Diterpene Formamides from the Tropical Marine Sponge *Cymbastela hooperi* and Their Antimalarial Activity in Vitro[§]

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Further investigations of the VLC (vacuum-liquid chromatography) fractions obtained from the dichloromethane solubles of the tropical marine sponge *Cymbastela hooperi* led to the isolation and characterization of five new diterpene formamides, **1**−**5**. Compound **1** is one of the very few examples of a natural product that contains both formamide and isonitrile functionalities within the same molecule. In in vitro antiplasmodial bioassays, **1** was found to have moderate activity (IC_{50} 0.5 μg/mL), **2** had weak activity (IC_{50} 14.8 μg/mL), and **3**−**5** were inactive. The pattern of activity found for the metabolites investigated in the current study is consistent with previous findings for these classes of molecules.

The sponge *Cymbastela hooperi* van Soest, Desqueyroux-Faúndez, Wright, König[1] is interesting not only taxonomically[1,2] but also chemically and biologically.[3−7] It was the predominantly isonitrile-containing diterpenes isolated from this sponge that eventually enabled the antiplasmodial mechanism of action of these[4,7] and other isonitriles found in the marine environment to be elucidated.[8] Synthetic work undertaken on the basis of the natural product leads has shown clearly the potential of these diterpenes as important prototype structures in the search for new antimalarial agents.[9] In the current work, the structures and antiplasmodial activities of the remaining isolates from original VLC fraction 11,[4] mainly diterpene-based formamides, from the sponge *C. hooperi* are described.

Reversed-phase silica gel HPLC separation of original VLC fraction 11, obtained from the CH_2Cl_2 solubles of the sponge *C. hooperi*, yielded five new diterpene formamides, **1**−**5**.

Mass spectrometric analyisis of **1** showed it to have the molecular formula $C_{22}H_{34}N_2O$. After association of all ^{1}H and ^{13}C NMR resonances resulting from C−H one-bond interactions, it was possible to deduce that **1** contained four CH_3, six CH_2, and nine CH groups and three quaternary C atoms. These data, together with the IR and MS data, showed the molecule to contain isonitrile (2128 cm^{-1}, $[M - HCN]^+$) and formamide (3280, 1683 cm^{-1}, $[M - HCN - NH_2CHO]^+$ base peak) functionalities, which accounted for all of the multiple bonds within **1**; the molecule was thus a tetracyclic diterpene. The presence of the formamide functionality was further supported by the originally unexpected number of resonances in both the ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2). Due to the two possible geometries the formamide can adopt within one molecule, it is common to see two sets of NMR signals originating from the same molecule on the NMR time scale. Close comparison of the NMR data for **1** with those for all of the diterpene isonitriles previously isolated[4] led to the deduction that the isonitrile functionality resided at C-20 (δ_C 65.1 [t, J_{CN} = 4.4 Hz]) of an isocycloamphilectane, and the formamide resided at C-7 (δ_C 57.2 [*cis*], 55.6 [*trans*]), showing **1** to be the 7-formamido derivative of 7,20-diisocyanoisocycloamphilectane. The optical rotation of **1** is almost identical to that reported for (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7,20-diisocyanoisocycloamphilectane, as are the two sets of ^{1}H and ^{13}C NMR data for comparable centers.[4] Thus, it is most likely that these two molecules have the same absolute configuration, making **1** (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7-formamido-20-isocyanoisocycloamphilectane. Compound **1** is one of the very few examples of a natural product that contains both a formamide and an isonitrile functionality within the same molecule.

The second formamide isolated in this study, **2**, was found to have the molecular formula $C_{22}H_{36}N_2O_2$ by accurate mass measurement. The complexity of both the ^{1}H and ^{13}C NMR spectra of **2**, combined with the significant mass spectrometric fragmentation ions at *m/z* 315 ($[M - NH_2CHO]^+$) and 270 ($[M - NH_2CHO - NH_2CHO]^+$ base peak) as well as the IR absorbances at 3280 and 1683 cm^{-1}, pointed toward the molecule being a diformamide derivative of a diterpene. Two of the multiple-bond elements of unsaturation indicated by the molecular formula of **2** were taken up by the two formamide moieties as the only multiple bonds within the molecule; **2** had to be tetracyclic. In common with **1**, it was shown from its NMR data to contain four CH_3, six CH_2, and nine CH groups and three quaternary C atoms and is virtually identical to **1** except for the presence of an extra formamide group in place of the isonitrile functionality found at C-20 in **1**. This deduction was further supported specifically by the ^{13}C NMR data of **2** that showed C-20 now to be two distinct resonances (δ_C 57.1 [*cis*], 55.5 [*trans*]). This means **2** is best described as 7,20-diformamidoisocycloamphilectane. As the NMR data of **1** and **2** are virtually identical for comparable stereocenters, and the fact that the optical rotation of **2** is of similar magnitude and sign to that of **1** and (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7,20-diisocyanoisocycloamphilectane,[4] it is likely that these three molecules all have the same absolute configuration, making **2** (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7,20-diformamidoisocycloamphilectane.

The molecular formula of **3**, $C_{21}H_{33}NO$, established by accurate mass measurement, showed the molecule to have the equivalent of six elements of C=C bond unsaturation. Two of these unsaturation elements were present as a C=C (δ_C 135.3, 134.7 [*cis*], 130.7, 131.1 [*trans*]) and a C=O (δ_C 160.3 [*cis*], 162.7 [*trans*]) double bond as the only multiple bonds within **3**, making the molecule tetracyclic. These data coupled with the mass spectrometric ($[M - NH_2CHO]^+$ base peak) and IR data (3280, 1683 cm^{-1}) also showed **3** to be a formamide derivative of a tetracyclic diterpene, but this time not an isocycloamphilectane. Close inspection of the ^{1}H and ^{13}C NMR data of **3** revealed the presence of a *gem*-dimethyl constellation (δ_C 29.3, 31.5 [*cis*], 29.2, 31.5 [*trans*]), a doublet methyl (δ_C 19.8 [*cis*], 19.8 [*trans*]), and a methyl (δ_C 19.5, [*cis*], 19.4 [*trans*]) on a carbon (δ_C 57.5 [*cis*], 55.7 [*trans*]) bearing the formamide functionality, together with six CH_2 and seven CH. When this information was compared to the data in the earlier study,[4] it was

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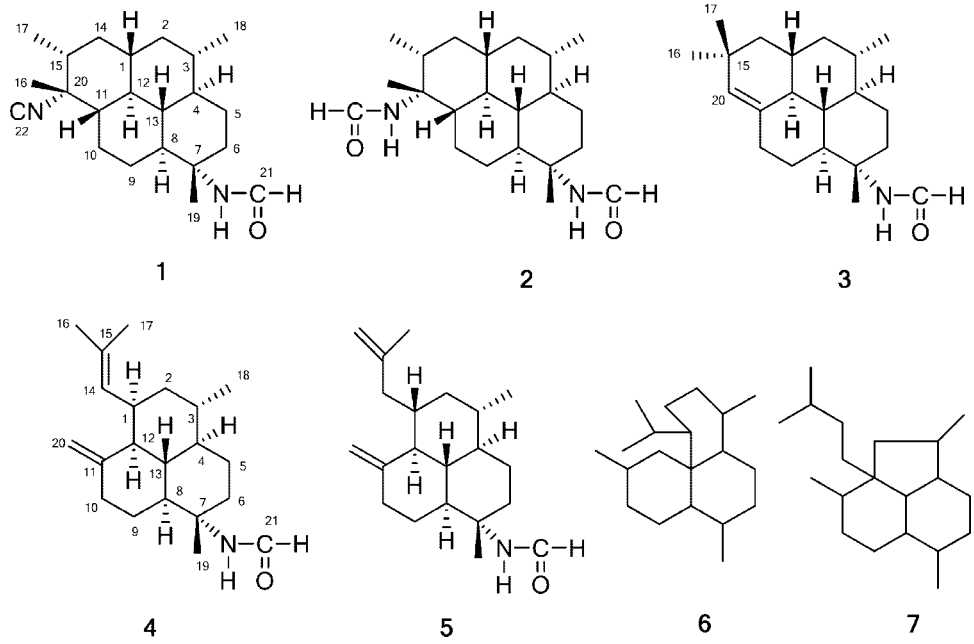
Chart 1



apparent that **3** is the 7-formamido derivative of (1*S**,3*S**,4*R**,7*S**, 8*S**,12*S**,13*S**)-7-isocyanocycloamphilect-11(20)-ene, with the same relative absolute configuration based on almost identical optical rotations and, as such, is best described as (1*S**,3*S**,4*R**,7*S**, 8*S**,12*S**,13*S**)-7-formamidocycloamphilect-11(20)-ene.

The molecular formula of **4**, $C_{21}H_{33}NO$, is identical to that found for **3**, but the two molecules were clearly structurally different. Apparent from the NMR, MS, and IR data of **4** was the presence in the molecule of a formamide function (δ_C 160.3, 162.7; 3280, 1680 cm^{-1}; $[M - NH_2CHO]^+$ base peak) and two C=C bonds, one exo (149.7, 107.5 [*cis*], 148.9, 108.1 [*trans*]) and one endo (126.6, 130.0 [*cis*], 126.6, 130.3 [*trans*]), as the only multiple bonds and functionality within the molecule; the molecule was tricyclic. With the identification of the aforementioned features of the molecule and the remaining NMR data (Tables 1 and 2), it was evident that **4** was an amphilectane derivative. Comparison of all of the spectroscopic data, mainly ^1H and ^{13}C NMR, for **4** with that for amphilectanes isolated and characterized in the original study[4] revealed it to be the 7-formamido derivative of 7-isocyanoamphilecta-11(20),14-diene. As the ^1H and ^{13}C NMR data for comparable stereocenters and the sign and magnitude of the optical rotation for **4** were similar to those of the original compound (+80.4, cf., +115.8), it was concluded that the molecule is (1*R**,3*S**,4*R**,7*S**, 8*S**,12*S**,13*S**)-7-formamidoamphilecta-11(20),14-diene.

The final diterpene formamide isolated and fully characterized (**5**) analyzed for $C_{21}H_{33}NO$, by accurate mass measurement. The structural features of **5** were very similar to those of **4** except for the presence of a second exomethylene group (δ_C 144.7, 111.0 [*cis*], 144.6, 111.2 [*trans*]) and no evidence of the endo C=C moiety associated with **4**. In common with **4**, **5** was also concluded to be an amphilectane derivative, and the position of the formamido group was also concluded to be at C-7, making it a 7-formamidoamphilecta-11(20),15-diene. Close comparison of all of the spectroscopic and physical data for **5** with those from similar molecules of the previous study[4] revealed it to be not just the double-bond isomer of **4** but also the C-1 epimer. On the basis of these deductions **5** is best described as (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-formamidoamphilecta-11(20),15-diene.

Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Data for Compounds **1**−**5** (δ in ppm)

carbon	**1** (*cis*)	**1** (*trans*)	**2** (*cis*)	**2** (*trans*)	**3** (*cis*)	**3** (*trans*)	**4** (*cis*)	**4** (*trans*)	**5** (*cis*)	**5** (*trans*)
1	40.6 d[a]	40.7 d	41.0 d	40.9 d	37.0 d	36.9 d	34.7 d	34.6 d	34.2 d	34.1 d
2	42.3 t	42.2 t	42.4 t	42.3 t	42.6 t	42.5 t	42.6 t	42.5 t	41.0 t	40.9 t
3	36.6 d	36.5 d	36.6 d	36.5 d	37.7 d	37.6 d	31.9 d	31.8 d	35.9 d	35.8 d
4	47.7 d	47.8 d	47.9 d	48.0 d	48.3 d	48.3 d	49.5 d	49.7 d	50.3 d	50.2 d
5	25.4 t	25.2 t	25.5 t	25.1 t	26.4 t	25.9 t	26.6 t	26.4 t	26.4 t	26.2 t
6	37.4 t	41.6 t	37.2 t	41.3 t	37.2 t	41.7 t	37.7 t	42.3 t	37.7 t	42.1 t
7	57.2 s	55.6 s	57.1 s	55.5 s	57.5 s	55.7 s	57.9 s	56.0 s	not obsd	56.0 s
8	46.0 d	49.6 d	46.3 d	49.6 d	46.1 d	50.1 d	46.7 d	51.3 d	46.7 d	51.4 d
9	26.4 t	26.3 t	26.3 t	26.2 t	26.7 t	26.5 t	27.0 t	26.6 t	30.0 t	29.6 t
10	25.3 t	24.9 t	24.8 t	24.8 t	33.7 t	33.5 t	36.3 t	36.1 t	37.3 t	37.5 t
11	48.9 d	48.8 d	50.2 d	50.2 d	135.3 s	134.7 s	149.7 s	148.9 s	151.2 s	150.9 s
12	46.2 d	46.0 d	46.5 d	46.2 d	48.7 d	48.9 d	48.5 d	48.5 d	51.7 d	51.7 d
13	45.9 d	46.0 d	45.8 d	45.9 d	47.1 t	47.1 d	41.7 d	41.7 d	48.4 d	48.7 d
14	37.8 t	37.7 t	37.3 t	37.2 t	44.5 t	44.4 t	126.9 d	126.6 d	43.3 t	43.3 t
15	39.9	39.9 d	41.0 d	40.9 d	32.4 s	32.4 s	130.0 s	130.3 s	144.7 s	144.6 s
16	23.9 q	23.8 q	22.2 q	22.2 q	29.3 q	29.2 q	26.0 q	26.0 q	111.0 t	111.2 t
17	16.1 q	16.1 q	15.5 q	15.5 q	31.5 q	31.5 q	17.7 q	17.7 q	22.7 q	22.6 q
18	19.7 q	19.7 q	19.7 q	19.7 q	19.8 q	19.8 q	20.2 q	20.2 q	19.9 q	19.7 q
19	19.1 q	19.2 q	19.7 q	19.7 q	19.5 q	19.4 q	19.9 q	19.2 q	20.3 q	20.2 q
20	65.1 t[c]	65.1 t[c]	57.1 s	55.5 s	130.7 d	131.1 d	107.5 t	108.1 t	105.0 t	105.5 t
21	160.4 d	162.7 d	160.4 d	162.9 d	160.3 s	162.7 s	160.3 d	162.7 d	160.3 d	162.7 d
22	155.0 t[c]	155.1 t[c]	163.8 d	163.9 d						

[a] Implied multiplicity by DEPT (s = C, d = CH, t = CH_2, q = CH_3). [b] br = broad singlet or a poorly resolved triplet. [c] CN coupling, J = 4.4 Hz.

Table 2. ^{1}H NMR (300 MHz, $CDCl_3$) Data for Compounds **1**−**5** (δ in ppm, mult. *J* in Hz)[a]

proton	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
1	1.13 m	1.14 m	1.41 m	2.88 m	1.77 m
2	0.82 m, 1.54 m	0.77 m, 1.54 m	0.90 m, 1.60 m	1.27 m, 1.62 m	0.65 m, 1.82 m
3	1.12 m	1.12 m	1.26 m	1.27 m	1.06 m
4	0.77 m	0.70 m	1.32 m	0.85 m *cis*, 0.78 m *trans*	0.92 m
5	1.03 m, 1.89 m	1.01 m, 1.78 m	1.04 m, 1.74 m	1.08 m, 1.89 m	0.98 m, 1.87 m
6	1.93 m, 2.08 m *cis*	1.96 m *cis*	1.32 m, 2.04 m *cis*	1.93 m, 2.23 m *cis*	1.56 m, 1.85 m
	1.56 m, 1.83 m *trans*	1.57 m, 1.80 m *trans*	1.58 m, 1.86 m *trans*	1.56 m, 1.88 m *trans*	
8	1.11 m *trans*	0.98 m *trans*	1.15 m *trans*	1.23 m *trans*	1.61 m
	1.62 m *cis*	1.57 m *cis*	1.81 m *cis*	2.03 m *cis*	
9	0.98 m, 1.88 m	0.99 m, 1.86 m	1.04 m, 1.90 m	1.08 m, 1.81 m	1.23 m, 1.95 m
10	1.25 m, 1.98 m	1.00 m, 1.89 m	1.95 m, 2.23 m	2.00 m, 2.34 m	1.90 m, 2.33 m
11	0.95 m	0.96 m			
12	0.94 m	0.75 m	0.81 m	1.88 m	1.45 m
13	0.77 m trans	0.70 m	0.79 m	1.31 m	0.75 m
14	1.13 m, 1.41 m	0.90 m, 1.40 m	1.08 m, 1.32 m	5.27 m	1.46 m, 2.61 (brd, *J* 14.8)
15	1.43 m	1.46 m			
16	1.35 s	1.34 s	0.96 s	1.71 s	4.65 s, 4.74 s
17	1.02 (d, *J* 6.4)	0.86 (d, *J* = 6.4)	0.93 s	1.65 s	1.71 s
18	0.86 (d, *J* 6.4) *cis*	0.85 (d, *J* = 6.4)	0.90 (d, *J* 6.1) *cis*	0.82 (q, *J* 5.7) *cis*	0.86 (d, *J* 5.6) *cis*
	0.88 (d, *J* 6.4) *trans*		0.92 (d, *J* 6.1) *trans*	0.84 (q, *J* 5.7) *trans*	0.88 (d, *J* 5.6) *trans*
19	1.21 s *trans*	1.17 s *trans*	1.20 s trans	1.24 s *cis*	1.19 s *cis*
	1.26 s *cis*	1.22 s *cis*	1.23 s cis	1.25 s *trans*	1.20 s *trans*
20			5.02 (q, *J* 2.0) *cis*	4.52 (q, *J* 1.5),	4.80 s, 4.58 s *cis*
				4.70 (q, *J* 1.5) *trans*	
			5.04 (q, *J* 2.0) *trans*	4.50 (q, *J* 1.5),	4.83 s, 4.60 s *trans*
				4.68 (q, *J* 1.5) *cis*	
21	8.06 (d, *J* 2.3) *cis*	8.02 (d, *J* 1.8) *cis*	8.06 (d, *J* 2.0) *cis*	8.06 (d, *J* 2.0) *cis*	8.05 (d, *J* 2.0) *cis*
	8.26 (br, *J* 12.4) *trans*	8.06 (brd, *J* 12.1) *trans*	8.28 (brd, *J* 12.2) *trans*	8.28 (brd, *J* 12.4) *trans*	8.27 (d, *J* 12.2) *trans*
22		8.22 (brd, *J* 12.1) *trans*			
NH *trans*	5.72 (brd, *J* 12.4)	6.25 (brd, *J* 12.1)	5.57 (brd, *J* = 12.2)	5.72 (brd, *J* 12.4)	5.54 (brd, *J* 12.2)
		5.92 (brd, *J* 12.1)			
NH *cis*	5.14 brs	5.31 brs	5.10 brs	5.13 brs	5.06 brs

[a] All assignments are based on extensive 1D and 2D NMR experiments, including COSY90, HMQC, and HMBC.

Table 3. Growth Inhibitory Effects (IC_{50} values) of **1** and Atovaquone on Three Strains of *Plasmodium falciparum* and KB Cells and of **2**−**5** on a Single Strain of *P. falciparum*

compound	FCR3F86	W2	D6	mean of all strains	KB cells
atovaquone[a]	0.4 ± 0.3 (n = 5)[b]	0.8 (n = 2)	0.2 (n = 2)	0.5 ± 0.4 (n = 9)	>5[c]
1[c]	0.2 (n = 2)	0.6 (n = 2)	0.8 (n = 2)	0.5 ± 0.3 (n = 6)	>5
2[c]	14.8 (n = 2)				
3[c]	>100.0 (n = 2)				
4[c]	63.0 (n = 1)				
5[c]	>90.0 (n = 2)				

[a] IC_{50} values in ng/mL. [b] n = number of replicates. [c] IC_{50} values in μg/mL.

In a paper concerning antimalarial activity of a series of isonitriles, isocyanates, and isothiocyanates the rationalization of the naming of a series of amphilectane-type compounds was discussed,[4] and in so doing, the structure published by Sharma et al.[10] was overlooked. Clearly, if the comments made then are to be consistent, the compound reported by Sharma and his colleagues will be the fourth type of amphilectane and, as the authors proposed, the class will be known as neoamphilectanes, based on the hypothetical alkane **6**, and our compound, originally named as a neoamphilectane, will be the first member of a fifth type of amphilectane, for which the new name [1(14)*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*R**,13*R**]-7-isocyanoisoneoamphilecta-1(14),15-diene, based on the hypothetical alkane isoneoamphilectane (**7**), is proposed.

Compounds **1**−**5** were tested for their in vitro biological activity against three strains (FCR3F86, W2, and D6) of the malaria parasite, *Plasmodium falciparum*. The results of these assays (Table 3) showed only **1** to have antiplasmodial activity that can be regarded as significant, and somewhat selective, based on its lack of cytotoxicity toward KB cells. Compound **2** has weak activity, and as the only difference between **1** and **2** is the presence of the isonitrile in **1** instead of the formamide found in **2**, it is likely that this functionality, the isonitrile, is important for the observed activity. The antiplasmodial activity of **4** showed it to be approximately 4 times less active than **2**. This result reveals that there is probably some molecular feature, probably lone pairs of electrons behaving as H-bond acceptors or donors, which may well be responsible for the observed activity, albeit weak. This observation is further supported by the fact that compounds lacking this level of activity (**3** and **5**) either lack the critical electron density in this region or may be sterically hindering a favorable interaction. Compounds **3** and **5** were essentially inactive in the antiplasmodial assay using the FCR3F86 strain of *P. falciparum*, a finding that supports the idea that the formamide functionality has little effect on the activity observed for **1**. The pattern of activity found for the metabolites investigated in the current study (**1**−**5**) is consistent with previous findings for these classes of molecules,[4] in that there is clearly a relationship between the observed activity and the ability of the active molecule to interact with the iron within heme.

Experimental Section

General Experimental Procedures. As previously published.[4,6]

Animal Material. As previously reported.[4]

Extraction and Isolation. Original VLC fraction 11[4] was further fractionated by HPLC (RP C_{18} Si60, various MeOH, CH_3CN, and H_2O mixtures; typically MeOH−H_2O, 20:80, and CH_3CN−H_2O, 20:80) to yield **1**−**5**. All remaining details as previously reported.[4]

(1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7-Formamido-20-isocyanoisocycloamphilectane (1): yellow oil (9.5 mg, 0.007%); $[\alpha]_D^{25}$ +44.0 (*c* 0.48, $CHCl_3$); IR (film) ν_{max} 2920, 2128, 1683, 1660, 1540, 1455, 1385 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS

m/z 343 $[M + H]^+$ (1), 342 $[M]^+$ (2), 316 (2), 315 (10), 301 (2), 300 (10), 297 (8), 271 (22), 270 (100), 256 (10), 255 (43); HREIMS *m/z* 342.267 (calcd for $C_{22}H_{34}N_2O$, $[M^+]$ 342.267).

(1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7,20-Diformamidoisocycloamphilectane (2): clear oil (3.2 mg, 0.002%); $[\alpha]_D^{25}$ +17.4 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3265, 2924, 2863, 1668 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m/z* 361 ($[M + H]^+$, 4), 360 ($[M]^+$, 10), 316 (10), 315 (38), 297 (10), 270 (100), 255 (30), 149 (14); HREIMS *m/z* found 360.277 ($[M]^+$), calcd for $C_{22}H_{36}N_2O_2$ 360.277.

(1*S,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-Formamidocycloamphilect-11(20)-ene (3):** yellow oil (4.9 mg, 0.004%); $[\alpha]_D^{25}$ +13.7 (*c* 0.49, $CHCl_3$); IR (film) ν_{max} 3280, 2920, 1676, 1454, 756 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m/z* 315 ($[M]^+$, 2), 300 (6), 270 (100), 255 (80), 214 (4), 145 (2); HREIMS *m/z* found 315.256 ($[M]^+$), calcd for $C_{21}H_{33}NO$ 315.256.

(1*R,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-Formamidoamphilecta-11(20),14-diene (4):** clear oil (20.0 mg, 0.015%); $[\alpha]_D^{25}$ +80.4 (*c* 0.50, $CHCl_3$); IR (film) ν_{max} 3280, 2920, 1680, 890, 756 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m/z* 315 ($[M]^+$, <1), 270 (24), 255 (18), 246 (15), 227 (12), 201 (100), 145 (20); HREIMS *m/z* found 315.256 ($[M]^+$), calcd for $C_{21}H_{33}NO$ 315.256.

(1*S,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-Formamidoamphilecta-11(20),15-diene (5):** clear oil (2.2 mg, 0.001%); $[\alpha]_D^{25}$ +15.5 (*c* 0.22, $CHCl_3$); IR (film) ν_{max} 3280, 2924, 1665, 1028, 889, 757 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m/z* 315 ($[M]^+$, 1), 313 (8), 270 (100), 255 (50), 214 (18), 162 (10); HREIMS *m/z* found 315.256 ($[M]^+$), calcd for $C_{21}H_{33}NO$ 315.256.

Antiplasmodial Activity. For each inhibitor, the concentration that inhibited 50% of parasite growth (IC_{50}) was determined by measuring the incorporation of $[^3H]$hypoxanthine in a 48 h assay as previously described.[11] All assays were done in triplicate, and the mean IC_{50} was determined from a plot of the mean percentage of inhibition at each concentration. Each experiment included an assay of atovaquone as a positive internal control for parasite inhibition. The *P. falciparum* strains used for these assays were FCR3F86, W2, and D6.

Cytotoxicity Activity. The cytotoxicity of each inhibitor was determined for human KB cells by measuring the incorporation of $[^3H]$-hypoxanthine in a 48 h assay. Briefly, radioactivity was present during the final 6 h of incubation of the cells with the inhibitor. The cells were lysed and the nucleic acid in each sample was precipitated with trichloroacetic acid and harvested onto glass fiber filters. The mean radioactivity precipitated from samples in which it was added immediately before cell lysis and nucleic acid precipitation was determined to estimate the level of unincorporated radioactivity that coprecipitated with the experimental samples. This unincorporated radioactivity was subtracted from the mean radioactivity precipitated from wells exposed to $[^3H]$-hypoxanthine for 6 h. The percent inhibition for each drug concentration was determined by comparing radioactivity incorporated in the presence versus the absence of drug. The percent inhibition at each drug concentration was plotted, and the IC_{50} was determined from the curve.

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