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The new bioactive diterpenes cyanthiwigins E–AA from the Jamaican sponge *Myrmekioderma styx*

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Abstract—Twenty-seven diterpenes, cyanthiwigins A–AA (**1**–**27**), were isolated from the Jamaican sponge *Myrmekioderma styx*. Cyanthiwigins E–AA (**5**–**27**) are unreported natural products and their structures were elucidated by detailed analysis of ^{1}H, ^{13}C, DEPT, COSY, NOESY, HMQC and HMBC NMR spectra. Cyanthiwigins are 5,6,7-tricarbocyclic diterpenes, in which the chemical shift of the β-olefinic carbon of the cyclopentenone resonates at a low field of 195 ppm. Cyanthiwigins A (**1**), B (**2**), C (**3**) and D (**4**) were assayed against hepatitis B virus (HBV), human immunodeficiency virus (HIV-1), and *Mycobacterium tuberculosis* (Mtb). Cyanthiwigin C (**3**) exhibited activity against HBV and Mtb with an EC_{50} of 43 μg/mL and 50% inhibition at 6.25 μg/mL, respectively. Cyanthiwigin B (**2**) exhibited activity against HIV-1 with an EC_{50} of 42.1 μM. Cyanthiwigins A, C–F, I and Z (**1**, **3**–**6**, **10**, **26**) are active against human primary tumor cells (IC_{50}<18 μM) with cyanthiwigin F exhibiting the strongest activity (IC_{50} 3.1 μM).

1. Introduction

Marine secondary metabolites represent an outstanding resource for diversity in both structure and bioactivity. In our investigation of antiinfective and anticancer leads from deep reef Jamaican sponges,[1,2] we studied the chemical constituents of *Myrmekioderma styx*. Twenty-seven cyanthiwigin type diterpenes were isolated and identified. Cyanthiwigins are 5,6,7-tricarbocyclic diterpenes, of which only six were reported previously. Four cyanthiwigins were first isolated from the Jamaican sponge *Epipolasis reiswigi* by Green et al. in 1992.[3] Since they have the same tricarbocyclic skeleton as the cyanthins, metabolites from the bird's nest fungus *Cyanthus* sp.,[4] they were named cyanthiwigins A–D. Tricyclic diterpenes from the sponge *Higginsia* sp. were also reported with the same skeleton.[5] Sennett et al. reported cyanthiwigin C and two epoxide analogs from a Venezuelan sponge *Myrmekioderma styx* and their cytotoxicity against P-388 and A549 cancer cell line.[6]

2. Results and discussion

The sponge *Myrmekioderma styx* was collected in July 2000 using closed circuit rebreathers at Discovery Bay Jamaica and the freeze-dried sponge was extracted with methanol. The extract was dissolved in acetone, and subjected to silica gel vacuum-liquid chromatography followed by column chromatography, preparative thin layer chromatography and reverse phase HPLC to yield the 27 diterpenes cyanthiwigins A–AA. These diterpenes can be divided into four classes according to differences in the seven-membered ring system. The first class includes cyanthiwigins A–G (**1**–**7**), which have a C-12, 13 double bond. The second class of cyanthiwigins have an epoxy group at C-11, 12, or C-12, 13, and includes cyanthiwigins H, J, K, M–Q (**8**, **10**, **11**, **13**–**17**). The third class with a double bond between C-13 and C-14 includes cyanthiwigins R–X (**18**–**24**). The final class has an α,β-unsaturated ketone in the seven-membered ring and consists of cyanthiwigins Y–AA (**25**–**27**).

Cyanthiwigin E (**5**) was obtained as a white powder and the molecular formula $C_{20}H_{30}O_2$ was determined by HRESMS 325.2136 [M+Na] (calcd 325.2138). The ^{1}H NMR (Table 1) presented two olefinic methine protons at δ 5.31 (m) and 5.76 (s) without correlation to each other in the COSY spectrum indicating two double bonds. Two methyl protons at δ 1.12 (d, J=6.8 Hz) and 1.22 (d, J=6.8 Hz) and a methine at δ 2.75 (m, J=6.8 Hz) suggested an isopropyl

Keywords: diterpenes; *Myrmekioderma styx*; cyanthiwigin; sponge; anticancer; antituberculosis; anti-HIV-1; anti-HBV.

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Table 1. ^{1}H and ^{13}C NMR data of cyanthiwigin A, E–G (**1**, **5**–**7**)

	1		**5**		**6**		**7**	
	^{1}H (mult, *J* in Hz)[a]	^{13}C (mult)[b]	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1α		213.9 (s)		217.3 (s)	1.87 (dd, 2.8, 16.0)	43.1 (t)		211.6 (s)
1β					2.56 (d, 16.0)			
2	5.80 (s)	123.8 (d)	5.76 (s)	124.5 (d)	5.35 (br)	120.3 (d)	5.89 (s)	124.6 (d)
3		193.3 (s)		195.5 (s)		156.9 (s)		192.3 (s)
4α	2.25 (d, 9.7)	56.0 (d)	2.32 (d, 9.0)	58.6 (d)	2.13 (d, 10.8)	60.2 (d)	2.50 (d, 10.5)	55.6 (d)
5β	1.01 (m)	58.8 (d)	1.08 (m)	60.0 (d)	1.58 (dt, 2.4, 10.8)	55.6 (d)	1.34 (m)	58.4 (d)
6		35.2 (s)		37.0 (s)		38.1 (s)		37.9 (s)
7α	1.07 (m)	40.1 (t)	1.65 (dd, 4.4, 12.8)	49.5 (t)	1.95 (d, 14.4)	55.0 (t)	5.56 (d, 10.0)	141.6 (d)
7β	1.16 (m)		1.28 (m)		2.48 (d, 14.4)			
8α	1.41 (dt, 4.4, 14.0)	27.8 (t)	3.82 (brd, 9.6)	73.6 (d)		215.8 (s)	5.71 (d, 10.0)	127.6 (d)
8β	2.15 (m)							
9		51.8 (s)		54.8 (s)		54.7 (s)		54.1 (s)
10α	1.34 (m)	27.5 (t)	1.26 (m)	26.9 (t)	1.22 (m)	26.8 (t)	1.36 (m)	26.3 (t)
10β	1.75 (m)		1.19 (m)		1.81 (m)		1.73 (m)	
11α	1.96 (dd, 6.5, 14.8)	34.2 (t)	1.95 (m)	34.1 (t)	1.98 (m)	33.6 (t)	1.98 (dd, 14.8, 7.0)	33.7 (t)
11β	2.11 (m)		2.10 (t, 13.1)		2.21 (m)		2.20 (m)	
12		140.5 (s)		140.7 (s)		142.1 (s)		141.2 (s)
13	5.33 (m)	123.2 (d)	5.31 (m)	122.7 (d)	5.31 (m)	121.8 (d)	5.40 (m)	122.4 (d)
14α	1.70 (m)	43.6 (t)	1.79 (dd, 8.4, 14.8)	43.6 (t)	1.70 (m)	42.6 (t)	1.90 (dd, 8.5, 14.5)	41.8 (t)
14β	1.88 (14.1)		1.95 (m)		2.11 (m)		2.16 (m)	
15	1.72 (3H, s)	25.8 (q)	1.71 (3H, s)	25.9 (q)	1.71 (3H, s)	25.5 (q)	1.76 (3H, s)	25.4 (q)
16	0.79 (3H, s)	17.3 (q)	0.80 (3H, s)	17.9 (q)	0.67 (3H, s)	17.7 (q)	0.78 (3H, s)	20.0 (q)
17	1.03 (3H, s)	31.0 (q)	1.36 (3H, s)	28.4 (q)	1.07 (3H, s)	23.2 (q)	1.18 (3H, s)	28.7 (q)
18	2.72 (m)	32.9 (d)	2.75 (m)	33.5 (d)	2.45 (m)	30.7 (d)	2.73 (m)	34.0 (d)
19	1.22 (3H, d, 6.5)	21.24 (q)	1.22 (3H, d, 6.8)	21.03 (q)	1.12 (3H, d, 6.8)	22.03 (q)	1.33 (3H, d, 7.0)	20.63 (q)
20	1.12 (3H, d, 6.5)	22.25 (q)	1.12 (3H, d, 6.8)	22.18 (q)	0.96 (3H, d, 6.8)	22.81 (q)	1.16 (3H, d, 7.0)	23.37 (q)

[a] ^{1}H NMR at 400 MHz, referenced to residual solvent $CDCl_3$.
[b] ^{13}C NMR at 100 MHz, referenced to $CDCl_3$, multiplicities inferred from DEPT and HMQC experiment.

group. An oxygenated methine proton appeared at δ 3.82 (bd, J=9.6 Hz). The ^{13}C NMR and DEPT spectra showed 20 carbon signals, including five methyls, five methylenes, and five methines, leaving five quaternary carbons, and are in agreement with the molecular formula established by MS. A carbonyl carbon signal appeared at δ 217.3 ppm and an oxygenated methine was observed at δ 73.6 ppm. The odd number of signals in the olefinic region (δ 124.5 (C-2), 140.7 (C-11), and 122.7 (C-12)) suggested the signal at δ 195.5 (C-3) was an olefinic carbon to provide the required two double bonds. The HMBC experiment showed that the olefinic proton δ 5.76 (H-2) correlated with δ 195.5 (C-3) and the methine protons at δ 2.32 (H-4) and 2.75 (H-18) correlated with δ 195.5 (C-3) and 124.5 (C-2), confirming that the δ 195.5 signal is an olefinic carbon. The protons at δ 5.76 (H-2) and 2.32 (H-4) also correlated with carbons at δ 217.3 (C-1) and 54.8 (C-9) establishing the presence of an α,β-unsaturated ketone five-membered ring system. Detailed analyses of 2D NMR spectra (COSY, HMQC, HMBC) led to the establishment of the six and seven-membered rings. The long-range heteronuclear correlations (see Fig. 1) of the methine proton δ 2.32 (H-4) and three methyl groups at δ 0.80 (H-16), 1.35 (H-17) and 1.71 (H-15) were important for establishing the structure. A C-8–OH group was determined by a long-range correlation of H-17 (δ 1.36) with C-8 (73.6 ppm) in the HMBC spectrum. The relative stereochemistry was determined by the NOESY spectrum. NOE effects were clearly observed between H-4 (δ 2.32, d, J=9.0 Hz), H-16 (δ 0.80, 3H, s) and H-17 (δ 1.36, 3H, s) establishing the configuration of five and six-membered ring as *cis*, and that of six and seven-membered ring as *trans*. The NOE correlation between H-8 (δ 3.82, d, J=9.6 Hz) and H-17 (δ 1.36) indicated 8-OH is β in configuration. All chemical shift values for α,β protons in the rings were assigned using the NOESY spectrum (see Fig. 1). Only one of the two methyls of the isopropyl group at δ 1.22 (H-19) showed an NOE with δ 5.76 (H-2) indicating the rotation of the isopropyl was hindered. The

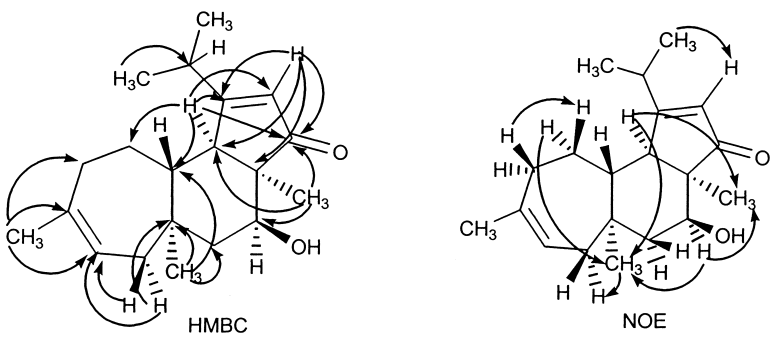
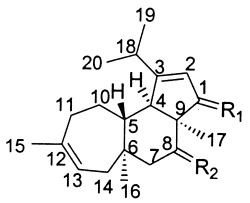
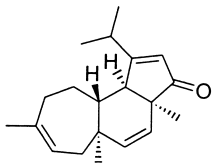


Figure 1. Important HMBC and NOE correlations.

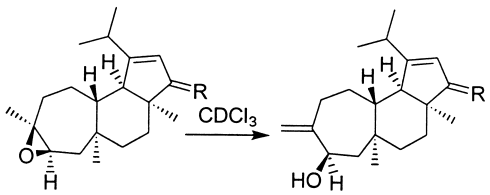
absolute stereochemistry of cyanthiwigin A (**1**) was reported as 4*S*, 6*S*, 9*S* and 5*R* by the Mosher's method.[3] In this study, cyanthiwigin E has the same positive Cotton effect in the CD spectrum as cyanthiwigin A suggesting the same absolute configuration. In general, the chemical shift of the β-carbon of a cyclopentenone is at about 160 ppm[7] with some examples resonating as far down field as 187.9 ppm.[8] The unusual chemical shift of the olefinic carbon at δ 195.5 in cyanthiwigin E is clearly attributed to the fact that it is part of an α,β-unsaturated cyclopentenone combined with the substitution effect of an isopropyl group.



cyanthiwigin A (**1**): R_1=O R_2=H
cyanthiwigin B (**2**): R_1=R_2=O
cyanthiwigin C (**3**): R_1=α-OH R_2=H
cyanthiwigin D (**4**): R_1=α-OH R_2=β-OH
cyanthiwigin E (**5**): R_1=O R_2=β-OH
cyanthiwigin F (**6**): R_1=H R_2=O

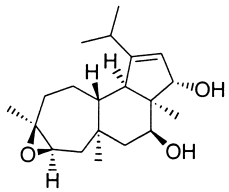


cyanthiwigin G (**7**)

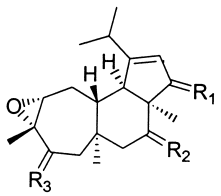


cyanthiwigin H (**8**): R=O
cyanthiwigin K (**11**): R=α-OH

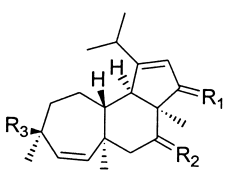
cyanthiwigin I (**9**): R=O
cyanthiwigin L (**12**): R=α-OH



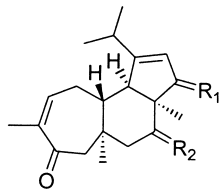
cyanthiwigin J (**10**)



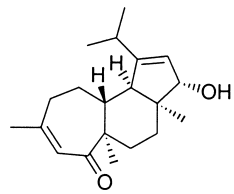
cyanthiwigin M (**13**): R_1=α-OH R_2=H R_3=β-OH
cyanthiwigin N (**14**): R_1=O R_2=O R_3=β-OH
cyanthiwigin O (**15**): R_1=O R_2=O R_3=O
cyanthiwigin P (**16**): R_1=O R_2=H R_3=β-OH
cyanthiwigin Q (**17**): R_1=O R_2=H R_3=O



cyanthiwigin R (**18**): R_1=O R_2=O R_3=OOH
cyanthiwigin S (**19**): R_1=O R_2=O R_3=OH
cyanthiwigin T (**20**): R_1=O R_2=β-OH R_3=OOH
cyanthiwigin U (**21**): R_1=O R_2=H R_3=OH
cyanthiwigin V (**22**): R_1=α-OH R_2=O R_3=OH
cyanthiwigin W (**23**): R_1=α-OH R_2=H R_3=OH
cyanthiwigin X (**24**): R_1=α-OH R_2=β-OH R_3=OH



cyanthiwigin Y (**25**): R_1=α-OH R_2=H
cyanthiwigin AA (**27**): R_1=R_2=O



cyanthiwigin Z (**26**)

Cyanthiwigin F (**6**) was isolated as a white powder and the ^{1}H and ^{13}C NMR spectra are similar to those of cyanthiwigin A (**1**). A carbonyl carbon signal appeared at δ 215.8 ppm, however the chemical shifts of the olefinic carbon C-3 (δ 156.9) and proton H-2 (δ 5.35) are shifted upfield, indicating the ketone group is not conjugating with the double bond. The proton signals at δ 1.07 (3H, s, H-17), 1.95 (d, J=14.4 Hz, H-7α) and 2.48 (d, J=14.4 Hz, H-7β) have long-range correlations with this ketone (δ 215.8 ppm) in the HMBC experiment indicating that it's position is at C-8. Absence of the C-1 ketone provided signals of a methylene that appeared at δ 1.87 (dd, J=2.8, 16.0 Hz, H-1α), 2.56 (d, J=16.0 Hz, H-1β) and δ 43.1 (C-1).

Cyanthiwigin G (**7**) is a minor component in this sponge and the ^{1}H and ^{13}C NMR spectra are similar to those of cyanthiwigin A, except for an additional pair of olefinic signals at δ 5.56 (d, J=10.0 Hz) and δ 5.71 (d, J=10.0 Hz) in the ^{1}H NMR spectrum, and δ 141.6 and 127.6 in the ^{13}C NMR spectrum. This additional double bond was assigned between C-7 and C-8 based on the long-range heteronuclear correlations between δ 0.78 (H-16) and δ 141.6 (C-7), as well as δ 1.18 (H-17) and δ 127.6 (C-8).

The second class of epoxy containing cyanthiwigins and related derivatives includes cyanthiwigins H–Q (**8**–**17**). In cyanthiwigins H, J and K (**8**, **10**, **11**), the epoxy group is between C-12 and C-13. The epoxy protons H-13 of cyanthiwigins H, J and K appeared at δ 2.73, 2.66 and 2.76, respectively, in the ^{1}H NMR spectra. The epoxy carbon C-12 appeared at δ 60.5, 59.5 and 60.6, and C-13 resonates at δ 61.0, 59.8 and 61.1 in the ^{13}C NMR spectra of cyanthiwigins H, J and K, respectively. The position of the epoxy group was determined by the HMBC experiments: H-15 (δ 1.34, 1.25, and 1.34 for cyanthiwigins H, J and K, respectively) showed correlations with epoxy carbon C-12, and C-13; H-14 (δ 1.94, 1.92 and 1.97, respectively) also correlated with C-13 and C-12. In the NOESY spectra, the epoxy proton H-13 (δ 2.73, 2.66 and 2.76, respectively) showed an NOE with H-15 (δ 1.34, 1.25, and 1.34, respectively) and H-16 (δ 0.95, 0.95 and 0.93, respectively) indicating a β configuration for the epoxy group has. Cyanthiwigin H (**8**), like **1**, **5** and **7**, has a ketone group (δ

Table 2. ^{1}H and ^{13}C NMR data of cyanthiwigin H–K (**8**–**11**)

	8		**9**		**10**[a]		**11**	
	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1β		213.4 (s)		213.8 (s)	5.05 (br)	76.9 (d)	4.79 (br)	79.1 (d)
2	5.81 (s)	124.3 (d)	5.78 (s)	123.7 (d)	5.29 (br)	126.0 (d)	5.31 (br)	125.6 (d)
3		192.9 (s)		192.6 (s)		157.2 (s)		159.9 (s)
4α	2.28 (d, 10.0)	55.8 (d)	2.24 (d, 9.7)	55.3 (d)	2.04 (o)	57.2 (d)	1.93 (d, 11.0)	55.8 (d)
5β	0.84 (m)	59.1 (d)	0.91 (m)	52.9 (d)	1.04 (m)	55.6 (d)	1.02 (m)	55.6 (d)
6		36.9 (s)		35.2 (s)		37.9 (s)		36.8 (s)
7α	1.06 (m)	39.8 (t)	1.06 (m)	40.4 (t)	1.56 (m)	47.4 (t)	1.27 (m)	38.8 (t)
7β	1.22 (m)		1.22 (m)		1.56 (m)		1.49 (m)	
8α	1.40 (m)	26.8 (t)	1.40 (m)	26.7 (t)	3.88 (t, 9.2)	72.9 (t)	1.44 (m)	28.0 (t)
8β	2.13 (m)		2.08 (m)				1.73 (m)	
9		51.9 (s)		51.5 (s)		53.1 (s)		49.0 (s)
10α	1.44 (m)	27.2 (t)	1.55 (m)	30.8 (t)	1.29 (m)	26.0 (t)	1.26 (m)	26.6 (t)
10β	1.83 (m)		1.80 (m)		1.74 (m)		1.75 (m)	
11α	1.39 (m)	35.6 (t)	2.23 (m)	32.7 (t)	1.31 (m)	35.5 (t)	1.42 (m)	35.6 (t)
11β	2.00 (m)		2.38 (m)		1.89 (m)		1.95 (m)	
12		60.5 (s)		153.8 (s)		59.5 (s)		60.6 (s)
13	2.73 (t, 8.0)	61.0 (d)	4.29 (dd, 4.4, 10.8)	71.1 (d)	2.66 (t, 7.2)	59.8 (d)	2.76 (m)	61.1 (d)
14α	1.94 (dd, 6.4, 14.4)	44.5 (t)	1.78 (dd, 4.4, 13.6)	52.4 (t)	1.19 (m)	44.7 (t)	1.97 (m)	44.8 (t)
14β	1.18 (m)		1.31 (m)		1.92 (m)		1.29 (m)	
15	1.34 (3H, s)	22.8 (q)	4.89 (s) 5.08 (s)	111.6 (t)	1.25 (3H, s)	22.1 (q)	1.34 (3H,s)	22.3 (q)
16	0.95 (3H, s)	16.3 (q)	0.89 (3H, s)	18.0 (q)	0.95 (3H, s)	15.9 (q)	0.93 (3H,s)	15.6 (q)
17	1.04 (3H, s)	31.0 (q)	1.03 (3H, s)	30.9 (q)	1.05 (3H, s)	21.3 (q)	0.90 (3H,s)	24.0 (q)
18	2.71 (m)	33.3 (d)	2.67 (m)	32.1 (d)	2.44 (m)	31.2 (d)	2.36 (m)	31.7 (d)
19	1.22 (3H, d, 6.8)	21.2 (q)	1.22 3H, (d, 6.8)	21.2 (q)	1.09 (3H, d, 6.8)	21.1 (q)	1.12 (3H, d, 7.0)	21.5 (q)
20	1.13 (3H, d, 6.8)	22.4 (q)	1.13 (3H, d, 6.8)	22.1 (q)	1.03 (3H, d, 6.8)	22.0 (q)	1.04 (3H, d, 7.0)	22.6 (q)

[a] ^{1}H and ^{13}C NMR of **10** were measured in acetone-d_6, **8**, **9** and **11** were taken in $CDCl_3$.

213.4) at C-1. A C-1-OH was apparent in cyanthiwigins J (**10**) and K (**11**) by signals of H-1 (δ 5.05, 4.79), H-2 (δ 5.29, 5.31), C-1 (δ 76.9, 79.1), C-2 (δ 126.0, 125.6), and their correlations to each other in the HMBC spectra. NOE correlations were not observed between H-1 (δ 5.05, 4.79) and H-17 (δ 1.05, 0.90), suggesting that H-1 is on the opposite side of the ring as compared with H-17 and the C-1–OH is in the same α configuration as H-17. Cyanthiwigin J (**10**) has an 8β-OH (^{1}H and ^{13}C NMR Table 2), which was determined by HMQC, HMBC and NOESY spectral data. During the acquisition of the 2D NMR spectra of cyanthiwigins H (**8**) and K (**11**), we found the proton NMR of these two compounds had changed. Integration of the epoxy proton H-13 and methyl proton H-15 showed they became smaller while the signals for two olefinic protons (δ 4.89 and 5.08 for cyanthiwigin I, δ 4.93 and 5.06 for cyanthiwigin K) and an oxygenated methine proton (δ 4.29 and 4.31, respectively) appeared. This corresponds with the rearrangement of the epoxy group to form an exocyclic double bond between C-12 and C-15, and a hydroxyl group at C-13 (cyanthiwigins I and L). Cyanthiwigins L (**12**) and I (**9**) were obtained by PTLC, their ^{13}C NMR, HMQC and HMBC agreed well with the rearranged structures and it is likely that the trace HCl in the NMR solvent chloroform catalyzed the reaction.

Cyanthiwigins M–Q (**13**–**17**) are also epoxide containing diterpenes. The methine signals at δ 2.64–3.55 in the ^{1}H NMR spectra, δ 59.8–62.2 in the ^{13}C NMR spectra, and the quaternary carbons at δ 62.6–64.7 were assigned to the epoxy groups. The position of the epoxy group was determined to be C-11 and C-12 by long-range heteronuclear correlations in the HMBC spectra: H-15 (δ 1.19, 1.23, 1.32, 1.18, 1.28, respectively) to the two epoxy carbons C-11 (δ 60.4, 59.8, 62.5, 60.2, 64.4, respectively) and C-12 (δ 62.7, 62.7, 64.7, 62.6, 64.4, respectively); epoxy protons H-11 (δ 2.64, 2.94, 3.55, 2.78, 3.37, respectively) to C-10 (δ 30.3, 31.7, 28.8, 31.6, 29.9, respectively); H-10β (δ 1.97, 2.29, 2.66, 2.13, 2.46, respectively) to the two epoxy carbons C-11 and C-12. The C-1 ketone in cyanthiwigins N–Q (**14**–**17**) and C-1 hydroxyl in cyanthiwigin M (**13**) were assigned based on the chemical shifts of H-2 and C-1 (Tables 3 and 4), and confirmed by the HMBC experiment. The H-2 proton resonating at lower field δ 5.7–5.8 (**14**–**17**) indicated a C-1 ketone group, while an H-2 at higher field δ 5.30 (**13**) suggested a C-1–OH group. Cyanthiwigins M, N and P (**13**, **14** and **16**) each has an additional hydroxyl group (δ 3.71, 3.75, 3.74 in the ^{1}H NMR spectra, δ 70.5, 70.0, 70.5 in the ^{13}C NMR spectra). These hydroxyl groups were assigned as C-13 based on the long-range heteronuclear correlations of H-15 (δ 1.19, 1.23, 1.18, respectively) and H-14α (δ 1.30, 1.27, 1.33, respectively) to C-13 (δ 70.5, 70.0, 70.5, respectively). Since H-13 (δ 3.71, 3.75, 3.74, respectively) showed correlations with H-16 (δ 0.87, 0.84, 0.92, respectively) in the NOESY spectra, the 13-OH was assigned as β in configuration. Cyanthiwigins O and Q (**15** and **17**) each has a ketone group resonating at δ 207.0 and 207.2, respectively. The ketone groups were assigned as C-13 by its long-range heteronuclear correlations to H-15 (δ 1.32, 1.28), H-14α (2.77, 2.60) and H-14β (2.01, 2.05). The C-8 ketone group was assigned in cyanthiwigins N (**14**) and O (**15**) by comparable data with cyanthiwigin G (**6**) and HMBC experiments.

The third class of cyanthiwigins possess a double bond moiety between C-13 and C-14 and include cyanthiwigins R–X (**18**–**24**). Their ^{1}H NMR spectra showed a pair of

Table 3. ^{1}H and ^{13}C NMR Data of cyanthiwigin L–O (**12**–**15**)

	12[a]		**13**		**14**		**15**	
	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1β	4.79 (br)	79.3 (d)	4.74 (br)	77.6 (d)		203.9 (s)		203.3 (s)
2	5.29 (br)	125.1 (d)	5.30 (br)	126.8 (d)	5.84 (s)	122.8 (d)	5.85 (s)	123.6 (d)
3		159.7 (s)		156.9 (s)		192.2 (s)		191.7 (s)
4α	1.87 (d, 11.0)	55.6 (d)	1.93 (d, 11.6)	54.2 (d)	2.81 (d, 10.4)	57.3 (d)	2.91 (d, 10.4)	56.1 (d)
5β	1.03 (m)	52.2 (d)	1.04 (m)	46.6 (d)	1.75 (t, 10.0)	47.4 (d)	1.50 (t, 10.0)	50.9 (d)
6		35.2 (s)		35.2 (s)		39.6 (s)		37.9 (s)
7α	1.27 (m)	39.7 (t)	1.25 (m)	39.1 (t)	1.96 (d, 13.6)	55.9 (t)	2.13 (d, 14.8)	53.1 (t)
7β	1.49 (m)		1.48 (m)		2.18 (d, 13.6)		2.23 (d, 14.8)	
8α	1.44 (m)	28.1 (t)	1.70 (m)	28.0 (t)		204.3 (s)		204.1 (s)
8β	1.73 (m)		1.45 (m)					
9		48.7 (s)		48.2 (s)		63.4 (s)		64.0 (s)
10α	1.26 (m)	31.6 (t)	1.13 (m)	30.3 (t)	1.49 (m)	31.7 (t)	1.97 (ddd, 3.9, 10.4, 16.4)	28.8 (t)
10β	1.81 (m)		1.97 (dd, 6.8, 15.6)		2.29 (dd, 6.4, 14.8)		2.66 (dd, 7.6, 16.4)	
11α	2.24 (m)	32.5 (t)	2.64 (dd, 6.8, 8.8)	60.4 (d)	2.94 (dd, 6.4, 8.8)	59.8 (d)	3.55 (dd, 3.9, 7.1)	62.5 (d)
11β	2.33 (m)							
12		154.7 (s)		62.7 (s)		62.7 (s)		64.7 (s)
13	4.31 (dd, 4.8, 10.8)	71.7 (d)	3.71 (bd, 11.6)	70.5 (d)	3.75 (bd, 10.8)	70.0 (d)		207.0 (s)
14α	1.82 (dd, 10.0, 16.0)	51.4 (t)	1.30 (m)	51.5 (t)	1.27 (m)	49.3 (t)	2.77 (overlaped)	47.5 (t)
14β	1.48 (m)		1.64 (m)		1.87 (t, 12.0)		2.01 (overlaped)	
15	4.93 (s)	112.3 (t)	1.19 (3H, s)	15.6 (q)	1.23 3H, s)	15.6 (q)	1.32 (3H, s)	16.3 (q)
	5.06 (s)							
16	0.85 (3H,s)	16.3 (q)	0.87 (3H, s)	16.1 (q)	0.84 (3H, s)	17.6 (q)	0.84 (3H, s)	19.9 (q)
17	0.88 (3H,s)	24.1 (q)	0.86 (3H, s)	24.2 (q)	1.20 (3H, s)	24.3 (q)	1.21 (3H, s)	24.6 (q)
18	2.40 (m)	31.0 (d)	2.45 (m)	30.5 (d)	2.95 (m)	30.8 (d)	3.05 (m)	31.7 (d)
19	1.13 (3H, d, 7.0)	21.5 (q)	1.13 (3H, d, 6.8)	21.3 (q)	1.31 (3H, d, 6.4)	20.9 (q)	1.29 (3H, d, 6.4)	20.9 (q)
20	1.03 (3H, d, 7.0)	22.6 (q)	1.04 (3H, d, 6.8)	21.4 (q)	1.24 (3H, d, 6.4)	21.0 (q)	1.23 (3H, d, 6.4)	21.3 (q)

[a] ^{1}H and ^{13}C NMR of **12** were measured in $CDCl_3$, **13**, **14** and **15** were taken in acetone-d_6.

coupled olefinic protons at δ 5.35–5.49 (H-13) and 5.06–5.35 (H-14) (Tables 4–6). The ^{13}C NMR and DEPT also showed a pair of methine carbons at δ 131.8–138.6 (C-13) and δ 137.8–143.3 (C-14) (Tables 4–6). The position of this double bond was determined by the long-range heteronuclear correlations of H-15 (δ 1.22–1.29) to C-13, and H-16 (δ 0.90–1.08) to C-14. Each ^{13}C NMR spectrum for these seven compounds has a quaternary oxygenated carbon signal. They appeared at approximately δ 72 ppm (Tables 4–6) for cyanthiwigins T–X, and at δ 83.3, 82.9 for cyanthiwigins R (**18**) and T (**20**). In the HMBC experiments, correlations between H-15, H-14 and these oxygenated

Table 4. ^{1}H and ^{13}C NMR Data of cyanthiwigin P–S (**16**–**19**)

	16		**17**		**18**		**19**	
	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1β		211.5 (s)		210.9 (s)		203.7 (s)		203.6 (s)
2	5.76 (s)	123.3 (d)	5.78 (s)	123.9 (d)	5.84 (s)	122.5 (d)	5.82 (s)	122.4 (d)
3		191.5 (s)		192.1 (s)		193.0 (s)		193.2 (s)
4α	2.42 (10.2)	53.7 (d)	2.53 (d, 10.4)	53.9 (d)	2.80 (d, 10.8)	58.9 (d)	2.79 (d, 9.2)	59.0 (d)
5β	0.97 (m)	48.8 (d)	0.85 (m)	51.9 (d)	2.23 (m)	49.8 (d)	2.25 (m)	50.1 (d)
6		35.5 (s)		35.5 (s)		44.2 (s)		44.1 (s)
7α	0.89 (m)	40.1 (t)	1.15 (m)	36.9 (t)	2.09 (d, 14.0)	54.8 (t)	2.06 (d, 13.2)	55.0 (t)
7β	1.30 (m)		1.29 (m)		2.25 (d, 14.0)		2.22 (d, 13.2)	
8α	1.43 (m)	26.58 (t)	1.43 (dt, 4.8, 14.0)	26.9 (t)		204.2 (s)		204.3 (s)
8β	2.10 (m)		2.13 (ddd, 2.4, 4.8, 14.4)					
9		50.8 (s)		51.1 (s)		63.4 (s)		63.4 (s)
10α	1.34 (m)	31.6 (t)	1.71 (m)	29.9 (t)	1.89 (m)	28.8 (t)	1.84 (m)	28.9 (t)
10β	2.13 (m)		2.46 (dd, 7.2, 16.0)		2.20 (m)		2.16 (m)	
11α	2.78 (dd, 6.8, 9.2)	60.2 (d)	3.37 (dd, 4.4, 6.8)	62.2 (d)	1.87 (m)	36.2 (t)	1.81 (m)	41.3 (t)
11β					2.10 (m)		1.91 (m)	
12		62.6 (s)		64.4 (s)		83.3 (s)		71.5 (s)
13	3.74 (d, 11.6)	70.5 (d)		207.2 (s)	5.49 (d, 12.8)	133.4 (d)	5.48 (d, 12.4)	137.9 (d)
14α	1.33 (m)	50.6 (t)	2.60 (d, 11.2)	50.1 (t)	5.29 (d, 12.8)	140.9 (d)	5.11 (d, 12.4)	137.8 (d)
14β	1.56 (m)		2.05 (d, 11.2)					
15	1.18 (3H, s)	15.6 (q)	1.28 (3H, s)	16.5 (q)	1.29 (3H, s)	25.6 (q)	1.29 (3H, s)	30.9 (q)
16	0.92 (3H, s)	16.6 (q)	0.89 (3H, s)	18.2 (q)	0.94 (3H, s)	20.0 (q)	0.93 (3H, s)	20.1 (q)
17	1.00 (3H, s)	30.2 (q)	1.00 (3H, s)	30.0 (q)	1.21 (3H, s)	24.0 (q)	1.21 (3H, s)	24.0 (q)
18	2.87 (m)	31.5 (d)	2.92 (m)	32.3 (d)	2.95 (m)	30.5 (d)	2.98 (m)	30.5 (d)
19	1.26 (d, 6.4)	20.8 (q)	1.25 (d, 6.8)	20.8 (q)	1.33 (d, 6.8)	21.0 (q)	1.30 (d, 6.8)	21.0 (q)
20	1.20 (d, 6.4)	21.1 (q)	1.20 (d, 6.8)	21.4 (q)	1.22 (d, 6.8)	21.4 (q)	1.21 (d, 6.8)	21.4 (q)

^{1}H and ^{13}C NMR were measured in acetone-d_6, solvent as the internal standard.

Table 5. ^{1}H and ^{13}C NMR data of cyanthiwigin T–W (**20**–**23**)

	20		**21**[a]		**22**		**23**	
	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1β		216.5 (s)		214.0 (s)	5.02 (br)	80.3 (d)	4.73 (br)	77.9 (d)
2	5.78	124.7 (d)	5.78 (s)	123.8 (d)	5.37 (br)	127.0 (d)	5.27 (br)	126.5 (d)
3		194.9 (s)		192.8 (s)		158.0 (s)		157.6 (s)
4α	2.52 (d, 9.2)	58.3 (d)	2.27 (d, 9.7)	55.7 (d)	2.44 (d, 8.4)	61.5 (d)	1.94 (d, 10.0)	55.9 (d)
5β	1.70 (m)	51.8 (d)	1,48 (dt, 2.5, 10.4)	51.6 (d)	1.99 (m)	52.2 (d)	1.48 (m)	50.5 (d)
6		41.3 (s)		39.8 (s)		44.8 (s)		39.6 (s)
7α	1.28 (m)	48.7 (t)	1.17 (m)	39.5 (t)	2.10 (d, 11.2)	54.8 (t)	1.32 (m)	38.9 (t)
7β	1.73 (m)		1.32 (m)		2.78 (d, 11.2)		1.62 (m)	
8α	3.73 (m)	72.3 (d)	1.38 (m)	26.8 (t)		211.6 (s)	1.35 (m)	28.3 (t)
8β			2.07 (m)				1.68 (m)	
9		53.5 (s)		51.2 (s)		62.4 (s)		48.6 (s)
10α	1.69 (m)	27.3 (t)	1.66 (m)	28.2 (t)	1.65 (m)	27.1 (t)	1.48 (m)	26.9 (t)
10β	2.10 (m)		1.97 (m)		2.06 (m)		1.86 (m)	
11α	1.84 (m)	36.2 (t)	1.91 (m)	41.4 (t)	1.91 (m)	43.3 (t)	1.59 (m)	42.5 (t)
11β	1.99 (m)		1.72 (m)		1.73 (m)		1.83 (m)	
12		82.9 (s)		72.9 (s)		72.9 (s)		72.0 (s)
13	5.39 (d, 12.4)	131.8 (d)	5.38 (d, 12.4)	135.8 (d)	5.49 (d, 10.4)	138.6 (d)	5.38 (d, 12.8)	136.8 (d)
14	5.35 (d, 12.4)	143.3 (d)	5.22 (d, 12.4)	142.3 (d)	5.06 (m)	138.0 (d)	5.12 (d, 12.8)	140.4 (d)
15	1.26 (3H, s)	26.0 (q)	1.29 (3H,s)	31.4 (q)	1.26 (3H,s)	30.6 (q)	1.23 (3H, s)	30.4 (q)
16	1.08 (3H, s)	19.4 (q)	0.99 (3H,s)	19.6 (q)	0.90 (3H,s)	20.4 (q)	0.95 (3H, s)	18.0 (q)
17	1.31 (3H, s)	27.1 (q)	1.02 (3H,s)	30.5 (q)	1.02 (3H,s)	17.5 (q)	0.85 (3H, s)	24.1 (q)
18	2.93 (m)	32.4 (d)	2.74 (m)	32.1 (d)	2.58 (m)	30.5 (d)	2.46 (m)	30.8 (d)
19	1.27 (d, 6.8)	20.5 (q)	1.21 (d, 6.8)	21.3 (q)	1.18 (d, 6.6)	22.4 (q)	1.10 (d, 6.8)	21.3 (q)
20	1.18 (d, 6.8)	21.5 (q)	1.13 (6.8)	22.1 (q)	1.09 (d, 6.6)	22.5 (q)	1.04 (d, 6.8)	21.8 (q)

[a] ^{1}H and ^{13}C NMR of **21** were measured in $CDCl_3$, **20**, **22** and **23** were taken in acetone-d_6.

carbons were observed indicating this oxygenation is at C-12. The molecular formula obtained from HRMS showed that the oxygenation is a hydroxyl group for cyanthiwigins S, U–X (**19**, **21**–**24**), and a hydroperoxyl group for cyanthiwigins R and T (**18**, **20**). The chemical shifts of the oxygenated carbons of cyanthiwigins R and T (**18**, **20**) were about 10 ppm lower than that of **19**, **21**–**24**, confirming the hydroperoxyl functionalities. In the NOE spectra, H-15 (δ 1.29, 1.29, 1.26, 1.29, 1.26, 1.23, 1.22, respectively) correlated with H-10α (δ 1.89, 1.84, 1.69, 1.66, 1.65, 1.48, 1.45, respectively) and H-11α (δ 1.87, 1.81, 1.84, 1.91, 1.91, 1.59, 1.62, respectively), which indicated the α configuration for these methyl groups and β configuration for these oxygenated groups. Other functional groups, including the C-1 ketone for cyanthiwigins R–U (**18**–**21**); 1α-OH for cyanthiwigins V–X (**22**–**24**); C-8 ketone for

Table 6. ^{1}H and ^{13}C NMR of cyanthiwigin X–AA (**24**–**27**)

	24		**25**		**26**		**27**	
	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1β	5.03 (br)	76.9 (d)	4.66 (br)	78.0 (d)	4.71 (br)	77.7 (d)		203.5 (s)
2	5.25 (br)	125.7 (d)	5.28 (br)	126.9 (d)	5.29 (br)	126.2 (d)	5.85 (s)	123.4 (d)
3		156.9 (s)		156.8 (s)		156.7 (s)		192.0 (s)
4	2.09 (d, 10.4)	57.9 (d)	2.08 (d, 11.2)	54.5 (d)	2.12 (d, 10.8)	53.6 (s)	2.93 (d, 10.4)	56.8 (d)
5	1.50 (m)	50.7 (d)	1.25 (m)	52.4 (d)	1.57 (m)	52.4 (d)	1.72 (m)	54.6 (d)
6		41.0 (s)		36.1 (s)		34.2 (s)		39.1 (s)
7α	1.67 (m)	47.2 (t)	1.37 (m)	36.2 (t)	1.42 (m)	46.6 (t)	2.15 (d, 14.8)	53.5 (t)
7β	1.67 (m)		1.62 (dt, 3.6, 10.4)		1.60 (m)		2.23 d, 14.8)	
8α	3.81 (dd, 5.6, 10.8)	72.9 (d)	1.46 (m)	28.8 (t)	1.47 (m)	28.0 (t)		204.4 (s)
8β			1.77 (m)		1.74 (m)			
9		52.7 (s)		48.8 (s)		48.1 (s)		64.0 (s)
10α	1.45 (m)	26.5 (t)	2.34 (m)	30.9 (t)	1.57 (m)	27.9 (t)	2.74 (m)	30.9 (t)
10β	1.86 (m)		2.43 (m)		1.77 (m)		2.78 (m)	
11α	1.62 (m)	42.4 (t)	6.51 (bd, 8.4)	142.2 (d)	2.15 (m)	37.8 (t)	6.67 (dd, 1.7, 8.4)	141.2 (d)
11β	1.82 (m)				2.33 (m)			
12		71.9 (s)		139.0 (s)		152.5 (s)		140.0 (s)
13	5.35 (dd, 0.8, 12.4)	136.8 (d)		202.4 (s)	5.71 (s)	127.2 (d)		201.3 (s)
14α	5.12 (d, 12.4)	139.6 (d)	2.56 (d, 12.2)	58.8 (t)		208.8 (s)	2.68 (d, 12.8)	56.7 (t)
14β			2.27 (d, 12.2)				2.34 (d, 12.8)	
15	1.22 (3H, s)	30.3 (q)	1.79 (3H, s)	18.8 (q)	1.83 (3H, s)	25.7 (q)	1.74 (3H, s)	18.7 (q)
16	0.97 (3H, s)	18.9 (q)	0.94 (3H, s)	18.0 (q)	1.06 (3H, s)	15.3 (q)	0.94 (3H, s)	19.6 (q)
17	1.05 (3H, s)	21.8 (q)	0.88 (3H, s)	23.7 (q)	0.88 (3H,s)	23.7 (q)	1.25 (3H, s)	24.4 (q)
18	2.49 (m)	30.5 (d)	2.31 (m)	30.8 (d)	2.43 (m)	30.0 (d)	2.94 (m)	31.6 (d)
19	1.11 (d, 6.8)	21.3 (q)	1.10 (d, 6.8)	20.2 (q)	1.07 (d, 6.4)	21.0 (q)	1.29 (d, 7.2)	20.9 (q)
20	1.05 (d, 6.8)	21.5 (q)	1.04 (d, 6.8)	21.9 (q)	1.23 (d, 6.4)	21.3 (q)	1.22 (d, 7.2)	21.5 (q)

^{1}H and ^{13}C NMR were measured in acetone-d_6, solvent as the internal standard.

Table 7. Bioactivity of cyanthiwigins

Compound	Cytotoxicity[a] IC_{50} (μM)	TB (Inh % at 6.25 μg/mL)	HBV (EC_{50}, μg/mL)	HIV (EC_{50}, μM)
Cyanthiwigin A (**1**)	6.8	25	>100	>100
Cyanthiwigin B (**2**)	NA[b]	9	>100	42.1
Cyanthiwigin C (**3**)	7.8	50	43	>100
Cyanthiwigin D (**4**)	5.0	30	–[c]	>100
Cyanthiwigin E (**5**)	9.1	–	–	–
Cyanthiwigin F (**6**)	3.1	–	–	–
Cyanthiwigin I (**10**)	18.1	–	–	–
Cyanthiwigin N (**14**)	NA	–	–	–
cyanthiwigin P (**16**)	NA	–	–	–
Cyanthiwigin Q (**17**)	NA	–	–	–
Cyanthiwigin S (**19**)	NA	–	–	–
Cyanthiwigin U (**21**)	NA	–	–	>100
Cyanthiwigin Z (**26**)	5.6	–	–	–

[a] Tumor tissues were obtained from patients undergoing surgical resections for therapeutic purposes. The tissues were minced and digested with enzymes. The tumor cells were isolated by size, density, and negative immunoselection. The compounds were tested against primary tumor cells maintained in short-term culture, and cytotoxicity was measured by Alamar Blue.
[b] NA, no activity.
[c] –, not assayed.

cyanthiwigins R, S and V (**18**, **19**, **22**); 8β-OH for cyanthiwigins T (**20**) and X (**24**), were identified using comparable data with cyanthiwigins E, F and J (**5**, **6** and **10**) and HMBC experiments.

The fourth class of cyanthiwigins have an α,β-unsaturated ketone moiety in the seven-membered ring and include cyanthiwigins Y–AA (**25**–**27**). The ^{1}H NMR data (Table 6) of cyanthiwigins Y and AA provided olefinic proton signals at δ 6.51 (bd, J=8.4 Hz) and 6.67 (dd, J=1.7, 8.4 Hz) suggesting a methylene was connected to these olefinic methines. The ^{13}C NMR (Table 6) provided α,β-unsaturated ketone signals at δ 142.2, 139.0, 202.4 ppm for cyanthiwigin Y, and δ 141.2, 140.0, 201.3 for cyanthiwigin AA. The HMBC spectra yielded correlations between H-15 (δ 1.79 and 1.74) and the α,β-unsaturated ketone carbons (C-11, 12, 13); H-14 (δ 2.56 and 2.68) and the ketone carbons (δ 202.4 and 201.3) and H-10 (δ 2.43 and 2.78) and the olefinic methine (δ 142.2 and 141.2). These correlations confirmed the ketone was at C-13 and the double bond was between C-11 and C-12.

Cyanthiwigin Z (**26**) is a minor component from this sponge. A singlet olefinic proton signal at δ 5.71 (H-13) in the ^{1}H NMR spectrum suggested a double bond in the seven-membered ring. The signals at δ 152.5 and 127.2 ppm in the ^{13}C NMR spectra could be assigned to this double bond. The signal at δ 208.8 ppm is clearly a carbonyl carbon. The long-range heteronuclear correlations in the HMBC spectrum between δ 1.83 (H-15) and 152.5 and 127.2, and 5.71 (H-13) and 34.2 (C-6) suggested the double bond existed between C-12 and C-13. The correlations of δ 1.06 (H-16) and 5.71 (H-13) with δ 208.8 indicated the carbonyl group was at C-14.

The structures of previously reported cyanthiwigins A–D were determined by ^{1}H, ^{13}C, and 2D NMR and comparison with the literature data.[3]

Thirteen cyanthiwigins were tested for cytotoxicity against human primary tumor cells (Table 7). Cyanthiwigins A, C–F, I and Z (**1**, **3**–**6**, **10**, and **26**) showed activity against human primary tumor cells (IC_{50} 3.1–18.1 μM), while cyanthiwigins B, N, P, Q, S and U (**2**, **14**, **16**, **17**, **19**, and **21**) are not active (IC_{50}>30 μM). The three cyanthiwigins with a single oxygen (**1**, **3** and **6**) are all active, and cyanthiwigin F (**6**) with a C-8 ketone group is the most active lead (IC_{50} 3.1 μM). Most cyanthiwigins with two oxygen moieties (**4**, **5** and **26**) are active, while the cyanthiwigins with three oxygen moieties (**16**, **17**, **19** and **14**) are generally inactive. The double bond at C-12, 13 may also be necessary for activity and transformation of this double bond to an epoxide or moving it to C-13, 14 (**21**) diminished the activity.

Cyanthiwigins A (**1**), B (**2**), C (**3**) and D (**4**) were assayed for hepatitis B virus (HBV), human immunodeficiency virus (HIV-1), and *Mycobacterium tuberculosis* (Mtb) (Table 7). Cyanthiwigin B exhibited moderate activity against HIV-1 with an EC_{50} of 42.1 μM, while cyanthiwigins A, C and D did not show activity. Only cyanthiwigin C exhibited marginal activity against HBV and Mtb with an EC_{50} 43 μg/mL and 50% inhibition at 6.25 μg/mL, respectively. Like cytotoxicity, it also appears more oxidation yields less activity against HBV and Mtb. Cyanthiwigin C (**3**) has only one hydroxyl group at C-1 and showed the strongest activity against HBV and Mtb. Oxidation of the hydroxyl group to ketone (**1**) or introduction of a hydroxyl group at C-8 (**4**) decreases the activity against HBV and Mtb. The C-8 ketone group seems important to anti-HIV-1 activity since only cyanthiwigin B (**2**), which contains a C_8-ketone, exhibited anti-HIV-1 activity while cyanthiwigins A (**1**), C (**3**) and D (**4**) were inactive.

Cyanthiwigins A (**1**) and B (**2**) were also examined for antimalarial activity and both compounds were shown to be inactive.

3. Experimental

3.1. General experiment procedures

IR and UV spectra were obtained using an AATI Mattson Genesis Series FTIR and a Hewlett-Packard 8452A Diode Array spectrometer. Optical rotations were measured with a

JASCO DIP-370 digital polarimeter. CD spectra were recorded on a JASCO J-715 spectropolarimeter. NMR spectra were measured on Bruker Avance DRX-400 and 500 spectrometers. ^{1}H and ^{13}C NMR spectra were measured and reported in ppm by using the residual solvent peak as an internal standard. ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. HPLC was carried out on a Waters 510 model system, column 1: Phenomenex Ultracarb, 5 μm, ODS 30, 250×21.5 mm, column 2: Luna C8 (2), 5 μm, 250×21.5 mm; flow rate, 6 mL/min; detector wavelength, 240 nm.

3.2. Sponge collection and taxonomy

The sponge was collected from caves and vertical coral walls, between −50 and 60 m of depth, at Discovery Bay, Jamaica, on 13 July 2000. The sponge forms lobes arising from a semispherical mass and has a deeply grooved surface. The texture is tough but slightly elastic. The color in life is orange with a light orange interior. The skeleton consists of fine widely spaced tracts of oxea and large trichodragmata, with a surface crust of acanthoxea. The sponge is *Myrmekioderma styx* de Laubenfels, 1953 (Order: Halichondrida; Family: Desmoxyiidae). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 2001.7.20.2).

3.3. Extraction and isolation

1130 g of freeze-dried sponge *Myrmekioderma styx* was extracted three times with 2000 mL of MeOH in a blender. The combined extracts were concentrated in vacuo until dried. The residue (216 g) was then extracted with 3×150 mL of acetone. The acetone soluble part (40 g) was subjected to vacuum liquid chromatography using 1400 g of silica gel and eluting with chloroform; chloroform/acetone (95:5) and finally chloroform/acetone (90:10) to give 11 fractions (fr. 1–11).

Fraction 3 (3.12 g) was chromatographed using a silica gel column eluted with hexane/EtOAc (90:10) to obtain four fractions (fr. 3-1 to fr. 3-4). The fr. 3-3 was concentrated and crystallized in MeOH to obtain cyanthiwigin A (**1**, 15 mg). The fr. 3-4 was concentrated to yield 410 mg of cyanthiwigin C (**3**). Fraction 3-1 was rechromatographed using reverse phase HPLC (column 1, eluting with a gradient of acetonitrile/H_2O from 85 to 100% over 40 min) to obtain cyanthiwigin F (**6**, 7 mg).

Fraction 4 (10 g) was chromatographed using a silica gel column (300 g) and eluted with hexane/EtOAc (85:15) to obtain five fractions (fr. 4-1 to fr. 4-5). Fraction 4-1 was concentrated and crystallized in MeOH to yield cyanthiwigin A (**1**, 532 mg). Fraction 4-2 was purified with sephadex LH-20 eluting with acetone and then chromatographed using HPLC (column 2, gradient elution of 85–100% MeOH in H_2O over 40 min) to give cyanthiwigin G (**7**, 1.2 mg). Fraction 4-4 was concentrated and crystallized in hexane to yield cyanthiwigin B (**2**, 268 mg). Fraction 4-3 was rechromatographed using HPLC (column 2, 85–100% MeOH in H_2O over 40 min) to give cyanthiwigin E (**5**, 12.7 mg) and another fraction. Cyanthiwigins H and K (**8**, 1.1 mg; **11**, 2.1 mg) were obtained from this fraction by silica gel preparative thin layer chromatography ($CHCl_3$/EtOAc 9:1). After NMR measurement of cyanthiwigins H and K, the samples were recovered and separated by preparative thin layer chromatography (silica gel, hexane/EtOAc 7:3) to obtain cyanthiwigins H, I, K, and L (**8**, **9**, **11**, and **12**; 0.3, 0.6, 0.6, and 1.1 mg, respectively).

Fraction 5 (2.6 g) was subjected to silica gel (100 g) column chromatography eluted with hexane/EtOAc 80:20, to give six fractions (fr. 5-1 to fr. 5-6). Fraction 5-2 was chromatographed using HPLC (column 2, gradient elution of 60–100% acetonitrile in H_2O over 40 min) to give seven fractions (fr. 5-2-1 to fr. 5-2-7). Fraction 5-2-5 and 5-2-7 yielded cyanthiwigins Q (**17**, 7.8 mg) and Y (**25**, 3.7 mg). Cyanthiwigins V (**22**, 0.9 mg) and M (**13**, 1.1 mg) were obtained from fr. 5-2-2 after purification on PTLC (silica gel, $CHCl_3$/EtOAc 8:2). Fraction 5-2-6 was separated again on silica gel preparative thin layer chromatography (hexane/EtOAc 75:25) to yield cyanthiwigin Z (**26**, 0.3 mg). Fraction 5-3 was rechromatographed using reverse phase HPLC (column 2, 50–100% acetonitrile in H_2O over 40 min) to give eight fractions (fr. 5-3-1 to fr. 5-3-8). Fraction 5-3-3 yielded cyanthiwigin P (**16**, 9.6 mg). Fraction 5-3-5 gave cyanthiwigin W (**23**, 5 mg) after purification using preparative thin layer chromatography (silica gel, $CHCl_3$/EtOAc 9:1). Fraction 5-4 was chromatographed using HPLC using the same condition as fraction 5-3 to give six fractions (fr. 5-4-1 to fr. 5-4-6). Cyanthiwigin U (**21**, 21 mg) was obtained from fr. 5-4-6, and cyanthiwigin T (**20**, 0.2 mg) was obtained from fr. 5-4-3 by preparative thin layer chromatography (silica gel, $CHCl_3$/acetone 9:1). Fraction 5-5 was also chromatographed using reverse phase HPLC in the same condition as fr. 5-3 to give seven fractions (fr. 5-5-1 to fr. 5-5-7). Fraction 5-5-4 was purified again on HPLC using the same condition to obtain cyanthiwigin R (**18**, 5.3 mg). Cyanthiwigins N (**14**, 2.9 mg) and X (**24**, 1.1 mg) were obtained from fr. 5-5-2 and 5-5-3 by further purification using preparative thin layer chromatography (silica gel, $CHCl_3$/acetone 85:15). Fraction 5-5-5 was chromatographed again using HPLC to give three fractions (fr. 5-5-5-1 to fr. 5-5-5-3). Cyanthiwigin O (**15**, 0.5 mg) was obtained from fr. 5-5-5-1 using preparative thin layer chromatography (silica gel, hexane/EtOAc 7:3). Cyanthiwigins J, S and AA (**10**, **19** and **27**; 3.7, 3.2 and 1.7 mg, respectively) were obtained from fr. 5-5-5-2 using preparative thin layer chromatography (silica gel, $CHCl_3$/EtOAc 9:1).

Fraction 6 (1.3 g) was first chromatographed using a silica gel column with chloroform/acetone (90:10) as the eluting solvent, and then again chromatographed using hexane/acetone (75:25) to yield cyanthiwigin D (**4**, 42.5 mg).

3.3.1. Cyanthiwigin A (1). Colorless crystal; HRESIMS 287.2339 [M+H] (calcd for $C_{20}H_{31}O$ 287.2374); $[\alpha]_D$= +46° (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=10,756); IR (film) ν 2960, 2917, 1700, 1594, 1444, 1375, 1261, 1041 cm^{-1}; CD $[\theta]_{248}$=+41,544, $[\theta]_{327}$=−8721 (*c* 8.74×10^{-5}, MeOH); ^{1}H and ^{13}C NMR see Table 1.

3.3.2. Cyanthiwigin B (2). Colorless crystal; HRESIMS

301.2153 [M+H] (calcd for $C_{20}H_{29}O_2$ 301.2167); $[\alpha]_D$=−125° (*c* 0.10, MeOH); UV λ_{max} (nm) 234 (ε=10,769); IR (film) ν 2967, 2915, 2852, 1720, 1689, 1598, 1448, 1376, 1255 cm^{-1}; CD $[\theta]_{230}$=+57,085, $[\theta]_{238}$=+49,947 $[\theta]_{296}$=−75,390 (*c* 3.33×10^{-5}, MeOH).

3.3.3. Cyanthiwigin C (3). Colorless crystal; HRESIMS 311.2341 [M+Na] (calcd for $C_{20}H_{32}ONa$ 311.2351); $[\alpha]_D$=+37.5° (*c* 0.12, MeOH); UV λ_{max} (nm) 208 (ε=98,183); IR (film) ν 3270, 2960, 2915, 2873, 1440, 1375, 1270, 1074, 1018, 835 cm^{-1}; CD $[\theta]_{219}$=+3018, $[\theta]_{241}$=−2923 (*c* 1.04×10^{-4}, MeOH).

3.3.4. Cyanthiwigin D (4). Colorless crystal; HRESIMS 327.2257 [M+Na] (calcd for $C_{20}H_{32}O_2Na$ 327.2300); $[\alpha]_D$=+25° (*c* 0.12, MeOH); UV λ_{max} (nm) 208 (ε=8087); IR (film) ν 3396, 2960, 2921, 2854, 1456, 1376, 1255, 1068, 1024 cm^{-1}; CD $[\theta]_{219}$=+4705, $[\theta]_{266}$=−1991 (*c* 9.87×10^{-5}, MeOH).

3.3.5. Cyanthiwigin E (5). White powder; HRESIMS 325.2136 [M+Na] (calcd for $C_{20}H_{30}O_2Na$ 325.2143); $[\alpha]_D$=+90° (*c* 0.10, MeOH); UV λ_{max} 238 (ε=5903); IR (film) ν 3434, 2964, 2925, 2869, 1680, 1596, 1454, 1378, 1253, 1031, 862, 746 cm^{-1}; CD $[\theta]_{250}$=+8694, $[\theta]_{333}$=−4559 (*c* 9.11×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 1.

3.3.6. Cyanthiwigin F (6). Colorless crystal; HRESIMS 309.2202 [M+Na] (calcd for $C_{20}H_{30}ONa$ 309.2194); $[\alpha]_D$=−128° (*c* 0.03, MeOH); UV λ_{max} (nm) 216 (ε=2437); IR (film) ν 2960, 2925, 2869, 1700, 1446, 1378, 1286, 1122, 1072, 809 cm^{-1}; CD $[\theta]_{214}$=+4477, $[\theta]_{227}$=−14,273 $[\theta]_{292}$=−28,735 (*c* 8.74×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 1.

3.3.7. Cyanthiwigin G (7). White powder; HRESIMS 307.2047 [M+Na] (calcd for $C_{20}H_{28}ONa$ 307.2038); $[\alpha]_D$=−9° (*c* 0.10, MeOH); UV λ_{max} (nm) 236 (ε=7343); IR (film) ν 2962, 2925, 2869, 1702, 1598, 1456, 1376, 1263, 870, 763 cm^{-1}; CD $[\theta]_{225}$=−11,233, $[\theta]_{234}$=−10,611, $[\theta]_{336}$=−2396 (*c* 8.80×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 1.

3.3.8. Cyanthiwigin H (8). White powder; HRESIMS 325.2138 [M+Na] (calcd for $C_{20}H_{30}O_2Na$ 325.2143); $[\alpha]_D$=+37° (*c* 0.03, MeOH); UV λ_{max} (nm) 236 (ε=16,280); IR (film) ν 2963, 2924, 1705, 1598, 1378 cm^{-1}; CD $[\theta]_{236}$=+62,361, $[\theta]_{287}$=+13,606, $[\theta]_{334}$=−4513 (*c* 2.48×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 2.

3.3.9. Cyanthiwigin I (9). White powder; HRESIMS 325.2137 [M+Na] (calcd for $C_{20}H_{30}O_2Na$ 325.2143); $[\alpha]_D$=+17° (*c* 0.06, MeOH); UV λ_{max} (nm) 238 (ε=11,164); IR (film) ν 3300, 2958, 2921, 1701, 1598, 1455, 1381, 1261, 1175 cm^{-1}; CD $[\theta]_{236}$=+68,623, $[\theta]_{281}$=+4931, $[\theta]_{330}$=−5193 (*c* 4.96×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 2.

3.3.10. Cyanthiwigin J (10). White powder; HRESIMS 343.2236 [M+Na] (calcd for $C_{20}H_{32}O_3Na$ 343.2249), 663.4631 [2M+Na] (calcd for $C_{40}H_{64}O_6Na$ 663.4601); $[\alpha]_D$=+50° (*c* 0.11, MeOH); UV λ_{max} (nm) 208 (ε=6550); IR (film) ν 3384, 2962, 2923, 2863, 1457, 1378, 1253, 1024, 734 cm^{-1}; CD $[\theta]_{210}$=+21,666, $[\theta]_{230}$=+1744, $[\theta]_{281}$=−1655 $[\theta]_{340}$=−1468 (*c* 8.59×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 2.

3.3.11. Cyanthiwigin K (11). White powder; HRESIMS 327.2297 [M+Na] (calcd for $C_{20}H_{32}O_2Na$ 327.2300); $[\alpha]_D$=33° (*c* 0.06, MeOH); UV λ_{max} (nm) 206 (ε=14,482); IR (film) ν 2958, 2922, 1597, 1459, 1376 cm^{-1}; CD $[\theta]_{204}$=+38,205, $[\theta]_{291}$=+7131 (*c* 4.93×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 2.

3.3.12. Cyanthiwigin L (12). White powder; HRESIMS 327.2341 [M+Na] (calcd for $C_{20}H_{32}O_2Na$ 327.2300); $[\alpha]_D$=+14° (*c* 0.11, MeOH); UV λ_{max} (nm) 206 (ε=10,236); IR (film) ν 3374, 2959, 2920, 1598, 1454, 1381 cm^{-1}; CD $[\theta]_{204}$=+49,216, $[\theta]_{290}$=+2744 (*c* 9.05×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 3.

3.3.13. Cyanthiwigin M (13). White gum; HRESIMS 343.2237 [M+Na] (calcd for $C_{20}H_{32}O_3Na$ 343.2249), 663.4705 [2M+Na] (calcd for $C_{40}H_{64}O_6Na$ 653.4600); $[\alpha]_D$=+45° (*c* 0.10, MeOH); UV λ_{max} (nm) 206 (ε=5540); IR (film) ν 3384, 2962, 2923, 2869, 1456, 1380, 1272, 1068, 1010, 887, 734 cm^{-1}; CD $[\theta]_{218}$=+3119, $[\theta]_{225}$=−2650 $[\theta]_{241}$=−3462 $[\theta]_{260}$=−3356 (*c* 7.81×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 3.

3.3.14. Cyanthiwigin N (14). Colorless crystal; HRESIMS 355.1876 [M+Na] (calcd for $C_{20}H_{28}O_4Na$ 355.1885), 687.3937 [2M+Na] (calcd for $C_{40}H_{56}O_8Na$ 687.3872); $[\alpha]_D$=−102° (*c* 0.10, MeOH); UV λ_{max} (nm) 236 (ε=7181); IR (film) ν 3440, 2967, 2929, 1724, 1685, 1602, 1452, 1386, 1261, 1047, 1008, 867 cm^{-1}, 732; CD $[\theta]_{237}$=+18,397, $[\theta]_{298}$=−14,792 (*c* 7.53×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 3.

3.3.15. Cyanthiwigin O (15). Colorless crystal; HRESIMS 353.1723 [M+Na] (calcd for $C_{20}H_{26}O_4Na$ 353.1728), 683.3627 [2M+Na] (calcd for $C_{40}H_{52}O_8Na$ 683.3560); $[\alpha]_D$=−142° (*c* 0.05, MeOH); UV λ_{max} (nm) 236 (ε=11,422); IR (film) ν 2969, 2929, 2869, 1722, 1699, 1600, 1452, 1369, 1261, 1172, 1105, 1047, 844 cm^{-1}; CD $[\theta]_{229}$=+20,127, $[\theta]_{236}$=+19,742, $[\theta]_{304}$=−22,730 (*c* 3.79×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 3.

3.3.16. Cyanthiwigin P (16). White powder; HRESIMS 341.2079 [M+Na] (calcd for $C_{20}H_{30}O_3Na$ 341.2092), 659.4354 [2M+Na] (calcd for $C_{40}H_{60}O_6Na$ 659.4288); $[\alpha]_D$=+50° (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=6681); IR (film) ν 3401, 2966, 2925, 1701, 1598, 1454, 1380, 1265, 865, 734 cm^{-1}; CD $[\theta]_{237}$=+22,483, $[\theta]_{326}$=−4818 (*c* 7.86×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 4.

3.3.17. Cyanthiwigin Q (17). White powder; HRESIMS 339.1923 [M+Na] (calcd for $C_{20}H_{28}O_3Na$ 339.1936), 655.3976 [2M+Na] (calcd for $C_{40}H_{56}O_6Na$ 655.3975); $[\alpha]_D$=+90° (*c* 0.08, MeOH); UV λ_{max} (nm) 236 (ε=10,228); IR (film) ν 2964, 2931, 2865, 1702, 1600, 1452, 1380, 1261, 1176, 1106, 1047, 848 cm^{-1}; CD $[\theta]_{231}$=+28,635, $[\theta]_{236}$=+28,320, $[\theta]_{299}$=+4686, $[\theta]_{333}$=−6999 (*c* 6.33×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 4.

3.3.18. Cyanthiwigin R (18). Colorless crystal; HRESIMS 355.1878 [M+Na] (calcd for $C_{20}H_{28}O_4Na$ 355.1885), 687.3889 [2M+Na] (calcd for $C_{40}H_{56}O_8Na$ 687.3873); $[\alpha]_D=-118°$ (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=9524); IR (film) ν 3388, 2966, 2929, 2869, 1724, 1685, 1602, 1454, 1373, 1263, 1168,736 cm^{-1}; CD $[\theta]_{238}$=+26,303, $[\theta]_{298}=-32{,}654$ (*c* 7.91×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 4.

3.3.19. Cyanthiwigin S (19). Colorless crystal; HRESIMS 339.1927 [M+Na] (calcd for $C_{20}H_{28}O_3Na$ 339.1936), 655.3981 [2M+Na] (calcd for $C_{40}H_{56}O_6Na$ 655.3975); $[\alpha]_D=-50°$ (*c* 0.090, MeOH); UV λ_{max} (nm) 236 (ε=6481); IR (film) ν 3440, 2964, 2927, 2871, 1724, 1687, 1602, 1454, 1375, 1261, 1170, 1116, 867, 746 cm^{-1}; CD $[\theta]_{238}$=+12,332, $[\theta]_{303}=-14{,}416$ (*c* 7.12×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 4.

3.3.20. Cyanthiwigin T (20). Colorless crystal; HRESIMS 357.2031 [M+Na] (calcd for $C_{20}H_{30}O_4Na$ 357.2041), 691.4197 [2M+Na] (calcd for $C_{40}H_{60}O_8Na$ 691.4186); $[\alpha]_D=+110°$ (*c* 0.02, MeOH); UV λ_{max} (nm) 238 (ε=12,689); IR (film) ν 3400, 2954, 2923, 1675, 1515, 1390, 1027, 869 cm^{-1}; CD $[\theta]_{245}$=+25,741, $[\theta]_{273}=-13{,}295$, $[\theta]_{340}=-13{,}141$ (*c* 1.48×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 5.

3.3.21. Cyanthiwigin U (21). Colorless crystal; HRESIMS 325.2136 [M+Na] (calcd for $C_{20}H_{30}O_2Na$ 325.2143); $[\alpha]_D=+131°$ (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=9621); IR (film) ν 3444, 2962, 2925, 2867, 1685, 1596, 1448, 1378, 1182, 1101, 873, 748 cm^{-1}; CD $[\theta]_{239}$=+64,790, $[\theta]_{329}=-6697$ (*c* 8.28×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 5.

3.3.22. Cyanthiwigin V (22). Colorless gum; HRESIMS 341.2075 [M+Na] (calcd for $C_{20}H_{30}O_3Na$ 341.2093), 659.4362 [2M+Na] (calcd for $C_{40}H_{60}O_6Na$ 659.4287); $[\alpha]_D=+8°$ (*c* 0.10, MeOH); UV λ_{max} (nm) 206 (ε=3533); IR (film) ν 3401, 2962, 2925, 1695, 1454, 1375, 1259, 1118, 1074, 1020, 736 cm^{-1}; CD $[\theta]_{220}$=+1080, $[\theta]_{225}=-917$, $[\theta]_{236}$=+636, $[\theta]_{241}=-713$ (*c* 8.22×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 5.

3.3.23. Cyanthiwigin W (23). White powder; HRESIMS 327.2281 [M+Na] (calcd for $C_{20}H_{32}O_2Na$ 327.2300), 631.4687 [2M+Na] (calcd for $C_{40}H_{64}O_4Na$ 631.4702); $[\alpha]_D=+97°$ (*c* 0.08, MeOH); UV λ_{max} (nm) 206 (ε=7184); IR (film) ν 3345, 2962, 2921, 2867, 1454, 1369, 1108, 1076, 1001, 921, 836, 740 cm^{-1}; $[\theta]_{216}$=+9670, $[\theta]_{259}=-3272$ (*c* 6.58×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 5.

3.3.24. Cyanthiwigin X (24). Colorless crystal; HRESIMS 343.2224 [M+Na] (calcd for $C_{20}H_{32}O_3Na$ 343.2249), 663.4641 [2M+Na] (calcd $C_{40}H_{64}O_6Na$ 663.4600); $[\alpha]_D=+87°$ (*c* 0.10, MeOH); UV λ_{max} (nm) 206 (ε=6050); IR (film) ν 3369, 2962, 2923, 2869, 1458, 1373, 1257, 1024, 734 cm^{-1}; CD $[\theta]_{205}$=+36,013, $[\theta]_{267}=-3371$ (*c* 7.80×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 6.

3.3.25. Cyanthiwigin Y (25). Colorless gum; HRESIMS 325.2144 [M+Na] (calcd for $C_{20}H_{30}O_2Na$ 325.2143), 627.4449 [2M+Na] (calcd for $C_{40}H_{60}O_4Na$ 627.4389); $[\alpha]_D=+24°$ (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=6914); IR (film) ν 3419, 2960, 2925, 2867, 1700, 1666, 1454, 1378, 1263, 1180, 1070, 1004, 734 cm^{-1}; CD $[\theta]_{229}$=+4267, $[\theta]_{330}=-5291$ (*c* 8.28×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 6.

3.3.26. Cyanthiwigin Z (26). Colorless gum; HRESIMS 325.2142 [M+Na] (calcd for $C_{20}H_{30}O_2Na$ 325.2143), 627.4458 [2M+Na] (calcd for $C_{40}H_{60}O_4Na$ 627.4389); $[\alpha]_D=-160°$ (*c* 0.03, MeOH); UV λ_{max} (nm) 238 (ε=13,828); IR (film) ν 3421, 2960, 2925, 2867, 1697, 1633, 1457, 1375, 1270, 1230, 1076, 1018, 836 cm^{-1}; CD $[\theta]_{241}$=+8534, $[\theta]_{320}=-10{,}582$ (*c* 4.14×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 6.

3.3.27. Cyanthiwigin AA (27). Colorless gum; HRESIMS 337.1762 [M+Na] (calcd for $C_{20}H_{26}O_3Na$ 337.1779), 651.3239 [2M+Na] (calcd $C_{40}H_{52}O_6Na$ 651.3661); $[\alpha]_D=-98°$ (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=9016); IR (film) ν 2964, 2927, 2873, 1724, 1687, 1604, 1452, 1384, 1259, 1170, 1108, 1068, 865 cm^{-1}; CD $[\theta]_{243}$=+28,523, $[\theta]_{320}=-15{,}114$ (*c* 7.96×10^{-5}, MeOH); ^1H and ^{13}C NMR see Table 6.

3.4. Bioassay

3.4.1. Cytotoxicity assay. PrimeCyte has a novel technique for screening for anti-cancer compounds. They obtain tumor and non-tumor tissues from surgical resection of cancer patients. These tissues are typed by a panel of medical pathologists to determine the kind of cancer and the amount of tumor versus normal cells. Primary cell cultures are generated from tumor cells. Anti-tumor screening is conducted using these primary cell cultures. Cell preparations that passed histological and cytological examination for diagnosis, grading, and cell purity were thawed at 37°C and resuspended in tissue culture medium designed to maintain the cells during the incubation period. The live and dead cells were counted, and the tumor cells were diluted in culture medium to 1.0×103 cells/well. The cells were added to microtiter plates and incubated at 37°C overnight with samples that were added at 1/10 the volume of the cell suspension. Alamar Blue (Accumed International, Westlake, OH) was then added to the cells at 1/10 the volume of the well, and the cells were further incubated at 37°C for various times. Alamar Blue dye measures cellular redox reactions (i.e. cellular respiration) whereby a spectral shift occurs upon reduction of the dye (excitation 530 nm; emission 590 nm). The kinetics of cellular redox reactions was subsequently measured at 3 h and 3 days post-dye addition. These measurements, in comparison with control cells (untreated with compound) and media controls (test wells without cells) were used to determine the percent inhibition of the test compound, as well as their IC_{50} determinations.

3.4.2. Anti-HIV assay. Anti-HIV-1 activity was determined in PBM cells as described previously.[9] Stock solutions (20 or 40 mM) of the compounds were prepared in sterile DMSO and then diluted to the desired concentration in growth medium. Cells were infected with the prototype HIV-1_{LAV} at a multiplicity of infection of 0.1. Details on the

infection of cells and assessment of antiviral effects was described previously.[10]

3.4.3. Antituberculosis assay. Primary assay is conducted at 6.25 μg/mL against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B MEDIUM using a broth microdilution assay (the Microplate Alamar Blue Assay).[11]

3.4.4. Anti-HBV assay. The anti-HBV assay method was described previously.[12]

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