20,510), 250 (17,180), 347 (8010), 402 (4740), 586 (1130) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 215 (−25.1), 258 (0), 343 (+8.0), 381 (0) nm; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.14 (1H, br s, *N*H-13), 10.36 (1H, br s, *N*H-9), 8.50 (1H, br s, *N*H-18), 7.36 (1H, s, H-14), 6.21 (1H, s, H-1), 5.56 (1H, d, *J*=7.8 Hz, OH), 5.20 (1H, d, *J*=2.6 Hz, H-8), 4.49 (1H, br s, H-3), 4.10 (1H, dd, *J*=12.8, 4.8 Hz, H-5), 3.89 (1H, br m, H-17a), 3.76 (1H, m, H-17b), 2.56 (1H, dd, *J*=12.1, 2.9 Hz, H-7a), 2.50 (under solvent peak H-16), 2.36 (1H, d, *J*=12.1 Hz, H-7b), 2.11 (1H, m, H-4a), 1.96 (1H, m, H-4b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.8 (C-11), 153.4 (C-19), 150.5 (C-10), 133.5 (C-2), 130.0 (C-1), 127.2 (C-14), 123.2 (C-12/21), 119.6 (C-15), 103.3 (C-20), 66.8 (C-3), 58.5 (C-8), 53.5 (C-5), 49.2 (C-6), 44.4 (C-17), 41.4 (C-7), 40.5 (C-4), 18.1 (C-16); ^{1}H NMR (D_2O, 400 MHz) δ 7.17 (1H, s, H-14), 6.52 (1H, d, *J*=1.9 Hz, H-1), 5.28 (1H, br d, *J*=2.6 Hz, H-8), 4.54 (1H, ddd, *J*=10.3, 5.7, 1.9 Hz, H-3), 4.21 (1H, dd, *J*=13.3, 4.6 Hz, H-5), 3.95 (1H, m, H-17a), 3.81 (1H, m, H-17b), 2.90 (2H, m, 2H-16), 2.68 (1H, dd, *J*=12.5, 3.7 Hz, H-7a), 2.47 (1H, d, *J*=12.6 Hz, H-7b), 2.27 (1H, m, H-4a), 2.03 (1H, m, H-4b); HRFABMS *m*/*z* $[M+H]^+$ 418.02248 (calcd for $C_{18}H_{17}{}^{79}BrN_3SO_2$, 418.02248), 420.02115 (calcd for $C_{18}H_{17}{}^{81}BrN_3SO_2$, 420.02044).

4.2.3. (+)-(6R,8S)-1-Thiomethyldiscorhabdin G/I trifluoroacetate salt, [(+)-**5**]*

$[\alpha]_D$ +640, $[\alpha]_{578}$ +480, $[\alpha]_{546}$ −160 (*c* 0.05, MeOH); IR (smear) ν_{max} 3122, 2911, 1671, 1614, 1523, 1414, 1330, 1306, 1173, 1120, 1018 cm^{-1}; UV (MeOH) λ_{max} (ε) 245 (16,190), 281 (10,130), 334 (8520), 407 (5070), 568 (760) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 230 (−7.9), 237 (0), 239 (+0.5), 240 (0), 260 (−12.0), 299 (−5.7), 317 (−8.7), 334 (0), 360 (+16.5), 438 (0) nm; ^{1}H NMR (CD_3OD-d_4, 600 MHz) δ 7.20 (1H, s, H-14), 6.40 (1H, d, *J*=1.1 Hz, H-2), 6.12 (1H, d, *J*=0.6 Hz, H-4), 5.58 (1H, dd, *J*=3.8, 1.2 Hz, H-8), 3.96 (1H, td, *J*=14.1, 6.4 Hz, H-17a), 3.85 (1H, m, H-17b), 2.96 (1H, d, *J*=12.0 Hz, H-7a), 2.89 (2H, m, H-16), 2.66 (1H, dd, *J*=12.0, 3.9 Hz, H-7b), 2.56 (3H, s, H-22); ^{13}C NMR (CD_3OD-d_4, 150 MHz) δ 181.6 (C-3), 170.7 (C-5), 166.0 (C-11), 164.8 (C-1), 156.7 (C-19), 153.4 (C-10), 128.0 (C-14), 125.6 (C-12), 124.2 (C-21), 123.3 (C-2), 122.0 (C-15), 119.2 (C-4), 100.1 (C-20), 60.6 (C-8), 52.9 (C-6), 45.9 (C-17), 45.2 (C-7), 19.2 (C-16), 14.6 (C-22); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.28 (1H, br s, *N*H-13), 10.83 (1H, br s, *N*H-9), 8.02 (1H, br s, *N*H-18), 7.39 (1H, d, *J*=2.4 Hz, H-14), 6.31 (1H, s, H-2), 6.17 (1H, s, H-4), 5.60 (1H, d, *J*=3.0 Hz, H-8), 3.93 (1H, m, H-17a), 3.80 (1H, m, H-17b), 2.82 (1H, d, *J*=11.8 Hz, H-7a), 2.80 (2H, m, H-16), 2.54 (1H, dd, *J*=11.8, 4.1 Hz, H-7b), 2.54 (3H, s, H-22); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 179.0 (C-3), 167.1 (C-5), 164.7 (C-11), 160.0 (C-1), 153.8 (C-19), 151.4 (C-10), 127.5 (C-14), 123.6 (C-21), 122.9 (C-2), 122.7 (C-12), 121.6 (C-21), 120.4 (C-15), 118.4 (C-4), 98.2 (C-20), 50.7 (C-6), 44.6 (C-17), 17.7 (C-16), 143.8 (C-22); HRFABMS *m*/*z* $[M+H]^+$ 382.06874 (calcd for $C_{19}H_{16}N_3O_2S_2$, 382.06840).

*4.2.4. $(-)_{578}$-(6S,6aS)-16a,17a-Dehydrodiscorhabdin W trifluoroacetate salt, [$(-)_{578}$-**6**]*

$[\alpha]_D$ 0, $[\alpha]_{578}$ −200, $[\alpha]_{546}$ −160 (*c* 0.025, MeOH); IR (smear) ν_{max} 3434, 1678, 1656, 1497, 1480, 1203, 1136 cm^{-1}; UV (MeOH) λ (ε) 204 (33,440), 226 (31,440), 283 (16,980), 296 (16,530), 357 (3840), 421 (10,320), 439 (11,810), 544 (2860) nm; ^1H NMR (CD_3OD-d_4, 600 MHz) δ 8.44 (1H, d, *J*=6.0 Hz, H-17a), 8.15 (1H, s, H-14a), 7.95 (1H, s, H-1a), 7.75 (1H, s, H-1), 7.73 (1H, d, *J*=6.0 Hz, H-16a), 7.25 (1H, s, H-14), 6.65 (1H, d, *J*=7.8 Hz, H-8a), 6.57 (1H, d, *J*=7.8 Hz, H-8), 6.15 (1H, s, H-4), 6.09 (1H, s, H-4a), 4.61 (1H, d, *J*=7.8 Hz, H-7), 4.31 (1H, m, H-17A), 4.11 (1H, d, *J*=7.8 Hz, H-7a), 3.91 (1H, m, H-17B), 3.18 (1H, m, H-16A), 3.06 (1H, m, H-16B); ^{13}C NMR (CD_3OD-d_4, 150 MHz) δ 178.3 (C-3a), 176.0 (C-3), 170.6 (C-5a), 166.4 (C-11), 165.5 (C-11a), 163.5 (C-5), 160.6 (C-19), 157.1 (C-1a), 149.6 (C-1), 148.4 (C-19a), 147.2 (C-10), 144.2 (C-17a), 142.5 (C-10a), 130.1 (C-14a), 129.1 (C-8a), 127.7 (C-14), 126.7 (C-15a), 126.6 (C-8), 125.9 (C-12), 125.6 (C-2), 123.3 (C-21), 121.5 (C-15), 121.2 (C-21a), 120.9 (C-12a), 120.7 (C-4), 119.9 (C-2a), 116.4 (C-4a), 116.3 (C-16a), 114.6 (C-7), 107.1 (C-20a), 104.2 (C-7a), 95.6 (C-20), 52.0 (C-6a), 49.6 (C-6), 46.5 (C-17), 19.4 (C-16); HRESIMS *m*/*z* 822.9413 $[M+H]^+$ (calcd for $C_{36}H_{21}{}^{79}Br_2N_6S_2O_4$, 822.9427), 824.9420 (calcd for $C_{36}H_{21}{}^{79}Br\ {}^{81}BrN_6S_2O_4$, 824.9409), 826.9433 (calcd for $C_{36}H_{21}{}^{81}Br_2N_6S_2O_4$, 826.9395).

*4.2.5. (−)-(6S,6aS)-16a,17a-Dehydrodiscorhabdin W free base, [(−)-**6**]*

$[\alpha]_D$ −120, $[\alpha]_{578}$ −160, $[\alpha]_{546}$ −320 (*c* 0.025, MeOH); UV (MeOH) λ_{max} (ε) 204 (49,630), 222 (shoulder 40,770), 283 (shoulder 25,540), 416 (9530), 438 (9910) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 217 (0), 232 (−32.7), 249 (0), 251 (+2.9), 252 (0), 270 (−16.2), 285 (0), 307 (+23.3), 336 (0), 345 (−1.11), 355 (0), 402 (+4.0) nm.

*4.2.6. $(+)_{578}$-(6R,6aR)-16a,17a-Dehydrodiscorhabdin W trifluoroacetate salt, [$(+)_{578}$-**6**]*

$[\alpha]_D$ 0, $[\alpha]_{578}$ +80, $[\alpha]_{546}$ +160 (*c* 0.025, MeOH); UV (MeOH) λ (ε) 203 (41,620), 221 (35,090), 284 (18,360), 295 (18,390), 356 (3850), 421 (11,840), 440 (13,720), 550 (3340) nm; ^1H NMR (CD_3OD-d_4, 600 MHz) δ 8.45 (1H, d, *J*=6.2 Hz, H-17a), 8.17 (1H, s, H-14a), 7.92 (1H, s, H-1a), 7.76 (1H, s, H-1), 7.72 (1H, d, *J*=6.2 Hz, H-16a), 7.25 (1H, s, H-14), 6.65 (1H, d, *J*=7.4 Hz, H-8a), 6.57 (1H, d, *J*=7.3 Hz, H-8), 6.15 (1H, s, H-4), 6.09 (1H, s, H-4a), 4.62 (1H, d, *J*=7.5 Hz, H-7), 4.32 (1H, m, H-17A), 4.10 (1H, d, *J*=7.5 Hz, H-7a), 3.91 (1H, m, H-17B), 3.19 (1H, m, H-16A), 3.05 (1H, m, H-16B); ^{13}C NMR (CD_3OD-d_4, 150 MHz) δ 178.3 (C-3a), 176.0 (C-3), 170.7 (C-5a), 166.4 (C-11), 165.5 (C-11a), 163.5 (C-5), 160.6 (C-19), 157.1 (C-1a), 149.6 (C-1), 148.5 (C-19a), 147.2 (C-10), 144.4 (C-17a), 142.4 (C-10a), 130.1 (C-14a), 129.1 (C-8a), 127.7 (C-14), 126.7 (C-15a), 126.6 (C-8), 125.9 (C-12), 125.6 (C-2), 123.3 (C-21), 121.5 (C-15), 121.2 (C-21a), 120.9 (C-12a), 120.6 (C-4), 119.9 (C-2a), 116.4 (C-4a), 116.3 (C-16a), 114.6 (C-7), 107.2 (C-20a), 104.0 (C-7a), 95.6 (C-20), 52.1 (C-6a), 49.6 (C-6), 46.5 (C-17), 19.4 (C-16); HRESIMS *m*/*z* 822.9416 $[M+H]^+$ (calcd for $C_{36}H_{21}{}^{79}Br_2N_6S_2O_4$, 822.9427), 824.9428 (calcd for $C_{36}H_{21}{}^{79}Br\ {}^{81}BrN_6S_2O_4$, 824.9409), 826.9450 (calcd for $C_{36}H_{21}{}^{81}Br_2N_6S_2O_4$, 826.9395).

*4.2.7. (+)-(6R,6aR)-16a,17a-Dehydrodiscorhabdin W free base, [(+)-**6**]*

$[\alpha]_D$ +80, $[\alpha]_{578}$ +80, $[\alpha]_{546}$ +120 (*c* 0.025, MeOH); ECD (MeOH) λ ($\Delta\varepsilon$) 231 (+26.4), 246 (0), 248 (−3.9), 252 (0), 270 (+17.0), 283 (0), 307 (−22.2), 331 (0), 343 (+2.5), 356 (0), 400 (−2.5), 453 (0) nm.

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Supplementary data

^1H, ^{13}C NMR and ECD spectra for compounds **5**, **6**, **7** and **8**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.06.012.

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