Cytotoxic Pyrroloiminoquinones from Four New Species of South African Latrunculid Sponges†

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An examination of organic extracts of four new species of South African latrunculid sponges, *Tsitsikamma pedunculata*, *T. favus*, *Latrunculia bellae*, and *Strongylodesma algoaensis*, yielded 13 known and eight new pyrroloiminoquinone alkaloids, 3-dihydro-7,8-dehydrodiscorhabdin C (**4**), 14-bromo-3-dihydro-7,8-dehydrodiscorhabdin C (**5**), discorhabdin V (**6**), 14-bromo-1-hydroxydiscorhabdin V (**7**), tsitsikammamine A N-18 oxime (**10**), tsitsikammamine B N-18 oxime (**11**), 1-methoxydiscorhabdin D (**12**), and 1-aminodiscorhabdin D (**13**). Standard spectroscopic methods provided the structures of the pyrroloiminoquinone metabolites, while chiral GC−MS analysis of the acylated ozonolysis products of **21** confirmed the stereochemistry of the L-histidine residue in this compound. The anticancer activity of 20 pyrroloiminoquinone compounds was explored in the HCT-116 cancer cell line screen, and the DNA intercalation of the tsitsikammamines, together with their ability to cleave DNA through topoisomerase I inhibition, is discussed.

Southern hemisphere marine sponges of the family Latrunculiidae are commonly found in the cold water environments prevalent off Antarctica, New Zealand, southwestern Australia, Tasmania, and South Africa.[1] Latrunculid sponges are relatively abundant on sheltered, rocky reefs down to a depth of 50 m off the temperate southeastern coast of South Africa[1,2] and are a rich source of bioactive alkaloid pigments.[3] The majority of these alkaloid pigments possess a characteristic pyrroloiminoquinone substructure and have been routinely isolated from sponges of the genera *Batzella*,[4−7] *Damiria*,[8] *Histodermella*,[9] *Latrunculia*,[10−15] *Negombata*,[15] *Prianos*,[12,16,17] and *Zyzzya*,[14,18−23] although there is much evidence to suggest that many species in this list are either *Latrunculia* or *Zyzzya* species.[24] Therefore, in continuation of our search for bioactive metabolites from South African marine sponges[3,25] we describe here the isolation, identification, and bioactivity of eight new and 13 known pyrroloiminoquinone metabolites occurring in extracts of four new species of South African Latrunculid sponges, *Tsitsikamma pedunculata*, *T. favus*, *Latrunculia bellae,* and *Strongylodesma algoaensis*.[1,2]

T. pedunculata yielded the known compounds 14-bromodiscorhabdin C (**1**),[3] 14-bromo-3-dihydrodiscorhabdin C (**2**),[3] and 3-dihydrodiscorhabdin C (**3**)[26] and four new minor metabolites, 3-dihydro-7,8-dehydrodiscorhabdin C (**4**), 14-bromo-3-dihydro-7,8-dehydrodiscorhabdin C (**5**), discorhabdin V (**6**), and 14-bromo-1-hydroxydiscorhabdin V (**7**). Our earlier investigation of the 1:1 methanol/chloroform extract of *T. favus* revealed that compounds **1** and **2** and the tsitsikammamines A and B (**8** and **9**) were the major pyrroloiminoquinone metabolites in this sponge.[3] Pyrroloiminoquinone metabolites have proved to be useful chemotaxonomic markers in support of a taxonomic revision of the sponge family Latrunculiidae.[1−3] Therefore, the rationale for our return to the original collection of this sponge was to search for minor pyrroloiminoquinone metabolites that could be used as chemotaxonomic markers for the genus *Tsitsikamma*. Accordingly, our reinvestigation of *T. favus* extracts yielded **2**, **4**, **7**, and the novel N-18 oxime analogues of tsitsikammamines A and B (**10** and **11**).

A new species of dark green, encrusting *Latrunculia* sponge, *L. bellae*, yielded the new discorhabdins 1-methoxydiscorhabdin D (**12**) and 1-aminodiscorhabdin D (**13**) and five known metabolites, damirone B (**14**),[9] makaluvic acid A (**15**),[23] makaluvamine C (**16**),[18] and discorhabdins G* and N (**17** and **18**).[24] Discorhabdins A (**19**),[11] D (**20**),[12] and H (**21**)[24] and compound **3** were the major pyrroloiminoquinone metabolites in polar extracts of a shallow-water, brown *S. algoaensis*.

Results and Discussion

The *T. pedunculata*, *T. favus*, and *L. bellae* sponges were similarly extracted. Frozen sponge material was thawed and steeped in either methanol or methanol/dichloromethane mixtures (1:1 or 2:1), and the aqueous organic extract was concentrated and sequentially partitioned with hexane, chloroform, and *n*-butanol. ^{1}H NMR analysis (DMSO-d_6) of the three partition fractions obtained from each sponge extract revealed characteristic pyrroloiminoquinone resonances [δ_H 2.8−4.0 (2 × t, H_2-16 and H_2-17); 7.2−7.4 (H-14); 7.8−8.5 (NH-18); 9.5−10.5 (NH-9); and 13−14 (NH-13)] in the chloroform and *n*-butanol fractions. Accordingly, NMR-guided C_{18} solid-phase extraction and exhaustive reversed-phase HPLC of the chloroform and *n*-butanol fractions were used to isolate the pyrroloiminoquinone metabolites. A paucity of ^{1}H NMR resonances

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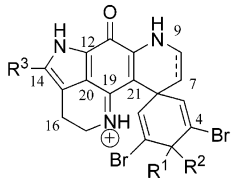
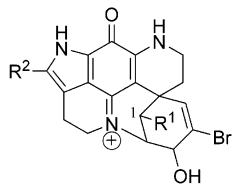
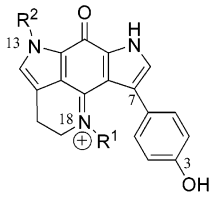
Chart 1



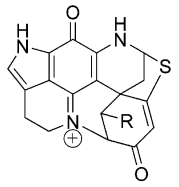
1 $R^1 + R^2 = O$, $R^3 = Br$
2 $R^1 = H$, $R^2 = OH$, $R^3 = Br$
3 $R^1 = H$, $R^2 = OH$, $R^3 = H$
4 $R^1 = H$, $R^2 = OH$, $R^3 = H$, Δ^7
5 $R^1 = H$, $R^2 = OH$, $R^3 = Br$, Δ^7
22 $R^1 + R^2 = O$, $R^3 = H$



6 $R^1 = R^2 = H$
7 $R^1 = OH$, $R^2 = Br$



8 $R^1 = R^2 = H$
9 $R^1 = H$, $R^2 = Me$
10 $R^1 = OH$, $R^2 = H$
11 $R^1 = OH$, $R^2 = Me$



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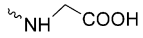
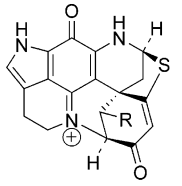
13 R = NH_2
18 R = 



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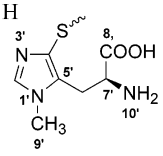
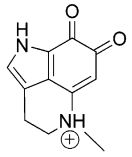
21 R = 



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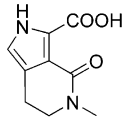

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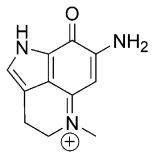

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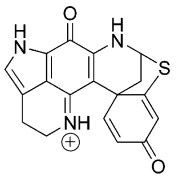

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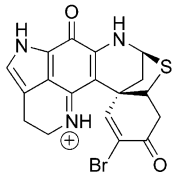

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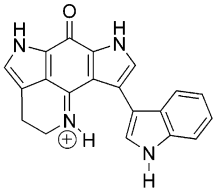

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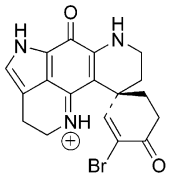

24

frequently hampers the structure elucidation of pyrroloiminoquinone metabolites, and comparison of both the ^1H and ^{13}C data of compounds **4**−**7** and **10**−**13** with those reported for known compounds in the discorhabdin[9−14] and tsitsikammamine[3] series provided the initial access to the structures of the new metabolites. Further support for the pyrroloiminoquinone structural motif that makes up the left-hand hemisphere of the compounds described here was routinely provided by a series of two- and three-bond HMBC correlations from the often clearly resolved protons (NH-9, NH-13, H-14, H_2-16, and H_2-17) to the six quaternary carbons (C-10−C-12, C-15, C-19−C-21). Additional COSY, HMBC, and HMQC data provided access to the right-hand hemisphere of the new pyrroloiminoquinone structures and enabled us to identify four general structural classes in the suite of eight new compounds isolated from the South African latrunculid sponges, viz., the pentacyclic discorhabdin compounds (**4** and **5**), the hexacyclic discorhabdin compounds (**6** and **7**), the heptacyclic discorhabdin compounds (**12** and **13**), and the pentacyclic bis-pyrroloiminoquinones (**10** and **11**).

The unusual stalked, golf ball-shaped sponge, *T. pedunculata*, was collected by scuba (−40m) in Algoa Bay in late summer 1999. The ^1H and ^{13}C NMR spectral data of the known compounds **1**−**3**, obtained from extracts of this sponge, were consistent with published data.[3,26] Although **3** has not been previously isolated from a natural source, it has been synthesized via reduction of discorhabdin C (**22**).[26,27] The molecular formula of **4** ($C_{18}H_{14}N_3O_2Br_2$), established from HRFABMS data, differed by two mass units from that of **3**. These mass data, together with the general compatibility of the ^1H and ^{13}C NMR spectra of **3** and **4**, suggested that **4** was an unsaturated analogue of **3**. Vicinal COSY couplings from the vinylic proton H-8 (δ 6.33, dd, 7.6, 4.3 Hz) to NH-9 (δ 10.59, brs) and H-7 (δ 4.72, d, 7.6 Hz) positioned the Δ^7 olefin in **4**. An additional ^5J COSY coupling from H-8 to H-1/H-5 (δ 6.64, s) was also observed. Long-range proton−proton couplings ($^5J \leq 1$ Hz) are reportedly fairly common in compounds where the coupling path contains one or more sp^2 carbons,[28] and we have observed an analogous coupling in the COSY spectra of the C-14 brominated analogue of **4**, 14-bromo-3-dihydro-7,8-dehydrodiscorhabdin C (**5**).

Comparison of the ^1H and ^{13}C NMR data of **5** with those of **2** and **4** initially indicated that **5** was the Δ^7 unsaturated homologue of **2**. Our initial assumptions were confirmed by the following evidence: a difference of two mass units between the molecular formulas of **2** ($C_{18}H_{16}N_3O_2Br_3$) and

Table 1. ^{1}H (DMSO-d_6, 400 MHz) and ^{13}C (DMSO-d_6, 100 MHz) NMR Data for Discorhabdin V (**6**) and 14-Bromo-1-hydroxydiscorhabdin V (**7**)

atom no.	**6**				**7**			
	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC	δ_C	δ_H (mult, *J*,Hz)	COSY	HMBC
1	34.6	1.82, dd, 2.3, 13.1	H-1	C-2, C-3, C-5	65.4	4.04, m		C-20
		2.28, d, 13.1		C-3, C-20				
2	60.6	4.24, dd, 3.8, 5.3	H-1	C-1, C-3, C-4, C-17	66.5	4.05, m	H-3	C-1, C-3, C-4, C-17, C-19
3	70.5	4.53, d, 5.3	H-5	C-4, C-5	70.5	4.63, s	H-2, H-5	
4	126.4				126.2			
5	136.6	6.31, s	H-3, H-1b	C-1, C-3, C-4, C-7	134.8	6.24, s	H-3	C-1, C-3, C-4, C-6, C-7
6	—[a]				36.8			
7a	29.1	1.51, m	H-7, H-8	C-5, C-20, C-8	23.9	1.68, d, 13.4	H-8	C-6
7b		1.95, d, 13.4		C-5, C-8		1.90, m	H-8	C-5, C-8
8	37.7	3.64, m	H-7		37.8	3.65, m	H-7, NH-9	
NH-9		9.50, s	H-8			9.65, s	H-8	
10	146.4				148.3			
11	166.7				165.5			
12	122.8				—[a]			
NH-13		12.99, s	H-14	C-14, C-15		13.85, s		C-14, C-15
14	126.5	7.28, d, 2.5	NH-13	C-15, C-21	118.1			
15	118.0				123.1			
16	19.2	2.93, m	H-17	C-15, C-17, C-21	18.7	2.81, m	H-17	C-14, C-17
		3.03, m		C-15, C-17		2.95, m	H-17	C-14, C-17
17a	52.7	3.99, m	H-16	C-15, C-16, C-19	53.0	3.88, m	H-16	
17b		4.14, m	H-16	C-15, C-16, C-19		4.11, m	H-16	
NH-18								
19	150.0				148.9			
20	96.6				94.0			
21	122.6				—[a]			
					OH-1	6.18, br s		
					OH-3	5.78, s		

[a] The signals due to quaternary carbons were not observed due to the small sample size.

5 ($C_{18}H_{14}N_3O_2Br_3$); the absence of the H-14 indole proton resonance in the ^{1}H NMR spectrum of **5** coupled with an upfield shift (Δ −15 ppm) of the C-14 carbon resonance indicative of bromination at C-14;[3] and finally the presence of a Δ^7 olefin in **5** clearly indicated from the COSY spectrum as argued previously for the structure of **4**. The configurations of the secondary alcohol functionalities in compounds **3**−**5** were not assigned.

High-resolution FABMS established the molecular formula of **6** as $C_{18}H_{17}N_3O_2Br$. The ^{1}H NMR spectrum revealed 14 nonexchangeable and two exchangeable protons (the secondary alcohol proton signal was not observed), while 17 well-resolved carbon resonances were evident in the ^{13}C NMR spectrum. Several attempts to locate the missing quaternary carbon resonance (C-6) by increasing the relevant ^{13}C relaxation delay time in the NMR acquisition parameters, or through HMBC correlations from the surrounding protons H-1, H-2, H-5, H-7, and H-8, were unsuccessful. A significant difference between the ^{1}H NMR data of **6** and those of compounds **1**−**5** was the absence of the H-18 proton signal (s, δ_H 7.8−8.5) in the ^{1}H NMR spectrum of **6**, which implied the presence of an additional ring in **6**, formed by a bond between N-18 and C-2 in the spiro ring, analogous to discorhabdin D (**20**).[12] Conclusive evidence for the N-18−C-2 bond was provided by the characteristic downfield shift (Δ 10 ppm)[12] of the C-17 resonance (cf. compounds **1**−**5**) and a three-bond HMBC correlation between H-2 and C-17. A combination of HMBC and COSY data (Table 1) established the hexacyclic structure of **6**. There are three major structural differences between **6** and **20**; namely, compound **6** has a C-3 allylic alcohol functionality and a C-4 bromine substituent and does not possess a thioether bridge between C-5 and C-8. Therefore, in accordance with the trend in discorhabdin nomenclature, **6** was deemed to be significantly different from **20** and the 16 other known discorhabdins and named discorhabdin V.[7] The configurations at C-2, C-3, and C-6 in discorhabdin V were not assigned.

The M + 1 peak (480.9638 Δ + 0.1 mmu) in the HRFABMS spectrum of **7** implied a molecular formula of $C_{18}H_{16}N_3O_3Br_2$ for this compound. Although our interpretation of the spectral data was again hampered by the absence of quaternary carbon signals (C-12 and C-21) in the ^{13}C NMR spectrum of **7**, the structural homology between **6** and **7** was clearly evident from comparison of their ^{1}H and ^{13}C NMR spectra. The prominent HMBC correlation between H-2 and C-17 confirmed that **7** was a discorhabdin V analogue with mass data suggesting the structural difference between these two compounds was limited to an additional bromine substituent and an extra hydroxyl moiety in **7**. The absence of the deshielded H-14 singlet in the ^{1}H NMR spectrum of **7** together with the upfield chemical shift (Δ −8 ppm) of the C-14 resonance (δ 118.1) observed in the ^{13}C NMR spectrum of **7** (cf. **6**) placed the additional bromine substituent at C-14.[3] The absence of a C-1 methylene signal (ca. δ 35) from the ^{13}C spectrum of **7**, and instead the appearance of an oxymethine resonance (δ 65.4), implied that the secondary alcohol functionality resided at C-1 in **7**. Two- and three-bond HMBC correlations from H-2 and H-5 to C-1 supported the assignment of a secondary alcohol functionality at C-1. The configurations at C-1−C-3 and C-6 in **7** were not assigned.

In addition to four compounds (**1**, **2**, **8**, and **9**) isolated previously by Hooper et al.[3] from *T. favus*, our reinvestigation of this sponge yielded the four minor metabolites **4**, **7**, **10**, and **11**. While the former two minor metabolites were also present in *T. pedunculata*, the two bis-pyrroloiminoquinone oximes, tsitsikammamine A N-18 oxime and tsitsikammamine B N-18 oxime (**10** and **11**), are novel. Close structural similarities between **8** and **10** and **9** and **11** were immediately apparent on comparison of their ^{1}H and ^{13}C NMR spectra. The molecular formulas of **10** ($C_{18}H_{14}N_3O_3$) and **11** ($C_{19}H_{16}N_3O_3$), proposed from HR-FABMS data, indicated that these two compounds differed by a single oxygen atom from **8** and **9**, respectively. A broad exchangeable proton singlet (δ 7.76) in the ^{1}H NMR spectra

Table 2. ^{1}H (DMSO-d_6, 400 MHz) and ^{13}C (DMSO-d_6, 100 MHz) NMR Data for Tsitsikammamine A N-18 Oxime (**10**) and Tsitsikammamine B N-18 Oxime (**11**)

	10				**11**			
atom no.	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC
1	114.5	6.75, d, 8.4	H-2/H-4	C-2, C-3, C-4, C-5, C-8	114.5	6.75, d, 8.4	H-2/H-4	C-3, C-5, C-20
2	129.7	7.55, d, 8.4	H-1/H-5	C-1, C-3, C-4, C-5, C-8	129.7	7.57, d, 8.4	H-1/H-5	C-1, C-3, C-4, C-5, C-6
3	156.6				156.6			
4	129.7	7.55, d, 8.4	H-1/H-5	C-1, C-2, C-3, C-5, C-8	129.7	7.57, d, 8.4	H-1/H-5	C-1, C-2, C-3, C-5, C-6
5	114.5	6.75, d, 8.4	H-2/H-4	C-1, C-2, C-3, C-4, C-8	114.5	6.75, d, 8.4	H-2/H-4	C-1, C-3, C-20
6	126.5				126.5			
7	121.2				121.2			
8	123.9	7.18, d, 2.8	NH-9	C-6, C-7, C-10, C-11	123.9	7.1, d, 2.4	NH-9	C-6, C-7, C-10
NH-9		12.67, s	H-8	C-6, C-7, C-10		12.63, s	H-8	C-6, C-7, C-10, C-20
10	133.2				133.2			
11	168.6				168.6			
12	129.4				129.4			
NH-13		12.47, s	H-14	C-15				
14	130.3	7.01, d, 2.0	NH-13	C-11, C-12/C-21, C-15	130.3	7.05, s	N-13-Me	C-12, N-13-Me, C-15, C-16, C-11, C-19, C-21
15	119.8				119.8			
16	23.5	2.97, m	H-17	C-15, C-17	23.5	2.95, m	H-17	C-14, C-15, C-17, C-21
17	38.7	3.04, m	H-16, N-18-OH	C-15, C-16	38.7	3.02, m	H-16, N-18-OH	C-16
NH-18-OH		7.76, s	H-17	C-16, C-17		7.76, s	H-17	
19	180.4				180.4			
20	123.9				123.9			
21	125.3				125.3			
C-3-OH		9.45, br s						
N-13-Me					35.8	3.19, s	H-14	C-14

of both **10** and **11** was absent in the ^{1}H NMR spectrum of **8** and **9** and provided tentative evidence for the incorporation of the extra oxygen atom into a hydroxyl moiety. The significant upfield chemical shift of the H_2-17 multiplet ($\Delta\delta$ 0.8 ppm) and downfield ^{13}C chemical shifts of C-16 ($\Delta\delta$ 0.8 ppm), C-17 ($\Delta\delta$ 0.8 ppm), C-19 ($\Delta\delta$ 0.8 ppm), and C-21 ($\Delta\delta$ 0.8 ppm) suggested that the hydroxyl group resided on N-18. Unequivocal confirmation of an N-18 oxime functionality in **10** was provided by a COSY cross-peak between the oxime proton and the H_2-17 protons in addition to two- and three-bond HMBC correlations between the oxime proton and C-16 and C-17 (Table 2). Analogous correlations were observed in the COSY and HMBC spectra of **11**.

Natural products containing either *N*-oxide or oxime moieties are rare in the marine environment. The reversed-phase HPLC purification of **10** and **11** required aqueous methanol eluents containing 0.05% trifluoroacetic acid, and it is therefore possible that these two compounds are isolation artifacts of the naturally occurring *N*-oxides of tsitsikammamines A and B. During the isolation of two *N*-oxide metabolites from a species of *Cribrochalina* sponge,[29] nonacidic chromatography solvents were used, whereas the isolation of purealidins A[30] (containing an oxime functionality) from the sponge *Psammaplysilla* (=*Psuedoceratina*) *purpurea* necessitated the use of chromatography solvents acidified with TFA. Attempts to convert **8** to its *N*-oxide derivative via either hydrogen peroxide or *meta*-chloroperbenzoic acid proved futile, suggesting that this conversion is not facile and may be of enzymatic origin in the sponge.

The co-occurrence of the metabolites **1**, **2**, **4**, and **7** in both *Tsitsikamma* species may provide some chemotaxonomic supporting evidence for both species belonging to this genus. Interestingly, the bis-pyrroloiminoquinone metabolites **8**−**11** found in *T. favus* are absent in extracts of *T. pedunculata*. Hence, the originally proposed[1,3] chemotaxonomic significance of this rare pyrroloiminoquinone skeleton, in defining species from the genus *Tsitsikamma*, appears to be misplaced.[3] From the evidence presented here bromination at C-14 in the discorhabdin template and the hexacyclic discorhabdin V structure may be more significant chemotaxonomic markers for the genus *Tsitsikamma*. The absence of the tsitsikammamines in *T. pedunculata* together with the obvious structural similarities between the tsitsikammamines and wakayin (**23**), previously isolated from a marine ascidian,[31] also tentatively suggests the intriguing possibility of a symbiont source for these bis-pyrroloiminoquinone metabolites.

The dark green encrusting sponge, *L. bellae*, was collected by scuba (−24 m) in Algoa Bay, South Africa. Interestingly, this sponge yielded a diverse range of pyrroloiminoquinone metabolites including the new discorhabdins 1-methoxydiscorhabdin D (**12**) and 1-aminodiscorhabdin D (**13**), in addition to the five known metabolites, damirone B, makaluvamine C, makaluvic acid A, and discorhabdins G* and N. Lill et al. have proposed a biosynthetic pathway linking the damirones, makaluvamines, and discorhabdins to a common pyrroloquinoline precursor.[32] Therefore, it is not surprising that while discorhabdins are the common metabolites found in *Latrunculia* sponges and makaluvamines are more frequently isolated from *Zyzzya* species, there is some chemotaxonomic overlap between *Latrunculia* and *Zyzzya*, with both discorhabdins and makaluvamines occurring in species from both genera.[24] The co-occurrence of discorhabdins, makaluvamines, and damirones is however less common, and

Table 3. ^{1}H (DMSO-d_6, 400 MHz) and ^{13}C (DMSO-d_6, 100 MHz) NMR Data for 1-Methoxydiscorhabdin D (**12**) and 1-Aminodiscorhabdin D (**13**)

	12				**13**			
atom no.	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC
1	75.8	4.30, d, 3.3	H-2	C-3, C-5, C-1′	66.6	4.55, d, 3.5	H-2	
2	62.1	4.57, d, 3.3	H-1, H-4	C-1, C-3, C-6, C-17, C-19	65.7	4.15, d, 3.5	H-1	
3	182.5				182.5			
4	112.8	6.19, s	H-2, H-7 (lr)	C-2/C-8, C-5, C-6	112.7	6.16, s	H-7b (lr)	C-2, C-5, C-6
5	168.9				169.0			
6	45.9				46.8			
7a	35.6	2.74, d, 3.5, 12.1	H-7b, H-8	C-5, C-6, C-20	35.9	2.77, dd, 3.5, 12.1	H-7b, H-8	C-1, C-5, C-6, C-17
7b		2.59, d, 12.1	H-7a, H-8, H-4 (lr)	C-5, C-6, C-2/ C-8, C-20		2.50, m	H-4 (lr), H-7a, H-8	C-1, C-5, C-6, C-17
8	62.1	5.65, d, 2.3	H-7a, H-7b, NH-9	C-5, C-6, C-10	62.3	5.66, d, 2.5	H-7a, H-7b	C-5, C-6
NH-9		10.84, br s	H-8			10.81, br s		
10	146.7				146.7			
11	166.1				166.2			
12	123.6				123.6			
NH-13		13.16, s	H-14			13.20, s	H-14	
14	126.9	7.30, d, 2.3	NH-13, H-16	C-15, C-12, C-21	126.8	7.27, s	NH-13	C-15
15	117.6				117.6			
16	19.1	3.05, m	H-14, H-17	C-14, C-15, C-17, C-21	19.1	3.01, m		
17a	51.0	4.05, m	H-16, H-17b	C-15, C-16, C-19	50.9	4.04, m	H-16, H-17b	C-5
17b		3.83, m	H-16, H-17a	C-16, C-19		3.78, m	H-16, H-17a	
19	147.4				147.5			
20	99.3				99.9			
21	121.0				121.1			
C1′	58.3	3.50, s		C-1	NH-1′	4.10, br s		

while not previously reported from a *Latrunculia* species, these three groups of metabolites have been isolated from *Zyzzya* cf. *marsailis*.[18]

Prominent molecular and pseudomolecular ions in the FABMS spectra of **12** (*m*/*z* 366.0912, Δ −0.1 mmu) and **13** (*m*/*z* 352.0995, Δ +0.1 mmu) suggested respective molecular formulas of $C_{19}H_{16}N_3SO_3$ and $C_{18}H_{15}N_4SO_2$ for these two new compounds. The absence of the NH-18 proton signal in the ^{1}H NMR spectra of **12** and **13** and the presence of a sulfur atom, supported by the characteristic chemical shifts of the deshielded thioether bridge quaternary carbon C-5 (δ_C 169) and thiomethine carbon C-8 (δ_C 62), implied that both these metabolites shared a similar discorhabdin D,[12] as opposed to a discorhabdin V, type skeleton. Comparison of the molecular formulas of **12** and **13** with those of discorhabdin D ($C_{18}H_{13}N_3SO_2$) suggested the presence of probable methoxy and amino substituents respectively in **12** and **13** (cf. **20**). Support for a methoxy substituent in **12** was clearly evident from the ^{1}H and ^{13}C NMR spectra (δ_H 3.5, s, 3H; δ_C 58.3, q), while a three-bond HMBC correlation between H-1 (δ_H 4.30, d, J = 3.3 Hz) and the O-methyl carbon unequivocally positioned this moiety at C-1 (Table 3). Similarly, the deshielded ^{13}C chemical shift of C-1 in **13** (δ_C 66.6) and the COSY correlation (Table 3) between the deshielded H-1 methine proton (δ_H 4.55, d, J = 3.5 Hz) and the H-2 proton (δ_H 4.15, d, J = 3.5 Hz) supported the placement of the amino moiety at C-1 in this compound.

Compounds **14** (0.05%), **13** (0.04%), **17** (0.03%) **16** (0.02%), and **18** (0.01%) were the five main pyrroloiminoquinone metabolites isolated from the *L. bellae* extracts. The mass and NMR data of **14** and **16** were consistent with those reported respectively by Stierle and Faulkner for damirone B isolated from a Palauan species of the sponge *Damiria*[8] and Radisky et al. for makaluvamine C from a Fijian *Zyzzya* sp.[18] The novelty of the structures of the two discorhabdins **17** and **18** is debatable. In their recent review of bioactive marine alkaloids, Munro and co-workers[24] note that although the spectral data for discorhabdins I, J, K, L, M, N, and O have been presented at conferences,[33] they are as yet unpublished. Accordingly, we are indebted to Professor Munro for kindly supplying us with the structures and ^{1}H NMR spectra of discorhabdins A−P, with which we were able to compare the ^{1}H NMR data of **17** and **18** and the other discorhabdin metabolites that we have isolated from South African latrunculid sponges. Although the structure of **17** together with its ^{1}H NMR spectrum matched that of Munro's original discorhabdin G, the structure of this compound does not appear in the review. The coincidental naming of a metabolite as discorhabdin G (**24**) from *L. apicalis* by Yang et al.[13] has probably contributed to the confusion that surrounds discorhabdin nomenclature. Therefore, from the evidence we have in hand we believe that **17** is not a new compound, and in deference to Munro's pioneering work in latrunculid sponge chemistry, we have ascribed the name discorhabdin G* to this compound. Further confusion surrounds the novelty of **18**. The structure of **18** originally provided to us by Professor Munro contains a Δ^4 olefin which may have inadvertently been omitted from the structure of discorhabdin N represented in the later review.[24] Our ^{1}H NMR data match completely those supplied to us by Professor Munro, where there is clear evidence for this olefin functionality (δ_H 6.2, s, H-4). In the absence of fully assigned published spectral data additional confirmation of the structures of **17** and **18** was provided by recourse to HRFABMS and 2D NMR data (Table 4). Compounds **12** (0.003%) and **15** (0.002%) were the two minor metabolites isolated from the *L. bellae* extracts. The ^{1}H and ^{13}C NMR data of **15** were consistent with those reported for makalu-

Table 4. ^{1}H (DMSO-d_6, 400 MHz) and ^{13}C (DMSO-d_6, 100 MHz) NMR Data for Discorhabdin G* (**17**) and Discorhabdin N (**18**)

	17				**18**			
atom no.	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC	δ_C	δ_H (mult, *J*, Hz)	COSY	HMBC
1	145.0	7.31, d, 9.9	H-2	C-3, C-5, C-6, C-7, C-20	56.5	3.63, d, 2.8	H-2	C-2, C-2′, C-3, C-5, C-20
2	132.5	6.53, dd, 9.9, 1.3	H-1, H-4 (^4J)	C-3, C-4, C-6	63.8	4.34, d, 2.8	H-1, H-4	C-1, C-3, C-4, C-6, C-17, C-19
3	181.0				182.9			
4	120.0	6.18, d, 1.3	H-2 (^4J)	C-2, C-5, C-6	113.1	6.17, s	H-2, H-7 (lr), H-8 (lr)	C-2, C-5, C-6, C-20
5	169.6				168.6			
6	48.3				45.9			
7a	41.9	2.73, d, 11.6	H-7b, H-8	C-1, C-5, C-6, C-8, C-20	36.2	3.10, m	H-7b, H-8	C-5, C-6, C-8, C-20
7b		2.33, dd, 3.5, 11.6	H-7a, H-8	C-5, C-6, C-8, C-20		2.50, m	H-4 (lr), H-7a, H-8	C-5, C-6, C-8, C-20
8	60.3	5.69, d, 3.5	H-7a, H-7b	C-5, C-6, C-7, C-10	62.2	5.68, d, 2.3	H-4 (lr), H-7a, H-7b	C-5, C-6, C-10
NH-9		10.65, br s				10.75, br s		
10	151.0				146.2			
11	165.1				166.3			
12	123.5				123.6			
NH-13		13.27, s	H-14			13.15, s	H-14	C-12, C-15, C-21
14	127.3	7.38, s	NH-13, H-16	C-12, C-15	126.7	7.28, s	NH-13	C-11, C-12, C-15, C-19, C-21
15	120.3				117.5			
16	17.6	2.80, m	H-17	C-15, C-17, C-21	19.1	3.00, m		C-14, C-15, C-17, C-21
17a	44.6	3.89, m	H-16, NH-18	C-15, C-16, C-19	51.1	4.02, m	H-16, H-17b	C-15, C-16, C-19
17b		3.75, m	H-16, NH-18	C-15, C-16, C-19		3.79, m	H-16, H-17a	C-15, C-16, C-19
NH-18		8.34, br s	H-17					
19	154.1				147.4			
20	97.1				100.6			
21	122.9				121.1			
C2′					47.8	3.52, dd, 17.9, 31.6		C-1, C-3′
C3′					173.4			
					NH-1′	5.30, br s		

vic acid A, a pyrrolecarboxylic acid previously obtained from *Zyzzya fuliginosa* by Schmitz and co-workers.[23]

The MeOH−CH_2Cl_2 (1:1) extract of the third latrunculid sponge species to be collected from Algoa Bay, *Strongylodesma algoaensis*, showed antibacterial activity and was concentrated and partitioned between H_2O (acidified with 0.05% TFA, pH 3) and EtOAc. Lyophilization of the antibacterial, aqueous partition layer followed by chromatography on a C_{18} Sep Pak cartridge using a solvent gradient from H_2O (0.05% TFA) to MeOH gave several brightly colored fractions. Further purification of these fractions yielded discorhabdins A, D, and H and 3-dihydrodiscorhabdin C. The spectroscopic data of **19** and **20** were consistent with those previously published for these two compounds.[11,12] Both discorhabdins A and D are ubiquitous pyrroloiminoquinone metabolites occurring in the latrunculid sponge genera *Latrunculia*,[11,12] *Prianos*,[16,17] and *Zyzzya*.[18] We have also isolated **19** as the major pyrroloiminoquinone metabolite from two other South African *Latrunculia* sponges, *L. lunaviridis* and *L. microacanthoxea*.[2,34]

High-resolution FABMS data established a molecular formula of $C_{25}H_{23}N_6O_4S_2$ for **21**, and although the enhanced structural complexity of **21** (cf. **19** and **20**) was immediately apparent from the ^{1}H and ^{13}C NMR spectra of this compound, there was sufficient congruency between the NMR data of **20** and **21** to suggest that these two compounds possessed similar nonbrominated discorhabdin skeletons differing only in substitution at C-1 in **21** (δ_C 51.0, d). The ^{1}H NMR spectrum of **21** was identical to the ^{1}H NMR spectrum we had in hand of discorhabdin H previously isolated from a New Zealand latrunculid sponge,[33] which, together with a complete assignment of all the ^{1}H and ^{13}C NMR resonances from 2D NMR data, provided substantive confirmation of the structure of **21**.[35] The relative stereochemistry of four of the five chiral centers of **21** (isolated from *S. algoaensis*) was explored with a ROESY NMR experiment (CD_3OD). Both H-2 (δ_H 4.41) and H-7b (δ_H 2.71) showed strong ROESY correlations to a signal resonating at δ_H 4.0 (H-1), suggesting that these protons reside on the same side of the molecule. Further ROESY correlations from H-8 (δ_H 5.75) to both the H_2-7 methylene protons confirmed that **21** shared the same relative stereochemistry around the tetrahydrothiophene ring as **20**.[12]

The absolute stereochemistry of the chiral center in the histidine moiety of **21** was established by oxidative ozonolysis of **21** followed by esterification of the ozonolysis products with 2-propanol and acylation with pentafluoropropionic anhydride to give a reaction mixture suitable for GC analysis on a Chirasil-Val chiral capillary column. The GC chromatogram of the derivatized reaction mixture revealed one major peak (t_R = 25.66 min), which on EIMS analysis gave five prominent mass fragments (*m*/*z* 276, 262, 234, 216, and 189). The same mass fragmentation pattern was obtained from GC−MS analysis of the similarly derivatized authentic samples of D- and L-aspartic acids, which gave major peaks at t_R = 25.57 and 25.69 min in their respective GC chromatograms. Co-injection of the derivatized L-aspartic acid with the derivatized aspartic acid obtained from **3** gave a single major peak confirming the expected L- or *S*-configuration of the aspartic acid residue and thus also of the histidine moiety of discorhabdin H.

In the past, pyrroloiminoquinones have demonstrated strong cytotoxicity and were at one time considered potential candidates for the development of antitumor drugs.[11,12,14,16−18,21] The suite of pyrroloiminoquinones iso-

Table 5. Human Colon Tumor (HCT-116) Cytotoxicity Data for Compounds **1**−**20**

compound	HCT-116 (IC_{50} μM)	compound	HCT-116 (IC_{50} μM)
1	0.077	**11**	16.541
2	0.645	**12**	0.232
3	0.323	**13**	0.119
4	0.197	**14**	3.102
5	0.222	**15**	28.399
6	1.266	**16**	1.089
7	12.496	**17**	0.327
8	1.414	**18**	2.249
9	2.382	**19**	0.007
10	128.213	**20**	0.595

lated from South African latrunculid sponges gave us a unique opportunity to explore the comparative in vitro activity of these compounds against a common human colon tumor cell line, HCT-116 (Table 5). Discorhabdin A was clearly the most cytotoxic compound in the HCT-116 assay (IC_{50} 0.007 μM). Some interesting structure−activity relationships emerged from the HCT-116 data, where cytotoxicity appeared to be enhanced by the presence of a C-1 substituent (**12**, **13**, **18**, cf. **20**) and a Δ^7 olefin (**4**, **5**, cf. **3**, **2**). The contribution of the pyrroloiminoquinone core structure to cytotoxicity was exemplified by the relative inactivity of makaluvic acid A (IC_{50} 28.399 μM) and to a lesser extent damirone B (IC_{50} 3.012 μM). The inactivity of the tsitsikammamine A N-18 oxime and B N-18 oxime ((IC_{50} 128.213 and 16.541 μM, respectively) is also of interest.

The bis-pyrroloiminoquinone wakayin[32] has been shown to inhibit topoisomerase I-catalyzed relaxation of supercoiled DNA and also to be a strong DNA binder.[36] The structural homology between **23** and the tsitsikammamines suggested that compounds **8**−**11** might exhibit analogous topoisomerase I inhibition and DNA intercalation. The tsitsikammamines exhibited similar topoisomerase inhibition to wakayin, which required 10−100-fold the molar concentrations that camptothecin requires to elicit DNA cleavage through topoisomerase I inhibition.[36] The micromolar concentration (K_s) of a compound that decreases DNA-bound ethidium bromide fluorescence by 50% is routinely used to quantify DNA intercalation, and the strong intercalating ability of **23** was reflected in a K_S value of 20 μM.[36] Of the four tsitsikammamines, **8** (K_S = 15 μM) and **10** (K_S = 20 μM) exhibited similar DNA binding to wakayin, while **11** (K_S = 30 μM) and **9** (K_S = 45 μM) appeared to bind less strongly than **23** to DNA.

Experimental Section

General Experimental Procedures. Waters C_{18} Sep Pak cartridges were used for the initial chromatographic separations. High-performance liquid chromatography was performed on a Phenomenex C_{18} column (9 mm i.d., 25 cm, flow rate 3 mL min^{-1}) using a Spectra-Physics liquid chromatography pump and a Waters R401 differential refractometer. The NMR spectra were measured on a Bruker AVANCE 400 MHz spectrometer using standard pulse sequences. Chemical shifts are reported in ppm and referenced to residual undeuterated solvent resonances (CH_3OH δ_H 3.30, δ_C 49.05 and DMSO δ_H 2.50, δ_C 39.43), and coupling constants are reported in Hz. HRFABMS data were obtained by Prof. Louis Fourie of the Mass Spectrometry Unit at the University of Potchefstroom. CD spectra were recorded in methanol on a Hitachi 150-20 ORD/CD spectrometer by Professors Kobus Steenkamp and Vincent Brandt from the University of the Free State.

Animal Material. Type specimens of *T. favus* Samaai and Kelly 2002, *T. pedunculata* Samaai and Kelly 2003, *L. bellae* Samaai and Kelly 2003, and *S. algoaensis* Samaai and Kelly 2003 (Demospongiae: Poecilosclerida: Latrunculiidae) have been deposited in the Natural History Museum, London, under Holotype registration numbers BMNH 1997.7.3.2, BMNH 2003.1.10.2, BMNH 2003.1.10.1, and BMNH 1996.7.3.3, respectively.[1,2]

Extraction and Isolation. Specimens of *T. pedunculata* were collected with scuba (−40 m) from Thunderbolt Reef, Algoa Bay, South Africa (34°03′ S, 25°41′ E) during late summer 1999 and frozen directly after collection. The frozen sponge material (dry mass of sponge after extraction = 106 g) was extracted (2:1 MeOH−CH_2Cl_2) and the resultant extract sequentially partitioned with hexane, $CHCl_3$, *n*-butanol, and MeOH. Each of the partition fractions were concentrated under vacuum and examined by ^{1}H NMR spectroscopy. Initial C_{18} solid-phase extractions of both the $CHCl_3$ (3.2 g) and *n*-butanol (2.4 g) fractions using a stepwise gradient elution (H_2O−MeOH/0.05% TFA) followed by exhaustive isocratic C_{18} reversed-phase HPLC (H_2O−MeOH−TFA, 65:35:0.05, 60:40:0.05, and 55:45:0.05) yielded **1** (22 mg, 0.02%), **2** (19 mg, 0.02%), **3** (90 mg, 0.09%), **4** (1 mg, 0.001%), **5** (8 mg, 0.007%), **6** (4 mg, 0.004%), and **7** (4 mg, 0.004%).

Specimens of *T. favus* were collected with scuba (−22 m) from Rheeders Reef, Tsitsikamma National Park (34°10′ S, 25°34′ E) on the southeast coast of South Africa during late summer 1995. The frozen sponge (dry mass of sponge after extraction = 474 g) was soaked in methanol and the aqueous methanol extract partitioned between hexane, chloroform, and *n*-butanol. Each of the partition fractions was concentrated under vacuum and examined by ^{1}H NMR spectroscopy. Initial C_{18} solid-phase extractions of both the $CHCl_3$ (3.9 g) and *n*-butanol (4.6 g) fractions using a stepwise gradient elution (H_2O−MeOH−0.05% TFA) followed by exhaustive isocratic C_{18} reversed-phase HPLC (H_2O−MeOH−TFA, 72.5:27.5:0.05, 67:5:32.5:0.05, 60:40:0.05, and 55:45:0.05) yielded **2** (10 mg, 0.002%), **4** (2 mg, 0.0004%), **7** (4 mg, 0.001%), **8** (10 mg, 0.002%), **9** (37 mg, 0.008%), **10** (5 mg, 0.001%), and **11** (3 mg, 0.0006%).

Specimens of *L. bellae* were collected with scuba (−22 m) from Riy Banks, Algoa Bay, South Africa (33°59′ S, 25°51′ E) during early summer 1998. The frozen latrunculid sponge (dry mass of sponge after extraction = 138 g) was thawed and extracted three times with methanol and then once with methanol−dichloromethane (1:1). The extracts were combined, concentrated, and subjected to a modified Kupchan partition procedure to give hexane, chloroform, *n*-butanol, and aqueous methanol partition fractions. C_{18} solid-phase extraction of the chloroform and *n*-butanol fractions, followed by isocratic reversed-phase HPLC (H_2O−MeOH−TFA, 80:20:0.05, 70:30:0.05) and LH-20 (MeOH) chromatography, yielded **12** (4 mg, 0.003%), **13** (57 mg, 0.04%), **14** (70 mg, 0.05%), **15** (2.7 mg, 0.002%), **16** (23 mg, 0.02%), **17** (18 mg, 0.01%), and **18** (35 mg, 0.03%).

Specimens of *S. algoaensis* were collected with scuba (−15 m) from Algoa Bay, South Africa (33°50′ S, 25°45′ E) during autumn 1994. The freeze-dried sponge (49.5 g) was extracted with MeOH−CH_2Cl_2 (1:1) to give 5.77 g of a dark oil. The oil was partitioned between H_2O (0.05% TFA) and EtOAc to give a dark colored aqueous fraction that was concentrated and freeze-dried. A dark green powder (2.43 g) was obtained, containing a large amount of inorganic salts. Desalting and further fractionation of a portion of this crude extract (2.0 g) were achieved on a C_{18} Sep-Pak cartridge using a solvent gradient from H_2O to MeOH (containing 0.05% TFA). Eight different colored bands were collected (1−8). The fraction eluting with 40% MeOH (fraction 4) contained almost pure discorhabdin A. Fraction 3, which eluted with 30% MeOH, contained a number of interesting aromatic signals and was further purified on a C_{18} Sep Pak cartridge followed by HPLC to give **19** (131 mg, 0.32%), **20** (5 mg, 0.01%), **3** (10 mg, 0.02%), and **21** (11 mg, 0.03%).

14-Bromodiscorhabdin C (1): reddish-brown solid; NMR data consistent with published data;[3] HRFABMS *m*/*z* [M + 1] 540.8636 (calcd for $C_{18}H_{14}N_3O_2{}^{79}Br_3$, 540.8636).

14-Bromo-3-dihydrodiscorhabdin C (2): olive green solid; CD (MeOH), λ_{max} 297.0 nm ($\Delta\epsilon$ −1.27), 483.0 nm ($\Delta\epsilon$

+0.24); NMR data consistent with published data;[3] HRFABMS *m/z* [M + 1] 542.8792 (calcd for $C_{18}H_{16}N_3O_2{}^{79}Br_3$, 542.8793).

3-Dihydrodiscorhabdin C (3): red solid; NMR data consistent with published data;[26] CD (MeOH), λ_{max} 298.5 nm ($\Delta\epsilon$ −1.11), 482.0 nm ($\Delta\epsilon$ +0.11); HRFABMS *m/z* [M + 1] 464.9687 (calcd for $C_{18}H_{17}N_3O_2{}^{79}Br_2$, 464.9688).

3-Dihydro-7,8-dehydrodiscorhabdin C (4): olive green solid; CD (MeOH), λ_{max} 291.0 nm ($\Delta\epsilon$ −2.66); ^{1}H (DMSO-d_6, 400 MHz) δ 13.23 (1H, br s, NH-13), 10.59 (1H, br s, NH-9), 8.31 (1H, br s, NH-18), 7.37 (1H, s, H-14), 6.64 (1H, s, H-1), 6.64 (1H, s, H-5), 6.33 (1H, dd, J = 4.3, 7.6 Hz, H-8), 4.72 (1H, t, J = 7.6 Hz, H-7), 4.55 (1H, s, H-3), 3.82 (2H, t, J = 7.6 Hz, H-17), 2.88 (2H, t, J = 7.6 Hz, H-16); ^{13}C (DMSO-d_6, 100 MHz) δ 158.0 (C-19, s), 132.1 (C-1, d), 132.1 (C-5, d), 126.2 (C-14, d), 122.5 (C-12, s), 122.2 (C-8, d), 122.2 (C-2, s), 122.2 (C-4, s), 119.7 (C-15, s), 112.5 (C-7, d), 68.7 (C-3, d), 45.4 (C-6, s), 44.0 (C-17, t), 17.7 (C-16, t); HRFABMS *m/z* [M + 1] 462.9532 (calcd for $C_{18}H_{15}N_3O_2{}^{79}Br_2$, 462.9531).

14-Bromo-3-dihydro-7,8-dehydrodiscorhabdin C (5): green solid; CD (MeOH), λ_{max} 263.5 ($\Delta\epsilon$ −2.21), 297.5 nm ($\Delta\epsilon$ −1.64), 478.0 nm ($\Delta\epsilon$ +0.57); ^{1}H (DMSO-d_6, 400 MHz) δ 14.13 (1H, br s, NH-13), 10.64 (1H, br s, NH-9), 8.62 (1H, br s, NH-18), 6.52 (1H, s, H-1), 6.52 (1H, s, H-5), 6.25 (1H, dd, J = 4.8, 7.3 Hz, H-8), 4.82 (1H, t, J = 7.3 Hz, H-7), 4.59 (1H, s, H-3), 3.90 (2H, t, J = 7.6 Hz, H-17), 2.78 (2H, t, J = 7.6 Hz, H-16); ^{13}C (DMSO-d_6, 100 MHz) δ 165.2 (C-11, s), 156.4 (C-19, s), 144.2 (C-10, s), 133.9 (C-1, d), 133.9 (C-5, d), 124.5 (C-2, s), 124.5 (C-4, s), 122.6 (C-21, s), 122.5 (C-8, d), 122.4 (C-12, s), 119.1 (C-15, s), 114.6 (C-7, d), 111.6 (C-14, s), 97.3 (C-20, s), 71.0 (C-3, d), 44.7 (C-6, s), 44.3 (C-17, t), 17.3 (C-16, t); HRFABMS *m/z* [M + 1] 540.8635 (calcd for $C_{18}H_{14}N_3O_2{}^{79}Br_3$, 540.8636).

Discorhabdin V (6): dark green solid; CD (MeOH), λ_{max} 350.5 nm ($\Delta\epsilon$ +2.58), 403.5 nm ($\Delta\epsilon$ −1.89); ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 1; HRFABMS *m/z* [M + 1] 387.0584 (calcd for $C_{18}H_{18}N_3O_2{}^{79}Br$, 387.0582).

14-Bromo-1-hydroxydiscorhabdin V (7): brown solid; CD (MeOH), λ_{max} 290.5 nm ($\Delta\epsilon$ +0.33), 339.5 nm ($\Delta\epsilon$ −0.42); ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 1; HRFABMS *m/z* [M + 1] 480.9638 (calcd for $C_{18}H_{17}N_3O_3{}^{79}Br_2$, 480.9637).

Tsitsikammamine A (8): reddish-brown solid; NMR data consistent with published data;[3] HRFABMS *m/z* [M$^+$] 304.1087 (calcd for $C_{18}H_{14}N_3O_2$, 304.1086).

Tsitsikammamine B (9): dark red solid; NMR data consistent with published data;[3] HRFABMS *m/z* [M + 1] 319.1319 (calcd for $C_{19}H_{17}N_3O_2$, 319.1321).

Tsitsikammamine A N-18 oxime (10): bright orange-red solid; ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 2; HRFABMS *m/z* [M + 1] 321.1112 (calcd for $C_{18}H_{15}N_3O_3$, 322.1113).

Tsitsikammamine B N-18 oxime (11): bright orange-red solid; ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 2; HRFABMS *m/z* [M + 1] 335.1273 (calcd for $C_{19}H_{17}N_3O_3$, 336.1270).

1-Methoxydiscorhabdin D (12): brown solid; CD (MeOH), λ_{max} 311.0 nm ($\Delta\epsilon$ +3.75), 361.5 nm ($\Delta\epsilon$ −8.09); ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 3; HRFABMS *m/z* [M$^+$] 366.0911 (calcd for $C_{19}H_{16}N_3SO_3$, 366.0912).

1-Aminodiscorhabdin D (13): dark green solid; CD (MeOH), λ_{max} 353.6 nm ($\Delta\epsilon$ −0.10); ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 3; HRFABMS *m/z* [M + 1] 352.0995 (calcd for $C_{18}H_{16}N_4SO_2$, 352.0994).

Damirone B (14): reddish-brown solid; NMR data consistent with published data;[8] HRFABMS *m/z* [M$^+$] 203.0821 (calcd for $C_{11}H_{11}N_2O_2$, 203.0821).

Makaluvic acid A (15): brown solid; NMR data consistent with published data;[23] HRFABMS *m/z* [M + 1] 195.0760 (calcd for $C_9H_{11}N_2O_3$, 195.0770).

Makaluvamine C (16): reddish-brown solid; NMR data consistent with published data;[18] HRFABMS *m/z* [M$^+$] 202.0979 (calcd for $C_{11}H_{12}N_3O$, 202.0980).

Discorhabdin G* (17): dark yellow-brown solid; CD (MeOH), λ_{max} 306.5 nm ($\Delta\epsilon$ +0.95), 357.5 nm ($\Delta\epsilon$ −2.80); ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 4; HRFABMS *m/z* [M$^+$] 336.0809 (calcd for $C_{18}H_{14}N_3SO_2$, 336.0807).

Discorhabdin N (18): reddish-brown solid; CD (MeOH), λ_{max} 349.0 nm ($\Delta\epsilon$ −0.43), 357.5 nm ($\Delta\epsilon$ +2.80); ^{1}H and ^{13}C NMR data (400/100 MHz, DMSO-d_6), see Table 4; HRFABMS *m/z* [M$^+$] 409.0971 (calcd for $C_{20}H_{17}N_4SO_4$, 409.0971).

Discorhabdin A (19): dark green solid; CD (MeOH), λ_{max} 271.0 nm ($\Delta\epsilon$ −1.86), 335.2 nm ($\Delta\epsilon$ +2.42); NMR data consistent with published data;[11] HRFABMS *m/z* 416.0072 [M + 1] (calcd for $C_{18}H_{15}{}^{79}Br\ N_3O_2S$, 416.0068).

Discorhabdin D (20): dark green solid; CD (MeOH), λ_{max} 265.1 nm ($\Delta\epsilon$ −6.52), 364.6 nm ($\Delta\epsilon$ +5.70); NMR data consistent with published data;[12] LRMS *m/z* [M + 1] 436.4.

Discorhabdin H (21): dark green solid; CD (MeOH), λ_{max} 270.4 ($\Delta\epsilon$ −2.71), 300.4 nm ($\Delta\epsilon$ −3.99), 358.4 nm ($\Delta\epsilon$ +8.59); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.95 (1H, s, H-2′), 7.09 (1H, s, H-14), 6.19 (1H, s, H-4), 5.66 (1H, d, J = 3.6 Hz, H-8), 4.41 (1H, d, J = 3.2 Hz, H-2), 4.12 (1H, d, J = 3.2 Hz, H-1), 4.10 (1H, m, H-7′), 3.98 (1H, m, H-17a), 3.88 (1H, m, H-17b), 3.75 (3H, s, H-9′), 3.34 (1H, m H-6′), 3.25 (1H, m H-7a), 3.10 (2H, m, H-16), 2.80 (1H, d, J = 12.2 Hz, H-7b); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.2 (1H, br s, NH-13), 11.0 (1H, br s, NH-9), 7.84 (1H, s, H-2′), 7.29 (1H, s, H-14), 6.29 (1H, s, H-4), 5.75 (1H, d, 3.6, H-8), 4.41 (1H, d, 3.2, H-2), 4.00 (1H, m, H-7′), 4.00 (1H, m, H-17a), 4.00 (1H, m, H-1), 3.8 (1H, m, H-17b), 3.64 (3H, s, H-9′), 3.25 (2H, m, H-6′), 3.10 (2H, m, H-16), 3.05 (1H, d, 12.2, H-7a), 2.71 (1H, d, 12.2, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.4 (s, C-3), 169.6 (s, C-8′), 169.0 (s, C-5), 166.0 (s, C-11), 147.6 (s, C-19), 146.6 (s, C-10), 139.3 (d, C-3′), 129.7 (s, C-4′), 127.0 (s, C-5′), 127.0 (d, C-14), 123.6 (s, C-12), 121.2 (s, C-21), 117.7 (s, C-15), 113.2 (d, C-4), 99.6 (s, C-20), 64.9 (d, C-2), 62.5 (d, C-8), 51.0 (t, C-17), 51.0 (d, C-1), 48.9 (d, C-7′), 45.2 (s, C-6), 37.6 (t, C-7), 32.0 (q, C-9′) 24.5 (t, C-6′), 19.2 (t, C-16); HRFABMS *m/z* 535.1226 [M]$^+$ (calcd for $C_{25}H_{23}N_6O_4S_2$ 535.1222).

Data on the Absolute Stereochemistry of Discorhabdin H by Ozonolysis. Discorhabdin H (400 μg) in MeOH (400 μL) was cooled to −70 °C, and a stream of ozone was bubbled into the cooled solution (30 min). The solution was allowed to warm to room temperature, and hydrogen peroxide (200 μL, 50%) was added and allowed to react over 1 h. Excess reagent was removed under a stream of nitrogen, and the reaction products were then taken up in 5.0 N HCl (500 μL) and heated to 100 °C in a sealed Reactivial (16 h). The solvent was removed in a stream of dry nitrogen with heating, followed by further drying under a high vacuum. To the hydrolysate was added 2-propanol (400 μL) and acetyl chloride (100μL) in a 1 mL thick-walled Reactivial, which was securely capped. The solution was heated to 100 °C for 45 min and cooled, and the solvent was removed under dry nitrogen. Pentafluoropropionic anhydride (PFPA, 400 μL) in CH_2Cl_2 (400 μL) was added to the residue, the vial capped, and the solution heated (100 °C, 15 min). The solvent was removed under a stream of dry N_2, and the residue was dissolved in CH_2Cl_2 (100 μL) and analyzed by GC−MS using an Alltech Chirasil-Val capillary column (0.32 × 25 m). The oven temperature was ramped from 50 to 200 °C at 3 °C min^{-1} continuing to 210 °C at 5 °C min^{-1}, and a mass range of 50−600 Da was recorded every 1.96 s. The identity of the aspartic acid was confirmed by co-injection with a solution of authentic standard D- and L-aspartic acid, which had been derivatized in the same manner.

Human Colon Tumor (HCT-116) Cytotoxicity Assay. The HCT-116 Cytotox Assay is a useful tool to screen and identify potential anticancer compounds. The HCT-116 cell line is a robust, adherent human colon tumor cell line that has been used as a good indicator for cell cytotoxicity. The assay is routinely performed as a whole-cell in vitro bioassay using 96-well plates. Serial dilution of the sample (extracts, fractions, or pure compounds) yields IC_{50} values for each sample. HCT cells are plated and incubated overnight under standard conditions at 37 °C prior to sample addition. Etoposide (Sigma) and DMSO (sample solvent) are used as the positive and negative controls, respectively. The entire plate is serially diluted and incubated for an additional 72 h. Cell viability is

assessed at the end of this time period by the addition of a MTS/PMS solution (CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay, Promega) and incubated for an additional 3 h. MTS/PMS is a non-radioactive tetrazolium solution that is bioreduced by living cells into a medium-soluble formazan product. The quantity of formazan product measured in each well is directly proportional to the number of viable cells in that well. The entire plate is read using a standard microplate reader (absorbance set at 490 nm), and with the use of a SOFTMax analysis program, the IC_{50} values are determined from the serial dilution data.

Quantitation of DNA Cleavage. DNA cleavage assays were performed as described by Matsumoto et al.[37] Aliquot (20 μL) volumes containing Tris-HCl (50 mM, pH 7.5), KCl (85 mM), $MgCl_2$ (10 mM), EDTA (0.5 mM), bovine serum albumin (30 μg/mL), DTT (2 mM), radiolabled (500 ng) and supercoiled rf M13 mp 19 DNA, and purified topoisomerase 1 (80−120 ng) were treated with either the drug (9-aminocamptothecan) or the bis-pyrroloiminoquinone metabolite made up in DMSO and incubated at 30 °C for 30 min. The reactions were stopped by the addition of 1.5 mg/mL proteinase K (2 μL) in 0.5% SDS and incubated (37 °C, 60 min). The DNA was fractionated by electrophoresis in 0.8% agarose (containing 50 ng ethidium bromide/mL TAE) to separate nicked, linear, relaxed, and supercoiled DNA. DNA was visualized under UV light and sliced out of the gel. Gel slices were placed in scintillation vials with water (1 mL), melted in a microwave oven, and mixed with Opti-Fluor (10 mL, Packer Co.) while molten, and radioactivity was determined by standard scintillation counting. The percentage of cleaved DNA, represented by radioactivity in the combined nicked and linear bands, was determined relative to the total radioactivity in the reactions, following subtraction of radioactivity due to endogenously cleaved DNA. The concentration and average percentage cleaved band for 9-aminocamptothecan and the four bis-pyrroloiminoquinone metabolites were as follows: 9-aminocamptothecan (9 μM, 87%), **8** (500 μM, 59%), **9** (500 μM, 27%), **10** (500 μM, 45%), and **11** (500 μM, 26%).

Ethidium Bromide Displacement Assays. The K_S value is defined as the concentration of compound required to decrease DNA-bound EtBr fluorescence by 50%, and the assay has been described by Barrows et al.[38] and modified by Kokoshka et al.[36] Salmon testes sperm DNA (25 μg mL^{-1}) and ethidium bromide (0.5 μg mL^{-1}) were put in a 96-well plate in TE (100 μL). Controls consisted of ethidium bromide in TE only, DNA in TE only, and ethidium bromide + DNA in TE. Final concentrations in the wells ranged from 100, 10, 1, 0.1 to 0.01μM for each compound. Each plate consisted of $n = 4$ for each drug dose tested and $n = 8$ for the controls. The 96-well plates were read by a fluorescent plate reader. Ethidium bromide displacement was determined by subtracting the amount of background ethidium bromide fluorescence from all samples and then dividing by the amount of fluorescence in the DNA and ethidium bromide control. Compounds **8**−**11** were found to intercalate into DNA with the following K_S values: 15, 20, 30, and 45 μM, respectively.

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