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PAPER

Dysidaminones A–M, Cytotoxic and NF- κ B Inhibitory Sesquiterpene Aminoquinones from the South China Sea Sponge *Dysidea fragilis*†‡

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Dysidaminones A–M (**1–13**), thirteen new sesquiterpene aminoquinones, along with six known ones (**14–19**), were isolated from the South China Sea sponge *Dysidea fragilis*. The new structures were determined by extensive spectroscopic analyses, the absolute configurations of **1** and **2** were determined by single-crystal X-ray diffraction analysis, and the absolute configurations of **3–13** were assigned by comparing their CD spectra with those of **1** and **2**. Dysidaminones C (**3**), E (**5**), H (**8**), and J (**10**), 18-methylaminoavarone (**14**), and 18-aminoavarone (**16**) showed cytotoxicity against mouse B16F10 melanoma and human NCI-H929 myeloma, HepG2 hepatoma, and SK-OV-3 ovarian cancer cell lines. In addition, these six cytotoxic compounds also exhibited NF- κ B inhibitory activity with IC₅₀ values of 0.05–0.27 μ M. Preliminary structure-activity relationship analysis indicated that 18-amiosubstituted sesquiterpene quinones with exocyclic double bond ($\Delta^{4,11}$) are cytotoxic agents and NF- κ B inhibitors.

Introduction

Nuclear factor κ B (NF- κ B) regulates the expression of genes involved in many processes that play a key role in the development and progression of cancer such as proliferation, migration and apoptosis.¹ Therefore, NF- κ B has become an important target for anticancer drug discovery.² A large number of natural and synthetic compounds have been investigated for NF- κ B inhibitory activity, and the majority of the known NF- κ B inhibitors to date are plant-derived metabolites, such as isoprenoids and polyphenolics.³ Marine-derived aquamin has been reported to inhibit the NF- κ B signaling pathway *in vitro*,⁴ however, there are few pure marine natural products were reported to exhibit NF- κ B inhibitory activity.

Sesquiterpene quinones represent a large group of biologically active marine natural products.⁵ Although over 200 in number, these mixed biogenesis metabolites were mainly isolated from marine sponges and belong to just a few chemical motifs that as exemplified by spongiaquinone,⁶ ilimaquinone,⁷ isospongiaquinone,^{6,8} frondosin A,⁹ bolinaquinone,¹⁰ dysidavarone A,¹¹ and metachromin C.¹² These compounds have sparked interests of researchers by their broad spectrum of bioactivities, including anti-HIV,¹³ antibacterial,¹⁴ antifungal,¹⁵ antioxidative,¹⁶ antitumor,¹⁷ anti-inflammatory,^{17,18} activating hypoxia-inducible factor 1 (HIF-1),¹⁹ and protein tyrosine phosphatase 1B (PTP1B) inhibitory activities.¹¹

In our search for new marine anticancer agents and NF- κ B inhibitors from the South China Sea sponges, we have found many chemical motifs from these sponges showed cytotoxic activities,

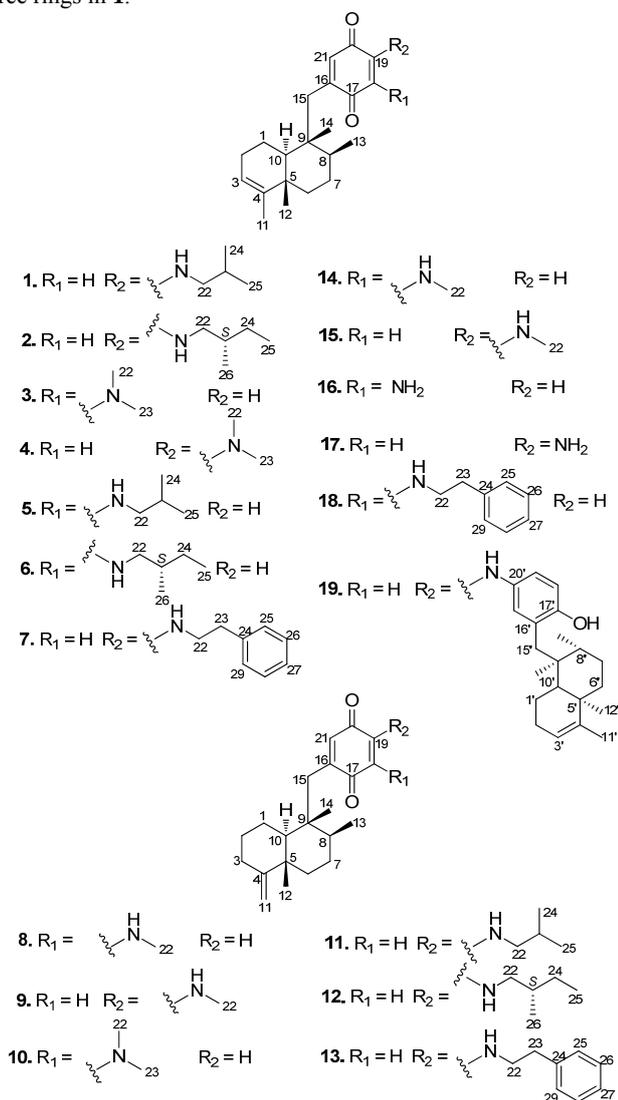
such as isomalabaricane triterpenes,²⁰ cyclopeptides,²¹ and polyketides,²² however, only the sesquiterpene quinone-containing extract of *Dysidea fragilis* showed NF- κ B inhibitory activity. Therefore we selected the sponge *D. fragilis* for a detailed investigation. Bioassay-guided fractionation of the extract of *D. fragilis* yielded a series of sesquiterpene aminoquinones, including thirteen new compounds, dysidaminones A–M (**1–13**), and six known ones. Herein, we described the isolation, structural determination, and absolute configuration assignments, as well as cytotoxic and NF- κ B inhibitory activities of these metabolites.

Results and discussion

The frozen specimen of *D. fragilis* was exhaustively extracted with EtOH to give the crude EtOH extract, which was dissolved in water and successively partitioned by *n*-hexane, CH₂Cl₂, and *n*-BuOH to yield three fractions. TLC analysis of the cytotoxic and NF- κ B inhibitory CH₂Cl₂-soluble fraction exhibited two red spots along with several UV-active spots (254 nm), which turned blue-purple by staining with anisaldehyde/sulfuric acid. Normal-phase silica gel flash separation of the CH₂Cl₂-soluble fraction followed by HPLC purification of selected fractions led to the isolation of thirteen new metabolites, dysidaminones A–M (**1–13**) as well as six known ones (**14–19**).

Dysidaminone A (**1**) was isolated as purple-red needles, and its molecular formula was assigned as C₂₅H₃₇NO₂ based on the HRESIMS positive ion at *m/z* 384.2900 [M + H]⁺ (calculated for

$C_{25}H_{38}NO_2$, 384.2903), implying eight degrees of unsaturation. The IR absorption at 1593 cm^{-1} and the UV characteristic absorptions at 227 and 292 nm indicated the presence of a benzoquinone group.²³ Meanwhile the IR absorption at 3392 cm^{-1} also suggested the presence of an amino group. The ^1H NMR spectrum of **1** showed one amino proton (δ_{H} 5.58), three olefinic protons (δ_{H} 6.35, 5.42, and 5.14), and six methyl signals (δ_{H} 1.53, 0.99, 0.93, 0.84, and 0.97×2). The ^{13}C NMR spectrum of **1** displayed resonances for two conjugated carbonyl carbons (δ_{C} 185.2 and 183.6), three olefinic quaternary carbons (δ_{C} 151.2, 146.2, and 144.0), three olefinic methines (δ_{C} 131.7, 120.7, and 98.3), six methyls (δ_{C} 16.8, 17.8, 18.1, 20.0, and 20.3×2), six methylenes (δ_{C} 49.9, 36.1, 35.7, 27.53, 26.5, and 19.4), three aliphatic methines (δ_{C} 47.2, 37.0, and 27.50), and two aliphatic quaternary carbons (δ_{C} 38.5 and 43.1) (Table 1). The two carbonyl carbons and six olefinic carbons accounted for five out of the eight degrees of unsaturation, suggesting the presence of three rings in **1**.



Interpretation of the ^1H - ^1H COSY and HSQC spectra of **1** led to the assignment of three isolated spin systems: (a) C_{10} - C_1 - C_2 - C_3 - C_4 - C_{11} , (b) C_6 - C_7 - C_8 - C_{13} , and (c) an isobutylamino group, as shown in

Fig 1. The connectivity of the three spin systems with the remaining atoms was deduced from the correlations observed in the HMBC spectrum. HMBC correlations from H_3 -12 to C-4, C-5, C-6, and C-10, from H_3 -13 to C-7, C-8, and C-9, and from H_3 -14 to C-8, C-9, C-10, and C-15 determined the decalin moiety with four methyl groups (H_3 -11, H_3 -12, H_3 -13, and H_3 -14) attached at C-4, C-5, C-8, and C-9, respectively (Fig 1). Further HMBC correlations of H_2 -15 with C-8, C-9, C-10, C-16, C-17, and C-21, H-18 with C-16 and C-20, as well as H-21 with C-17 and C-19 suggested that the benzoquinone ring was attached at C-15. In addition, the HMBC cross-peaks between H_2 -22 and C-19 indicated the isobutyl amino group was tethered at C-19, confirmed by the NOESY correlations of H_2 -22/H-18 and NH/H-18. Thus, the planar structure of **1** was determined as 19-isobutylaminoavarone.

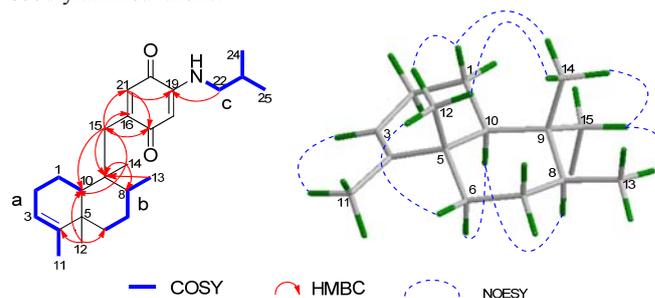


Fig. 1 Selected ^1H - ^1H COSY and HMBC correlations of dysidaminone A (**1**) (left). Selected NOESY correlations and relative configuration for the sesquiterpenoid moiety in **1** (right).

The relative configuration was established by the correlations observed in the NOESY spectrum of **1** (Fig 1). The NOESY correlations of H_3 -12 with H_3 -14 and H-10 with H-8 indicated the *trans* fusion of the rings A and B. The NOESY correlations of H_3 -13/ H_3 -14, H-15 β / H_3 -13, H_3 -12/H-6 β , and H-1 β / H_3 -14 indicated these methyl groups and protons are β -oriented, while the correlations of H-10/H-8/H-6 α and H-1 α /H-10/H-15 α revealed that these protons are α -oriented. The single crystal X-ray diffraction analysis with Cu K α irradiation confirmed the proposed structure of **1** and also determined the absolute configuration as 5*S*,8*S*,9*R*,10*S* (Fig 2). In addition, the CD spectrum of **1** showed a positive Cotton effect at 264 nm and a negative Cotton effect at 305 nm (Fig 5).

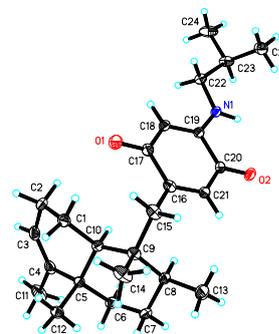


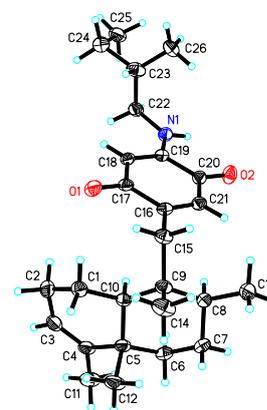
Fig. 2 ORTEP drawing of dysidaminone A (**1**).

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for Dysidaminones A–E (**1–5**) in CDCl_3 .^a

	1			2			3			4			5		
position	δ_{H} (<i>J</i> in Hz)	δ_{C}													
1 α	1.88 m ^b	19.4	1.87 m ^b	19.4	1.80 m	19.0	1.91 d (10.0)	19.4	1.04 m	19.4					
1 β	1.48 m		1.48 m		1.48 m		1.49 m		1.49 m						
2 α	1.87 m ^b	26.5	2.03 m	26.5	1.88 m	26.4	1.88 m	26.6	1.83 m	26.5					
2 β	2.01 m		1.90 m ^b		2.03 m		2.02 m		2.02 m						
3	5.14 brs	120.7	5.15 brs	120.7	5.12 brs	120.4	5.16 brs	120.8	5.12 brs	120.6					
4		144.0		144.0		144.0		144.1		144.0					
5		38.5		38.5		38.3		38.6		38.4					
6 α	1.04 m	36.1	1.06 m	36.1	1.03 m	36.0	1.05 m	36.2	1.06 m	36.0					
6 β	1.62 brd (13.2)		1.64 dt (13.0, 3.0)		1.64 dt (12.5, 3.0)		1.64 dt (12.6, 3.0)		1.63 m						
7	1.38 2H m	27.53	1.38 2H m	27.5	1.38 2H m	27.4	1.38 2H m	27.6	1.38 2H m	27.4					
8	1.29 m	37.0	1.29 m	37.1	1.16 m	37.1	1.30 m	37.0	1.27 m	36.6					
9		43.1		43.1		42.0		42.8		42.1					
10	1.11 d (12.0)	47.2	1.11 brd (11.5)	47.2	1.06 d (12.0)	46.8	1.11 brd (12.0)	47.2	1.02 m	46.8					
11	1.53 3H brs	18.1	1.54 3H brs	18.1	1.52 3H brs	18.0	1.54 3H brs	18.1	1.53 3H brs	18.0					
12	0.99 3H s	20.0	1.00 3H s	20.0	0.98 3H s	19.9	1.00 3H s	20.0	1.00 3H s	20.0					
13	0.93 3H d (6.6)	16.8	0.94 3H d (6.6)	16.8	0.88 3H d (7.5)	16.6	0.95 3H d (6.6)	16.8	0.93 3H d (6.5)	16.7					
14	0.84 3H s	17.8	0.84 3H s	17.8	0.81 3H s	17.6	0.84 3H s	17.9	0.83 3H s	17.7					
15 α	2.64 d (13.2)	35.7	2.65 d (13.5)	35.7	2.62 d (13.5)	35.4	2.61 d (13.2)	35.1	2.62 d (13.8)	35.0					
15 β	2.46 d (13.2)		2.47 d (13.5)		2.45 d (13.5)		2.44 d (13.2)		2.37 d (13.8)						
16		151.2		151.3		144.6		148.4		142.0					
17		185.2		185.2		185.7		185.1		184.0					
18	5.42 s	98.3	5.42 s	98.2		152.7	5.53 s	104.5		146.9					
19		146.2		146.2	5.49 d (2.5)	103.6		150.0	5.41 d (2.4)	97.7					
20		183.6		183.6		185.1		185.2		185.5					
21	6.35 s	131.7	6.36 s	131.7	6.29 d (2.5)	137.1	6.26 s	134.0	6.37 d (2.4)	139.8					
22a	2.88 2H t (6.6)	49.9	3.00 m	48.2	3.05 3H s	42.0	3.12 3H s	42.1	2.89 2H t (6.4)	50.2					
22b			2.87 m												
23	1.93 m	27.50	1.72 m	33.9	3.05 3H s	42.0	3.12 3H s	42.1	1.94 m	27.5					
24a	0.97 3H d (6.6)	20.3	1.46 m	27.2					0.97 3H d (6.7)	20.3					
24b			1.22 m												
25	0.97 3H d (6.6)	20.3	0.93 3H t (6.6)	11.2					0.97 3H d (6.7)	20.3					
26			0.96 3H d (7.0)	17.4											
NH	5.58 brs		5.57 brs						5.70 brs						

^a Assignments of the ^{13}C and ^1H signals were made on the basis of HSQC spectral data. ^b Overlapped signals.

Dysidaminone B (**2**) showed a pseudomolecular ion at m/z 398.3062 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{26}\text{H}_{40}\text{NO}_2$, 398.3059) in its HRESIMS, appropriate for a molecular formula of $\text{C}_{26}\text{H}_{39}\text{NO}_2$. The NMR data of **2** were nearly identical to those for **1** and supported an avarone core, except for the resonances of 2-methylbutyl amino group ($\delta_{\text{C}}/\delta_{\text{H}}$ 48.2/3.00 and 2.87, 33.9/1.72, 27.2/1.46 and 1.22, 11.2/0.93, and 17.4/0.96). The presence of the amino group was confirmed by the ^1H - ^1H COSY correlations of NH/ H_2 -22, H_2 -22/ H -23, H -23/ H -24a, H -24b/ H_3 -25, and H -23/ H_3 -26. In the HMBC spectrum of **2**, correlations from H_2 -22 to C-19 indicated that the amino group was attached at C-19. This assignment was supported by the NOESY correlations of H -18/ H_2 -22 and H -18/NH. Detailed NOESY analysis suggested that compound **2** had the same relative configurations at C-5, C-8, C-9, and C-10 as those of **1** (Fig S18 in ESI). Furthermore, the single crystal X-ray diffraction analysis revealed that **2** had the same absolute configurations at the four chiral centers as those of **1** (Fig 3), confirmed by the similarity of the

**Fig. 3** ORTEP drawing of dysidaminone A (**2**).

CD spectra of **1** and **2** (Fig 5). Additionally, the X-ray diffraction analysis of **2** also determined the absolute configuration of C-23 in the 2-methylbutyl amino group as *S*, which was identical with those of nakijiquinones K and L.²⁴

The positive HRESIMS of dysidaminone C (**3**) provided a pseudomolecular ion at m/z 356.2588 $[M + H]^+$, consistent with the molecular formula of $C_{23}H_{33}NO_2$. The similarity of the 1H and ^{13}C NMR data between **1** and **3** indicated compound **3** possesses the same avarone carbon skeleton, with the exception of two overlapped downfield methyl resonances (δ_H/δ_C 3.05 \times 2/40.2 \times 2) assigned as a unique *N,N*-dimethyl amino group.²⁵ In addition, the 1H NMR spectrum of **3** (Table 1) displayed two *m*-coupled protons at δ_H 5.49 ($J = 2.5$ Hz) and 6.29 ($J = 2.5$ Hz), which suggested the different substitution pattern of the amino group in the quinone unit from **1** and **2**. The HMBC correlations from H₃-22 and H₃-23 to C-18 and from H-21 to C-17 and C-19 placed the *N,N*-dimethyl amino group at C-18, instead of at C-19 in **1** and **2**. Detailed analysis of the NOESY data of **3** revealed the same relative configuration as those of **1** and **2**. Furthermore, the characteristic positive Cotton effect at 258 nm and negative Cotton effect at 310 nm in the CD spectrum suggested **3** may possess the same absolute configuration as those of **1** and **2** (Fig 5). Dysidaminone C (**3**) represents the first example of a sesquiterpene quinone that harbors a *N,N*-dimethyl amino group.

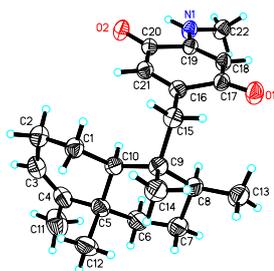


Fig. 4 ORTEP drawing of 19-methylaminoavarone (**15**).

Dysidaminone D (**4**) exhibited the same molecular formula of $C_{23}H_{33}NO_2$ ($[M + H]^+$ m/z 356.2586) as that of **3**. A detailed analysis of the 1D and 2D NMR spectroscopic data revealed that **4** bears the same avarone skeleton and *N,N*-dimethyl amino group as well, suggesting an isomer of compound **3**. However, two singlets for the quinone protons at δ_H 5.53 (H-18) and 6.26 (H-21) in the 1H NMR spectrum of **4** indicated the *N,N*-dimethyl amino group was located at C-19, instead of C-18 in **3**, which was confirmed by the HMBC correlations of both H₃-22 and H₃-23 with C-19. The NOESY correlations (Fig S40 in ESI) and the similarity of their CD spectra between **3** and **4** suggested compound **4** shares the same absolute configurations with those of **1–3** (Fig 5).

Dysidaminone E (**5**), an isomer of **1**, gave the same molecular formula of $C_{25}H_{37}NO_2$, as assigned by the HRESIMS ion at m/z 384.2903 $[M + H]^+$. The NMR data (Table 1) indicated that compound **5** possesses the same avarone skeleton and an isobutyl amino group as in **1**, and the major difference was the location of the isobutyl amino group. Again, the two doublet quinone protons at δ_H 5.41 (H-19) and 6.37 (H-21) with the same meta coupling constant of 2.4 Hz, combined with the HMBC correlation from H₂-22 with C-

18, and NOESY correlations of NH/H-19 and H-19/H₂-22 suggested that the isobutyl amino group was positioned at C-18. The relative and absolute configurations of dysidaminone E (**5**) were consistent with **1**, verified by the similar NOESY correlations and similar CD Cotton effects of **1** and **5**, as shown in Fig 5 and 6.

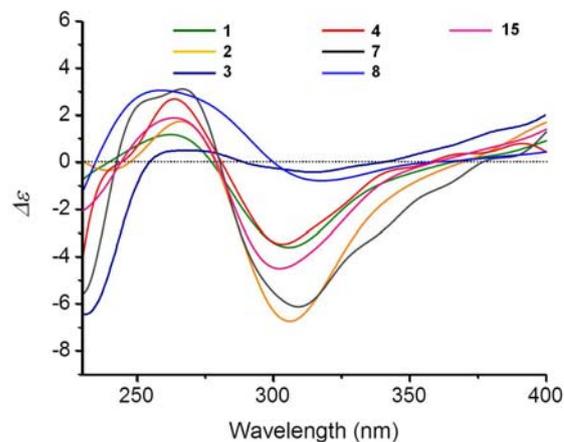


Fig. 5 Experimental CD spectra of dysidaminones A–D (**1–4**), G (**7**), H (**8**), and 19-methylaminoavarone (**15**).

Dysidaminone F (**6**) gave the same molecular formula $C_{26}H_{39}NO_2$ as that of **2** by the HRESIMS ion at m/z 420.2876 $[M + Na]^+$. The 1H and ^{13}C NMR spectroscopic data of **6** closely resembled those of **2**, indicative of the same avarone core and 2-methylbutyl amino group in **6**. Distinct from the two singlet quinone proton signals of **2**, two doublet quinone protons at δ_H 5.42 and 6.37 with the meta coupling constant of 2.5 Hz were observed for **6**, implying the amino group was placed at C-18 in **6** (Table 1) instead of C-19 in **2**, which was supported by the HMBC correlation of H₂-22/C-18 as well as the NOESY correlations from H₂-22 and NH to H-19. The similar Cotton effects in the CD spectra of **2** and **6** suggested the same stereochemistry at C-5, C-8, C-9, and C-10 of **6** as those of **1–5** (Fig 6). In view of the biosynthetic relationship, it is most likely that compound **6** possesses the same absolute configuration at C-23 as that of dysidaminone B (**2**).

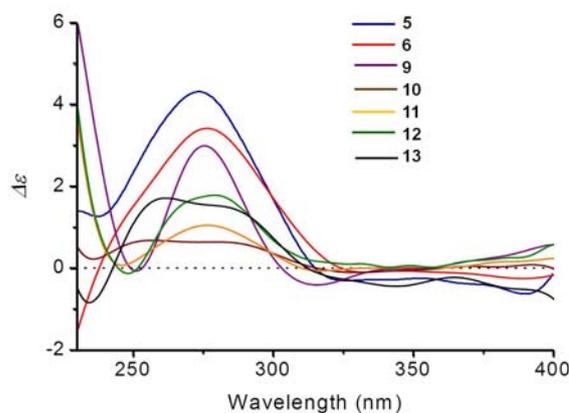


Fig. 6 Experimental CD spectra of dysidaminones E (**5**), F (**6**), and J–M (**10–13**).

Table 2. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for Dysidaminones F–J (**6–10**) in CDCl_3 .^a

	6		7		8		9		10	
position	δ_{H} (J in Hz)	δ_{C}								
1 α	1.86 m ^b	19.4	1.90 m ^b	19.4	1.92 m	22.7	2.01 m	22.7	1.82 m ^b	22.4
1 β	1.51 m		1.49 m		1.49 m		1.49 m		1.54 m	
2 α	2.02 m	26.5	1.87 m ^b	26.5	1.18 m	28.1	1.31 m	28.1	1.86 m ^b	28.2
2 β	1.81 m ^b		2.03 m		1.85 m		1.88 m		1.27 m	
3 α	5.13 brs	120.6	5.15 brs	120.7	2.08 m	32.9	2.09 m	32.9	2.29 m	32.8
3 β					2.31 td (13.5, 5.0)		2.32 td (13.5, 5.0)		2.07 m	
4		144.0		144.0		159.6		160.0		159.6
5		38.5		38.6		40.2		40.4		40.2
6 α	1.06 m	36.0	1.05 m	36.1	1.39 m	36.7	1.37 m	36.8	1.12 m	36.8
6 β	1.64 dt (13.0, 3.0)		1.64 dt (12.6, 3.0)		1.54 m		1.54 m		1.49 dd (13.2, 3.0)	
7	1.36 2H m	27.4	1.38 2H m	27.6	1.43 2H m	27.4	1.42 m	27.6	1.42 2H,m	27.5
8	1.27 m	36.6	1.29 m	37.1	1.26 m	36.9	1.26 m	37.4	1.39 m	37.4
9		42.1		43.1		42.5		43.5		42.5
10	1.04 d (11.5)	46.8	1.10 m	47.3	0.77 dd (12.0, 2.0)	49.0	0.88 m	49.4	0.79 dd (12.6, 2.4)	49.3
11a	1.53 3H brs	18.0	1.54 3H d (1.2)	18.0	4.45 s	103.1	4.46 s	102.9	4.46 s	103.1
11b					4.44 s		4.45 s		4.45 s	
12	1.00 3H s	20.0	1.00 3H s	20.0	1.04 3H s	20.6	1.05 3H s	20.6	1.04 3H s	20.6
13	0.93 3H d (6.6)	16.7	0.94 3H d (6.6)	16.8	0.92 3H d (7.5)	16.8	0.94 3H d (7.5)	17.0	0.89 3H d (6.6)	16.7
14	0.83 3H s	17.7	0.84 3H s	17.8	0.84 3H s	17.6	0.85 3H s	17.7	0.83 3H s	17.5
15 α	2.63 d (13.5)	35.0	2.64 d (13.2)	35.8	2.54 d (13.5)	34.9	2.58 d (13.0)	35.7	2.58 d (13.8)	35.4
15 β	2.38 d (13.5)		2.47 d (13.2)		2.34 d (13.5)		2.44 d (13.0)		2.39 d (13.8)	
16		142.0		151.2		142.0		151.2		144.6
17		184.0		185.3		183.8		185.1		185.0
18		147.0	5.47 s	98.6		147.8	5.42 s	98.2		152.8
19	5.42 d (2.5)	97.6		145.9	5.40, d (2.5)	97.6		147.1	5.49 d (2.5)	103.7
20		185.5		183.4		185.4		183.5		185.7
21	6.37 d (2.5)	139.8	6.35 s	131.8	6.32 d (2.5)	139.8	6.30 s	131.7	6.25 d (2.4)	137.0
22a	3.00 m	48.5	3.35 2H q (7.2)	43.5	2.83 3H d (5.5)	29.1	2.84 3H d (5.5)	28.9	3.05 3H s	41.9
22b	2.87 m									
23	1.73 m	33.8	2.93 2H t (7.2)	34.4					3.05 3H s	41.9
24a	1.43 m	27.2		137.9						
24b	1.22 m									
25	0.92 3H t (6.6)	11.1	7.20 d (7.8)	128.6						
26	0.95 3H d (7.0)	17.4	7.33 t (7.8)	128.8						
27			7.25 t (7.8)	126.9						
28			7.33 t (7.8)	128.8						
29			7.20 d (7.8)	128.6						
NH	5.69 brs		5.55 brs		5.67 brs		5.58 brs			

^a Assignments of the ^{13}C and ^1H signals were made on the basis of HSQC spectral data. ^b Overlapped signals.

The HRESIMS data of dysidaminone **7** suggested a molecular of $\text{C}_{29}\text{H}_{37}\text{NO}_2$, an isomer to 18-phenethylaminoavarone (**18**).²⁶ Detailed analysis of the 1D and 2D NMR spectroscopic data of **7** indicated that compound **7** has the same avarone skeleton and phenethyl amino group as those of **18**, expect for two singlet quinone protons at δ_{H} 5.47 and 6.35 in the ^1H NMR spectrum of **7**. This difference was verified by the HMBC correlation of H_2 -22/C-19 and the NOESY correlations of H-18/ H_2 -22 and H-18/NH, supporting the assignment of the phenethyl amino group at C-19. The relative and absolute configurations of dysidaminone **7** were determined the same as those of **1–6** by the identical NOESY correlations and the similar CD Cotton effects to those of **1–6** as shown in Fig 5.

Dysidaminone **8** was isolated as purple-red powders. The HRESIMS showed a quasimolecular ion at m/z 364.2252 [$\text{M} + \text{Na}$]⁺, consistent with a molecular formula of $\text{C}_{22}\text{H}_{31}\text{NO}_2$. The ^1H and ^{13}C NMR data and the information from the 2D NMR studies indicated that compound **8** was a sesquiterpene aminoquinone with a methyl amino group in the quinone unit. However, the olefinic methyl (CH_3 -11) and the olefinic methine (CH-3) observed for compounds **1–7** was missing in **8**, and instead, an exomethylene group and a methylene group were detected at δ_{C} 103.1/ δ_{H} 4.46, 4.45 (CH_2 -11) and δ_{C} 32.9/ δ_{H} 2.31, 2.08 (CH_2 -3). In the HMBC spectrum of **8**, correlations from the two exomethylene protons H_2 -11 to C-3, C-4, and C-5 suggested the presence of a exocyclic double bond at Δ .^{4,11}

Table 3. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for Dysidaminones K–M (**11–13**) in CDCl_3 .^a

	11		12		13	
position	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1 α	1.92 m ^b	22.7	1.92 m ^b	22.7	1.91 m ^b	22.7
1 β	1.48 m		1.49 m		1.49 dd (12.6, 3.0)	
2 α	1.16 m	28.1	1.18 m	28.1	1.16 m	28.1
2 β	1.86 m ^b		1.87 m ^b		1.88 m ^b	
3 α	2.09 m	32.9	2.09 m	32.9	2.08 m	32.9
3 β	2.30 td (13.8, 3.6)		2.31 td (13.8, 3.6)		2.30 m	
4		160.0		160.0		160.0
5		40.4		40.4		40.4
6 α	1.40 m	36.8	1.39 m	36.8	1.39 m	36.8
6 β	1.54 m		1.53 m		1.55 m	
7	1.42 2H m	27.62	1.41 2H m	27.6	1.43 2H m	27.6
8	1.26 m	37.4	1.26 m	37.4	1.26 m	37.4
9		43.5		43.5		43.51
10	0.88 m	49.4	0.88 m	49.4	0.86 m	49.4
11a	4.46 m	102.9	4.61 s	102.9	4.46 s	102.9
11b	4.45 m		4.45 s		4.45 s	
12	1.04 3H s	20.7	1.05 3H s	20.7	1.05 3H s	20.7
13	0.94 3H d (7.2)	17.0	0.94 3H d (7.0)	17.0	0.94 3H d (6.6)	17.0
14	0.85 3H s	17.7	0.85 3H s	17.7	0.85 3H s	17.7
15 α	2.57 d (13.2)	35.7	2.57 d (13.2)	35.7	2.57 d (13.2)	35.7
15 β	2.43 dd (13.2, 1.2)		2.43 d (13.2)		2.43 d (13.2)	
16		151.2		151.2		151.1
17		185.1		185.1		185.2
18	5.41 s	98.3	5.42 s	98.3	5.46 s	98.6
19		146.2		146.2		145.8
20		183.6		183.6		183.4
21	6.30 s	131.7	6.30 s	131.6	6.29 s	131.7
22a	2.89 2H t (6.0)	50.0	3.00 m	48.2	3.55 2H q (6.6)	43.46
22b			2.87 m			
23	1.94 m	27.56	1.72 m	33.9	2.93 2H t (7.2)	34.4
24a	0.98 3H d (6.6)	20.3	1.46 m	27.2		137.9
24b			1.24 m			
25	0.98 3H d (6.6)	20.3	0.93 3H t (6.6)	11.2	7.20 d (7.2)	128.6
26			0.96 3H d (7.0)	17.4	7.33 t (7.2)	128.9
27					7.25 t (7.2)	126.9
28					7.33 t (7.2)	128.9
29					7.20 d (7.2)	128.6
NH	5.58 brs		5.69 brs		5.55 brs	

^a Assignments of the ^{13}C and ^1H signals were made on the basis of HSQC spectral data. ^b Overlapped signals.

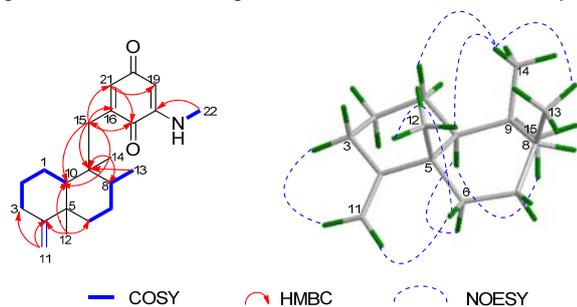


Fig. 7 Selected ^1H - ^1H COSY and HMBC correlations of dysidaminone H (**8**) (left). Selected NOESY correlations and relative configuration for the sesquiterpenoid moiety in **8** (right).

in **8** instead of the cyclohexenyl double bond $\Delta^{3,4}$ in **1–7**, which was further supported by the ^1H - ^1H COSY correlations of H-1 α /H-2 β , H-1 α /H-2 α , H-2 β /H-3 α , and H-2 α /H-3 β as well as HMBC correlations from H₃-12 to C-4, C-5, C-6, and C-10 (Fig 7). The regioisomerization of the carbon-carbon double bond at C-4 showed that **8** possesses a neoavarone skeleton instead of the avarone skeleton in **1–7**.²⁷ In addition, placement of the *N*-methyl at C-18 was supported by the HMBC correlation of *N*-methyl at δ_{H} 2.83 with C-18 at δ_{H} 147.8. The stereocenters of **8** were indirectly established on the basis of coupling constants, NOESY correlations (Fig 7), and close NMR similarity of **8** to **1–7**. The large coupling constant of H-10 ($J = 12$ Hz) coupled with the NOESY correlations of H₃-13/H₃-14, H₃-12/H₃-14, and H-10/H-8 revealed **8** had the same relative

configurations with those of **1–7**, as depicted in Fig 7. In the CD spectrum of **8**, the positive Cotton effect at 264 nm and negative Cotton effect at 315 nm as shown in Fig 5 was also identical to **1–7**, therein supporting the same absolute configuration *5S,8S,9R,10S* for **8**.

Dysidaminone I (**9**) bears the same molecular formula and the same neoavarone skeleton as compound **8** evidenced by its HRESIMS, 1D, and 2D NMR spectra, while the singlet quinone proton H-21 in the ¹H NMR spectrum of **9** different with the doublet corresponding proton (*J* = 2.5 Hz) of **8** (Table 2), suggested the methyl amino group was attached at C-19 in **9**, instead of C-18 in **8**. The similar NOESY correlations as well as the positive Cotton effect at 281 nm and negative Cotton effect at 304 nm in the CD spectrum of **9** established the same stereochemistry with that of **8** (Fig 6).

Dysidaminone J (**10**) showed a molecular formula of C₂₃H₃₃NO₂ as determined by the HRESIMS ion at *m/z* 356.2588 [M + H]⁺, different with that of **8** by addition of CH₂. Analysis of its ¹H and ¹³C NMR data (Table 2) implied that compound **10** is a congener of **8**, which possesses a neoavarone skeleton with a *N,N*-dimethyl amino group. The HMBC correlations from H₃-22 and H₃-23 to C-18 determined the placement of *N,N*-dimethyl amino group at C-18. The identical NOESY correlations and the similar CD absorptions (positive at 271 nm and negative at 312 nm) of **10** assigned the same relative and absolute configurations as those of **1–9** (Fig 6).

Dysidaminones K (**11**) and L (**12**) showed molecular formulas of C₂₅H₃₇NO₂ and C₂₆H₃₉NO₂, as determined by the HRESIMS ions at *m/z* 384.2096 [M + H]⁺ and *m/z* 420.2880 [M + Na]⁺, respectively, which suggested that they are respective isomers of dysidaminones A (**1**) and B (**2**). The ¹H and ¹³C NMR spectra of **11** and **12** (Table 3) were also similar to those of **1** and **2**, including the same isobutyl amino and 2-methylbutyl amino groups and their substitution patterns. The major differences were the positions of the C-4 double bonds, the olefinic methyls (CH₃-11) and the olefinic methines (CH-3) groups in **1** and **2** were replaced by exomethylene and methylene groups in **11** and **12** (Table 3), which was deduced on the basis of the corresponding HSQC, HMBC, and NOESY experiments of **11** and **12**. Therefore the structures of **11** and **12** were established as 19-isobutylaminoneoavarone and 19-2-methylbutylaminoneoavarone, respectively. Both of compounds **11** and **12** had the same stereochemistry at C-5, C-8, C-9, and C-10 as those of **1** and **2**, which were suggested by their similar CD absorptions with those as shown in Fig 6. The absolute configuration of C-23 in **12** might also be assigned as *S* on account of the biosynthetic relationship between compounds **2**, **6**, and **12**.

Dysidaminone M (**13**) gave a molecular formula of C₂₉H₃₇NO₂ established by the HRESIMS ion at *m/z* 432.2906 [M + H]⁺. Comparison of ¹H, ¹³C and HSQC NMR spectra indicated compound **13** possesses a neoavarone skeleton and a phenethyl amino group (Table 3). The singlet peaks of proton H-21 (δ_{H} 6.29, s) and H-18 (δ_{H} 5.46, s) combined with the HMBC correlation from H₂-22 (δ_{H} 3.55) to C-19 (δ_{C} 145.8) assigned the placement of the phenethyl amino group at C-19. The NOESY correlations and CD spectrum analysis revealed that **13** possesses the same relative and absolute configurations as those of **1–12** (Fig 6).

The two red spots exhibited on the TLC of CH₂Cl₂-soluble fraction were purified and finally determined as two major

metabolites, 18-methylaminoavarone (**14**)²⁸ and 19-methylaminoavarone (**15**).²⁹ Additional to the two known compounds, four other known ones, 18-aminoavarone (**16**), 19-aminoavarone (**17**),²⁶ 18-phenethylaminoavarone (**18**),²⁶ and popolohuanone D (**19**),³⁰ were also obtained, and their structures were determined by comparison of their MS and NMR spectroscopic data with the reported values in literature.

Table 4. Cytotoxicity against mouse B16F10 melanoma cells and human NCI-H929 myeloma, HepG2 hepatoma, and SK-OV-3 ovarian cancer cells as well as the inhibitory activity on NF- κ B of compounds **1–19**.

No.	IC ₅₀ (μ M)				
	NCI-H929	HepG2	B16F10	SK-OV-3	NF- κ B
1	15.6	21.8	65.7	73.4	>10
2	36.4	68.9	52.9	31.8	>10
3	0.57	0.45	8.71	5.25	0.22
4	5.78	15.4	32.7	18.5	>10
5	1.13	0.94	9.52	4.54	0.27
6	7.19	8.95	5.39	5.40	1.92
7	16.3	18.4	35.6	27.8	>10
8	0.71	0.62	2.45	9.65	0.23
9	1.39	0.88	6.28	6.22	3.14
10	0.87	0.45	1.43	2.89	0.11
11	4.58	7.86	8.14	9.41	8.62
12	5.18	5.81	7.56	8.41	>10
13	9.21	9.68	4.92	5.83	>10
14	0.63	1.42	4.51	6.42	0.06
15	2.70	7.65	3.26	9.38	>10
16	0.88	0.68	3.64	8.15	0.05
17	5.86	3.67	6.31	4.81	2.63
18	1.76	7.13	4.73	5.88	1.52
19	9.24	35.4	>100	>100	>10
5-fluorouracil ^a	0.39	7.64	1.23	1.72	–
rocaglamide	–	–	–	–	0.12

^a 5-Fluorouracil and rocaglamide were used as positive controls.

All compounds obtained in this study from *D. fragilis* were tested in terms of their cytotoxicity against mouse B16F10 melanoma cells and human NCI-H929 myeloma, HepG2 hepatoma, and SK-OV-3 ovarian cancer cell lines, using 5-fluorouracil as positive control. Compounds **3**, **5**, **8**, **10**, **14**, and **16** showed cytotoxicity toward these four cell lines (Table 4). Previous structure-activity relationship (SAR) analysis have shown that sesquiterpene quinones with *exo*-olefin ($\Delta^{4,11}$) exhibited more potent cytotoxicity than the *endo*-olefin ($\Delta^{3,4}$) ones,³¹ which was confirmed by the cytotoxicity of $\Delta^{3,4}$ *endo*-olefin compounds **1** and **2** to their corresponding $\Delta^{4,11}$ *exo*-olefin ones **11** and **12**. It is also interesting to note that the 18-amio group in quinone substructure is a key structural requirement for sesquiterpene aminoquinones to show cytotoxicity, such as compounds **3**, **5**, **6**, **14**, and **16** showed much more potent activity than the 19-amiosubstituted one, **4**, **1**, **2**, **15**, and **17**. In addition, we also observed that the activity decreases as the length of amino chain increases, such as **3**, **5**, **6**, and **14**.

To further investigate the bioactivity of these compounds, all the nineteen chemicals were tested in NF- κ B inhibition assay, using rocaglamide as positive control. It is intriguing to find that the six

most cytotoxic compounds **3**, **5**, **8**, **10**, **14**, and **16** showed inhibitory activity with IC₅₀ values of 0.05–0.27 μ M (Table 4).

Conclusions

In summary, nineteen sesquiterpene aminoquinones, including thirteen new ones, dysidaminones A–M (**1**–**13**), and six known ones (**14**–**19**) were isolated from the South China Sea sponge *D. fragilis*. The new structures were determined by extensive spectroscopic analyses, and their absolute configurations were assigned by single-crystal X-ray diffraction and CD spectra. Cytotoxicity of all these compounds were evaluated, and Dysidaminones C (**3**), F (**5**), J (**8**), L (**10**), 18-methylaminoavarone (**14**), and 18-aminoavarone (**16**) showed cytotoxicity against four cancer cell lines. In addition, these six compounds also showed NF- κ B inhibitory activity. Preliminary SAR analysis showed that sesquiterpene aminoquinones containing 18-amio group and $\Delta^{4,11}$ double bond in the decalin ring exhibited much more potent cytotoxicity and NF- κ B inhibitory activity than others. Therefore, dysidaminones appeared to offer a new type NF- κ B inhibitors, and further sesquiterpene aminoquinone NF- κ B inhibitors should contain both the exocyclic $\Delta^{4,11}$ double bond and the 18-amino group.

Experimental

General procedures

Optical rotation measurements were conducted on an Autopol I polarimeter (No. 30575, manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA) with a 10 cm length cell operating at $\lambda = 589$ nm, corresponding to the Sodium D line at room temperature, however, the dark purple-red solution of the test compounds made it difficult to obtain the accurate optical values, therefore the optical values of new compounds were not provided in this section. UV and IR (KBr) spectra were recorded on a Hitachi U-3010 spectrophotometer and Jasco FTIR-400 spectrometer, respectively. CD spectra were obtained on a Jasco J-715 spectropolarimeter. ¹H, ¹³C, DEPT135, ¹H-¹H COSY, HSQC, HMBC, and NOESY NMR spectra were recorded at room temperature on a Bruker Avance DRX-600 MHz NMR spectrometer with CDCl₃ as the solvent and internal standard. Spectra were referenced to residual solvent signals with resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 7.26/77.0 for CDCl₃. ESIMS were obtained using a Finnigan MAT 95 spectrometer, and HRESIMS were measured on an Agilent 6210 LC/MSD TOF mass spectrometer. Column chromatography was conducted using silica gel (65 \times 250 or 230 \times 400 mesh). Analytical thin-layer chromatography (TLC) systems were performed on percolated silica gel 60 F₂₅₄ plates. Sephadex LH-20 was purchased from Amersham Pharmacia Biotech AB, Uppsala, Sweden. Purification of the compounds was performed using a Waters Alliance 2695 separation module equipped with a Waters 2998 Photodiode Array (PDA) detector. All chemicals were of analytical grade, solvents for open column chromatography and MPLC were also analytical grade, whereas solvents for HPLC were chromatographic grade. MPLC and HPLC were performed with columns of 50 μ m and 5 μ m ODS, respectively.

Collection and identification of the animal material

Samples of *Dysidea fragilis* were collected along the coast of Yongxing Island in South China Sea on April 11th, 2011. The voucher number for this collection is XD10403, and a voucher sample is maintained at the Key Laboratory for Marine Drugs, Department of Pharmacy, Renji Hospital, Shanghai Jiao Tong University School of Medicine.

Extraction and isolation

The animals (1.2 kg, dry weight) were soaked in 95% EtOH repeatedly to give 92.9 g extract. The extract was dissolved in 1 L water, and partitioned with the same volume of CH₂Cl₂ four times to yield 32 g CH₂Cl₂-solvent extract, which was partitioned between 90% aqueous MeOH and *n*-hexane to give 12 g *n*-hexane fraction and 20 g aqueous MeOH fraction. After the aqueous MeOH fraction was dissolved in 1L 60% aqueous MeOH and then partitioned by the same volume of CH₂Cl₂ five times to afford 10.1 g CH₂Cl₂-solvent fraction. The 10.1 g CH₂Cl₂-solvent fraction was subjected to a silica gel chromatography column elucidated with gradient *n*-hexane and EtOAc, yielding 12 subfractions (DA–DL). The sixth fraction DF (1.58 g) was subjected to size-exclusion chromatography Sephadex LH-20 eluted with CH₂Cl₂/MeOH (1:1) to give five fractions DF1–DF5, and the fraction DF2 was further purified by reversed-phase HPLC (YMC, 10 \times 250 mm, 2 mL/min, 290 nm) with a elution of 95% MeOH, to give dysidaminones A (**1**, 7.5 mg), B (**2**) (3.3 mg), K (**11**, 5.0 mg), and L (**12**) and M (**13**) (2.1 mg). Fractions DF3 and DF4 were combined together to give DF34 on account of their consistent results in TLC analysis. DF34 was also separated by reversed-phase HPLC eluted by 90% MeOH to afford 18-methylaminoavarone (**14**, 56.1 mg), 19-methylaminoavarone (**15**, 48.2 mg), 18-aminoavarone (**16**, 7.9 mg), and 19-aminoavarone (**17**, 19.8 mg). The seventh fraction DG (2.81 g) was passed through ODS chromatography column eluted with gradient aqueous MeOH (from 30% to 100%) to give eight subfractions DG1–DG8. Following separation by ODS chromatography eluted with 90% MeOH, the fraction DG8 was passed through size-exclusion chromatography Sephadex LH-20 eluted with CH₂Cl₂/MeOH (1:1) to give four fractions DG8A–DG8D, and then the fraction DG8B was purified by reversed-phase HPLC with an elution of 92% MeOH at 280 nm, to give metabolites dysidaminones E (**5**, 7.5 mg), F (**6**) (4.1 mg), and M (**13**, 1.6 mg), while the reversed-phase HPLC purification of DG8C by 90% CH₃CN at the wavelength of 290 nm resulted in the isolation of dysidaminones G (**7**, 5.7 mg), I (**9**, 1.7 mg), and J (**10**, 2.8 mg). The fraction DK (1.2 g) was firstly separated by Sephadex LH-20 to give three fractions DK1–DK3, popolohuanone D (**19**, 21.3 mg) was crystallized from the first fraction DK1, and the other two fractions DK2 and DK3 were both isolated by reversed-phase HPLC eluted with 95% MeOH at 290 nm to afford dysidaminones C (**3**, 3.1 mg), D (**4**, 2.8 mg), H (**8**, 4.3 mg), and 18-phenethylaminoavarone (**18**, 15.2 mg).

DYSIDAMINONE A (**1**). purple-red needles; UV (MeOH) λ_{max} (log ϵ) 212 (3.95), 227 (3.75), 292 (3.55) nm; CD (MeOH) λ ($\Delta\epsilon$) 232 (-0.71), 264 (1.68), 305 (-3.91) nm; IR (KBr) ν_{max} 3392, 3357, 2959, 2926, 2855, 1667, 1630, 1593, 1514, 1467, 1221, 840 cm⁻¹; ¹H and

^{13}C NMR data, see Table 1 and Supplementary Information; ESIMS m/z 406.3 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 384.2900 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{25}\text{H}_{38}\text{NO}_2$, 384.2903).

DYSIDAMINONE B (2). purple-red needles; UV (MeOH) λ_{max} (log ϵ) 212 (4.13), 227 (4.08), 292 (3.87) nm; CD (MeOH) λ ($\Delta\epsilon$) 233 (-4.66), 273 (2.69), 305 (-7.77) nm; IR (KBr) ν_{max} 3357, 2960, 2927, 1730, 1667, 1631, 1593, 1514, 1463, 1220, 891 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1 and Supplementary Information; ESIMS m/z 398.3 $[\text{M} + \text{H}]^+$; HRESIMS m/z 398.3062 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{26}\text{H}_{40}\text{NO}_2$, 398.3059).

DYSIDAMINONE C (3). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 209 (3.92), 230 (3.79), 295 (3.43) nm; CD (MeOH) λ ($\Delta\epsilon$) 221 (-5.13), 232 (-9.13), 258 (0.45), 310 (-0.54) nm; IR (KBr) ν_{max} 3324, 2927, 2856, 1731, 1672, 1636, 1568, 1512, 1454, 1383, 1288, 800 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1 and Supplementary Information; ESIMS m/z 356.3 $[\text{M} + \text{H}]^+$; HRESIMS m/z 356.2588 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{23}\text{H}_{34}\text{NO}_2$, 356.2590).

DYSIDAMINONE D (4). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 210 (3.97), 227 (3.75), 292 (3.54) nm; CD (MeOH) λ ($\Delta\epsilon$) 235 (1.61), 241 (-2.22), 268 (1.03), 303 (4.11) nm; IR (KBr) ν_{max} 3356, 2926, 2855, 1731, 1667, 1628, 1593, 1515, 1451, 1224 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1 and Supplementary Information; ESIMS m/z 356.3 $[\text{M} + \text{H}]^+$; HRESIMS m/z 356.2586 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{23}\text{H}_{34}\text{NO}_2$, 356.2590).

DYSIDAMINONE E (5). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 212 (4.02), 226 (3.87), 294 (3.53) nm; CD (MeOH) λ ($\Delta\epsilon$) 221 (4.89), 228 (-0.14), 275 (4.51) nm; IR (KBr) ν_{max} 3395, 2959, 2926, 2855, 1732, 1669, 1634, 1589, 1511, 1463, 1247, 979, 802 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1 and Supplementary Information; ESIMS m/z 384.2 $[\text{M} + \text{H}]^+$; HRESIMS m/z 384.2905 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{25}\text{H}_{38}\text{NO}_2$, 384.2903).

DYSIDAMINONE F (6). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 212 (4.12), 227 (4.07), 293 (3.72) nm; CD (MeOH) λ ($\Delta\epsilon$) 235 (7.61), 251 (1.93), 272 (11.79), 327 (-0.49) nm; IR (KBr) ν_{max} 3392, 2960, 2928, 2873, 1738, 1671, 1634, 1589, 1510, 1463, 1249, 803 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2 and Supplementary Information; ESIMS m/z 398.4 $[\text{M} + \text{H}]^+$; HRESIMS m/z 420.2876 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{26}\text{H}_{39}\text{NO}_2\text{Na}$, 420.2878).

DYSIDAMINONE G (7). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 212 (4.17), 228 (4.11), 290 (3.85) nm; CD (MeOH) λ ($\Delta\epsilon$) 233 (-4.35), 268 (4.40), 306 (-6.94) nm; IR (KBr) ν_{max} 3357, 2960, 2926, 2856, 1730, 1666, 1630, 1593, 1513, 1464, 1222, 700 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2 and Supplementary Information; ESIMS m/z 432.2 $[\text{M} + \text{H}]^+$; HRESIMS m/z 432.2901 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{29}\text{H}_{38}\text{NO}_2$, 432.2903).

DYSIDAMINONE H (8). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 212 (4.04), 227 (3.94), 287 (3.62) nm; CD (MeOH) λ ($\Delta\epsilon$) 264 (3.34), 315 (-1.09) nm; IR (KBr) ν_{max} 3404, 3323, 2926, 2856, 1732, 1672, 1634, 1588, 1510, 1450, 1420, 1346, 1253, 1083, 892

cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2 and Supplementary Information; ESIMS m/z 364.2 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 364.2252 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{22}\text{H}_{31}\text{NO}_2\text{Na}$, 364.2252).

DYSIDAMINONE I (9). purple-red amorphous solid; UV (MeOH) λ_{max} (log ϵ) 211 (3.99), 227 (3.95), 289 (3.62) nm; CD (MeOH) λ ($\Delta\epsilon$) 232 (13.3), 246 (-2.02); 281 (4.01), 304 (-1.02), 315 (1.03) nm; IR (KBr) ν_{max} 3323, 2926, 2855, 1731, 1669, 1629, 1591, 1510, 1451, 1420, 1223, 1035, 798 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2 and Supplementary Information; ESIMS m/z 364.2 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 364.2250 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{22}\text{H}_{31}\text{NO}_2\text{Na}$, 364.2252).

DYSIDAMINONE J (10). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 213 (4.11), 228 (4.08), 294 (3.78) nm; CD (MeOH) λ ($\Delta\epsilon$) 210 (17.10), 222 (6.52), 226 (-3.08), 271 (2.07) nm; IR (KBr) ν_{max} 3312, 3083, 2926, 2856, 1738, 1673, 1634, 1578, 1512, 1450, 1258, 892 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2 and Supplementary Information; ESIMS m/z 356.2 $[\text{M} + \text{H}]^+$; HRESIMS m/z 356.2588 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{23}\text{H}_{34}\text{NO}_2$, 356.2590).

DYSIDAMINONE K (11). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 212 (4.06), 227 (3.91), 290 (3.70) nm; CD (MeOH) λ ($\Delta\epsilon$) 214 (15.55), 248 (-0.34), 275 (1.22) nm; IR (KBr) ν_{max} 3308, 3089, 2956, 2926, 2855, 1665, 1622, 1591, 1580, 1504, 1449, 1320, 1223, 894 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 3 and Supplementary Information; ESIMS m/z 384.3 $[\text{M} + \text{H}]^+$; HRESIMS m/z 384.2905 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{25}\text{H}_{38}\text{NO}_2$, 384.2903).

DYSIDAMINONE L (12). purple-red, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 212 (4.07), 227 (4.05), 291 (3.81) nm; CD (MeOH) λ ($\Delta\epsilon$) 211 (19.48), 251 (-0.33), 277 (1.85) nm; IR (KBr) ν_{max} 3357, 2956, 2927, 2855, 1731, 1667, 1631, 1590, 1514, 1463, 1220, 893 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 3 and Supplementary Information; ESIMS m/z 398.4 $[\text{M} + \text{H}]^+$; HRESIMS m/z 420.2880 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{26}\text{H}_{39}\text{NO}_2\text{Na}$, 420.2878).

DYSIDAMINONE M (13). purple-red amorphous solid; UV (MeOH) λ_{max} (log ϵ) 211 (4.16), 228 (4.09), 290 (3.91) nm; CD (MeOH) λ ($\Delta\epsilon$) 234 (-1.33), 269 (5.15), 326 (-0.59) nm; IR (KBr) ν_{max} 3311, 2958, 2926, 2855, 1729, 1665, 1626, 1593, 1508, 1454, 1322 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 3 and Supplementary Information; ESIMS m/z 432.2 $[\text{M} + \text{H}]^+$; HRESIMS m/z 432.2906 $[\text{M} + \text{H}]^+$ (calculated for $\text{C}_{29}\text{H}_{38}\text{NO}_2$, 432.2903).

X-ray Crystallographic Analysis Data of DYSIDAMINONE A (1). $\text{C}_{25}\text{H}_{37}\text{NO}_2$, $M = 383.56$, monoclinic, $P2_1$ (1), $a = 11.8655$ (2) \AA , $b = 6.34770$ (10) \AA , $c = 14.6161$ (2) \AA , $\alpha = \gamma = 90^\circ$, $\beta = 97.0310^\circ$ (10), $V = 1092.59$ (3) \AA^3 , $Z = 2$, $D_x = 1.166$ mg/m^3 , $F(000) = 420$, $\mu(\text{Cu-K}\alpha) = 0.559$ mm^{-1} , crystal dimensions $0.16 \times 0.05 \times 0.04$ mm^3 were used for measurement on a SMART CCD using graphite monochromated radiation ($\lambda = 1.54178$ \AA); 3165 unique reflections were collected to $\theta_{\text{max}} = 67.98^\circ$. The structure was solved by direct methods (Shelxs97) and refined by full-matrix least-squares on F^2 . Hydrogen atoms were located by the geometric calculation method and difference Fourier method. The final $R_1 = 0.0408$, $wR_2 = 0.1195$ ($w = 1/\sigma(F)^2$) and $S = 0.992$. The crystallographic data for dysidaminone A (1) have been

deposited at the Cambridge Crystallographic Data Centre with the deposition number 974398. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk).

X-ray Crystallographic Analysis Data of DYSIDAMINONE B (2). $C_{26}H_{39}NO_2$, $M = 397.58$, $P2_12_12_1$, $a = 11.9570$ (4) Å, $b = 12.5129$ (4) Å, $c = 15.4458$ (4) Å, $\alpha = \beta = \gamma = 90^\circ$, $V = 2310.95$ (12) Å³, $Z = 4$, $D_x = 1.143$ mg/m³, $F(000) = 872$, $\mu(\text{Cu-K}\alpha) = 0.544$ mm⁻¹, crystal dimensions $0.26 \times 0.20 \times 0.16$ mm³ were used for measurement on a SMART CCD using graphite monochromated radiation ($\lambda = 1.54178$ Å); 4200 unique reflections were collected to $\theta_{\text{max}} = 69.35^\circ$. The structure was solved by direct methods (Shelxs97) and refined by full-matrix least-squares on F^2 . Hydrogen atoms were located by the geometric calculation method and difference Fourier method. The final $R_1 = 0.0540$, $wR_2 = 0.1856$ ($w = 1/\sigma|F|^2$) and $S = 1.095$. The crystallographic data for dysidaminone B (2) have been deposited at the Cambridge Crystallographic Data Centre with the deposition number 974399. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk).

X-ray Crystallographic Analysis Data of 19-methylaminoavarone (15). $C_{22}H_{31}NO_2$, $M = 341.48$, Orthorhombic, $P2(1)$, $a = 7.2191$ (14) Å, $b = 7.6075$ (15) Å, $c = 35.255$ (7) Å, $\alpha = \beta = \gamma = 90^\circ$, $V = 1936.2$ (7) Å³, $Z = 4$, $D_x = 1.171$ mg/m³, $F(000) = 744$, $\mu(\text{Cu-K}\alpha) = 0.574$ mm⁻¹, crystal dimensions $0.21 \times 0.12 \times 0.08$ mm³ were used for measurement on a SMART CCD using graphite monochromated radiation ($\lambda = 1.54178$ Å); 3361 unique reflections were collected to $\theta_{\text{max}} = 66.99^\circ$. The structure was solved by direct methods (Shelxs97) and refined by full-matrix least-squares on F^2 . Hydrogen atoms were located by the geometric calculation method and difference Fourier method. The final $R_1 = 0.0500$, $wR_2 = 0.1203$ ($w = 1/\sigma|F|^2$) and $S = 1.045$. The crystallographic data for 19-methylaminoavarone (15) have been deposited at the Cambridge Crystallographic Data Centre with the deposition number 974400. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Bioassays

The MTT method was used for in vitro evaluation of the cytotoxic potential of the isolated compounds against mouse B16F10 melanoma and human NCI-H929 myeloma, HepG2 hepatoma, and SK-OV-3 ovarian cancer cell lines were used. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO₂ at 37 °C. The cytotoxicity assay was performed in 96-well microplates.³² Briefly, adherent cells (100 μL) were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each cancer cell line was exposed to the tested compound at concentrations of 1,

10, 50, and 100 μM in triplicate for 48 h with doxorubicin (for NCI-H929), 5-fluorouracil (for HepG2), and adriamycin (for B16F10 and SK-OV-3) (Sigma, USA) as the positive controls. The cells in each well were then solubilized with DMSO (100 μL for each well) and the optical density (OD) was recorded at 595 nm. IC₅₀ values were derived from the mean OD values of the triplicate tests versus drug concentration curves.

NF-κB luciferase assay

The NF-κB inhibitory activity assay was carried out according to the established protocol.^{33,34} NF-κB cells, which are HEK293 cells stably transfected with an NF-κB-responsive luciferase reporter plasmid, were generated. Other cells were purchased from Cell Bank (Chinese Academy of Sciences, Shanghai, China). A luciferase reporter plasmid containing an NF-κB binding site and pcDNA3.1 was co-transfected into HEK293 cells using Lipofectamine 2000 at a concentration of 10:1 (Invitrogen, Carlsbad, CA, USA). Stable recombinant cells were selected for resistance to 1 mg/mL G418 and for a strong luciferase signal. Stable HEK293/NF-κB cells were plated into 384-well plates at a concentration of approximately 3000 cells per well. After culture overnight, compounds were added to the medium at a final concentration of 2 μg/mL. After 6 h, the luciferase substrate was added to each well, and the released luciferin signal was detected using an EnVision microplate reader. HEK293/NF-κB cells were seeded into 96-well cell culture plates (Corning, NY, USA) and allowed to grow for 24 h.³⁵ The cells were then treated with compounds, followed by stimulation with TNF-α. Luciferase activity was determined using luciferase assay kits (Promega, Madison, WI, USA) according to the manufacturer's instructions.

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- † Electronic Supplementary Information (ESI) available: The ^1H NMR, ^{13}C NMR, DEPT135, ^1H - ^1H COSY, HSQC, HMBC and NOESY spectra of compounds **1**–**13**, the HRESIMS, UV, IR and CD spectra of **1**–**13**, as well as cif data for compounds **1**, **2**, and **15**. See DOI: 10.1039/b000000x/
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- § These authors contributed equally to this work.
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