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New anticancer bastadin alkaloids from the sponge *Dendrilla cactos*☆

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Abstract—Two new bastadin alkaloids, bastadins-22 (**1**) and 23 (**2**), together with six known bastadins, bastadins-6 (**3**), -12 (**4**) (formerly basatadin-9), -14 (**5**), -15 (**6**), -16 (**7**), -19 (**8**) and a common steroid cholesterol were isolated from the sponge *Dendrilla cactos*. The structures of the isolates were established by the study of extensive spectroscopic analysis (1D and 2D NMR and MS data). Anticancer activity of the isolates has been carried out against Sup-T_1 cancer cells (T cell lymphoma).

1. Introduction

Sponges are proven to be a rich source of secondary metabolites among the invertebrates. Many of these secondary metabolites are bioactive.[1] As part of our ongoing investigation on bioactive compounds from marine organisms[2] we describe the isolation of bastadin alkaloids from the sponge *Dendrilla cactos* which was collected at Mandapam coast, Tamilnadu, India, during November 2003. A literature survey revealed that the genus *Dendrilla* (family Darwinellidae, order Dendroceratida) has yielded several diterpenes,[3,4] lamellarin alkaloids,[5] and amino acid derivatives.[6] Further, so far bastadins have been reported from the sponges *Ianthella* sp.[7] and *Psammaplysilla purpurea*.[8] This is the new source for the presence of bastadin alkaloids in the sponge *D. cactos*. To date, 21 bastadin alkaloids have been described in the literature.[7–10] Bastadins are macrocyclic alkaloids derived by the oxidative phenolic coupling of two tyramino tyrosine units.[11] Bastadins exhibited wide variety of biological activities. Bastadins 1–7 showed potent in vitro anti-microbial activity against gram (+) organisms. 34-Sulfatobastadin-13 weakly inhibited binding to the endothelion A receptor (ET_A), while bastadin-13 inhibited growth of *Bacillus subtilis*.[7f] Bastadin-14 inhibited the enzymes topoisomerase-II and dehydrofolate reductase, with IC_{50} values of 2.0 and 2.5 μg/ml, respectively.[8a] Eight bastadin compounds bastadin-1, 2, 5, 6, 8, 10, 11, and 18 were evaluated for anticancer chemotherapeutics in ovarian tumor and leukemia cells, and were found moderate inhibitors of IMPDH (inosine 5[1]-phosphate dehydrogenase).

2. Results and discussions

In the present study, the dichloromethane/methanol (1:1) extract of the sponge *D. cactos* was partitioned between water and EtOAc. The water extract was freeze-dried, and the residue was triturated with MeOH. The methanol-soluble material was subjected to gel filtration [Sephadex LH-20, dichloromethane/methanol (1:1)], followed by silica gel column chromatography eluting with hexane/ethyl acetate to ethyl acetate. The fractions eluted with hexane/ethyl acetate (1:1) to ethyl acetate gradients were combined and purified on a preparative reversed-phase (C_{18}) HPLC column (25×250 mm) using CH_3OH/H_2O (80:20) to yield two new bastadin alkaloids bastadin-22 (**1**) and bastadin-23 (**2**) together with six known bastadin alkaloids bastadin-6 (**3**), -12 (**4**), -14 (**5**), -15 (**6**), -16 (**7**), and -19 (**8**).

Keywords: Bastadin alkaloids; Sponge; *Dendrilla cactos*; Anticancer activity; Sup-T_1 cancer cells (T cell lymphoma).
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Compound **1** was obtained as a colorless solid, mp 198–202 °C, and showed cluster ions at $(M-H)^-$ *m/z* 1088.66, 1090.65, 1092.65, 1094.65, 1096.65, 1098.65, 1100.65 in the ratio of 1:6:15:20:15:6:1 by negative ESI/HRMS indicated the presence of six bromine atoms. The molecular formula of compound **1** was deduced as $C_{34}H_{24}{}^{79}Br_6N_4O_8$ by the HRMS analysis. Its IR spectrum showed bands at 3600, 3410, 1990, 1515, 1410, 1225, 1090, and 1037 cm^{-1} indicating the presence of hydroxyl and amide groups, respectively. The structure of compound **1** was established by the studies of NMR spectral data (^1H, ^{13}C, ^1H–^1H, COSY, NOESY, HMQC, and HMBC).

The ^1H NMR spectrum of compound **1** (Table 1) revealed spin systems corresponding to four tetrasubstituted aromatic rings, in which two are symmetric [δ 7.50 (2H, s)] and [7.49 (2H, s)] and two are asymmetric [δ 7.01 (1H, d, J = 2.6 Hz, H-8); 6.33 (1H, d, J = 2.6 Hz, H-12)] and [δ 7.08 (1H, d, J = 2.6 Hz, H-27); 6.15 (1H, d, J = 2.6 Hz, H-31) HMBC correlations, Table 1] substituted benzene rings along with a disubstituted double bond signals at δ 6.96 (1H, d, J = 14.4 Hz, H-5) and 6.25 (1H, d, J = 14.4 Hz, H-6). A pronounced nuclear Overhauser effect revealed that the vinylic proton at δ 6.25 showed correlation with an aromatic proton δ 7.01 (1H, d, J = 2.6 Hz, H-8), which was coupled with the aromatic proton at δ 6.33 (1H, d, J = 2.6 Hz, H-12) suggesting the presence of one Ar–CH=CH– group in the molecule. Further, the ^1H NMR spectrum of compound **1** showed two linearly coupled methylene proton signals at δ 2.80 (2H, t, J = 6.5 Hz, H-20) and δ 3.49 (2H, t, J = 6.5 Hz, H-21). The signal at δ 2.80 showed NOESY correlations with two aromatic protons singlet at δ 7.49 (2H, s, H-17, H-19) and also exhibited three bond correlations with carbons at δ 136.7 (C-17, C-19) and two bond correlations with adjacent carbon at δ 43.5 (C-21) in its HMBC spectrum.

Further, in the HMBC spectrum the methylene protons at δ 3.49 (2H, s, C-21) showed three bond correlations with amide carbonyl (δ 167.8, C-23), which suggested

Table 1. Spectral data for compounds **1** and **2**

Sl. no.	Compound (**1**)				Compound (**2**)	
	^1H NMR[a] (J in Hz)	^{13}C NMR[b]	NOESY[a]	HMBC[a]	^1H NMR[c] (J in Hz)	NOESY[c]
1	3.92 (2H, s)	31.9	H-36, H-38	C_2, C_3, C_{36}, C_{38}	3.76 (2H, s)	H-36, H-38
2	—	154.2	—	—	—	—
3	—	165.8	—	—	—	—
4	—	—	—	—	10.18 (1H, d, J = 10.2 Hz)	H-5
5	6.96 (1H, d, J = 14.4 Hz)	124.6	H-6, H-12	C_3	6.80 (1H, dd, J = 14.4, 10.2 Hz)	H-4, H-6
6	6.25 (1H, d, J = 14.4 Hz)	117.5	H-5, H-8	C_8	6.26 (1H, d, J = 14.4 Hz)	H-5, H-8
7	—	138.8	—	—	—	—
8	7.01 (1H, d, J = 2.6 Hz)	129.5	H-6, H-12	C_{10}, C_{12}	7.01 (1H, d, J = 1.9 Hz)	H-12, H-6
9	—	111.5	—	—	—	—
10	—	144.4	—	—	—	—
11	—	149.4	—	—	—	—
12	6.33 (1H, d, J = 2.6 Hz)	111.3	H-8, H-5	C_8	6.11 (1H, d, J = 1.9 Hz)	H-8, H-5
14	—	118.9	—	—	—	—
15	—	146.2	—	—	—	—
16	—	118.9	—			—
17	7.49 (1H, s)	136.7	H-20, H-19	C_{20}, C_{18}, C_{15}	7.62 (1H, s)	H-19, H-20
18	—	129.8	—	—	—	—
19	7.49 (1H, s)	136.7	H-20, H-17	C_{20}, C_{17}, C_{15}	7.62 (1H, s)	H-17, H-20
20	2.80 (2H, t, J = 6.5 Hz)	37.5	H-21, H-17, H-19	C_{21}, C_{17}, C_{19}	2.75 (2H, t, J = 6.5 Hz)	H-21, H-17, H-19
21	3.49 (2H, t, J = 6.5 Hz)	43.5	H-20	C_{20}, C_{23}	3.30 (2H, m)	H-20, H-22
22	—	—	—	—	8.08 (1H, t, J = 5.7 Hz)	H-21
23	—	167.8	—	—	—	—
24	—	156.0	—	—	—	—
25	3.68 (2H, s)	31.2	H-27, H-31	C_{23}, C_{24}, C_{26} C_{27}, C_{31}	3.62 (2H, s)	H-27, H-31
26	—	130.1	—	—	—	—
27	7.08 (1H, d, J = 2.6 Hz)	130.8	H-25, H-31	C_{29}	7.24 (1H, d, J = 1.9 Hz)	H-25, H-31
28	—	108.6	—	—	—	—
29		141.8	—	—	—	—
30		147.4	—	—	—	—
31	6.15 (1H, d, J = 2.6 Hz)	117.0	H-27, H-25	C_{27}	6.60 (1H, d, J = 1.9 Hz)	H-25, H-27
33	—	126.5	—	—	—	—
34	—	144.6	—	—	—	—
35	—	126.5	—	—	7.38 (1H, d, J = 7.5 Hz)	H-36
36	7.50 (1H, s)	137.0	H-1, H-38	C_1, C_{34}, C_{38}	7.05 (1H, dd, J = 7.5, 1.9 Hz)	H-1, H-35, H-38
37	—	122.5	—	—	—	—
38	7.50 (1H, s)	137.0	H-1, H-36	C_1, C_{36}	6.58 (1H, d, J = 1.9 Hz)	H-36, H-1

[a] Measured in CD_3OD, 400 MHz.
[b] Measured in CD_3OD, 125 MHz.
[c] Measured in DMSO-d_6, 600 MHz.

the presence of Ar–CH_2–CH_2–NH–CO-group in the molecule. The ^{1}H NMR spectrum showed two isolated benzylic protons at δ 3.68 (2H, s, H-25) (δ_c 31.2 C-25) and 3.92 (2H, s, H-1) (δ_c 31.9, C-1). In the HMBC spectrum former benzylic protons showed correlations with an amide carbonyl and oxime carbons at δ 167.8 (C-23) and δ 156.0 (C-24), respectively; and the latter benzylic protons showed correlations with the second amide carbonyl and oxime carbons at δ 165.8 (C-3) and 154.2 (C-2), respectively, and also showed correlations with the aromatic carbons at δ 137.0 (C-36, C-38). The forgoing spectral data revealed that the compound **1** belongs to bastadin group of alkaloids[7] and closely related to bastadin-6[7a] except for a double bond between C5–C6 positions. Further, the structure of bastadin-22 and was established from the NOESY correlations of its tetramethyl ether derivative (**1a**).[8a] In the NOESY spectrum of tetramethyl derivative (**1a**), the two aromatic methoxyl groups at δ 3.87 (3H, s) and 3.85 (3H, s) did not show any correlation with any of the aromatic protons in the four benzene rings of the molecule. Hence, its structure was established as bastadin-22 (**1**), which is a new bastadin. The ^{13}C NMR chemical shifts of C-1 (δ 31.9) and C-25 (δ 31.3) implied that both oximes have *E* geometry.[12]

Compound **2** was obtained as colorless solid, mp 235–238 °C, and showed molecular ion ($M^{+\cdot}$) clusters at *m*/*z* 1012–1022 in the ratio of 1:5:10:10:5:1 in ESI/HRMS indicating the presence of five bromine atoms, and its molecular formula was deduced as $C_{34}H_{25}{}^{79}Br_5N_4O_8$. The IR spectrum showed bands at 3385, 3219, 1651, 1586, 1495, 1282, and 1189 cm^{-1} indicating the presence of hydroxyl, amide groups and aromatic nature of the compound.

The ^{1}H NMR spectrum of compound **2** resembled to that of bastadin alkaloids.[7,8] Its ^{1}H NMR spectrum displayed signals for the presence of three tetrasubstituted benzene rings and one trisubstituted benzene ring, two linearly coupled methylene groups, two isolated methylenes, and two vinylic protons (Table 1). Further ^{1}H NMR spectrum of compound **2** in DMSO-d_6 showed the presence of six D_2O exchangeable signals at δ 12.23 (1H, br s, –C═N–OH), 11.81 (1H, br s, –C═N–OH), 10.50 (1H, br s, Ph–OH), 9.50 (1H, s, Ph–OH), 10.18 (1H, d, J = 10.2 Hz, NH-4) and 8.08 (1H, t, J = 5.7 Hz, NH-22). The linear connectivities of the proton signals and their dispositions were deduced by the study of ^{1}H–^{1}H COSY and NOESY spectral correlations. In its ^{1}H–^{1}H COSY spectrum, the signals at δ 10.18 showed correlations with the vinylic proton at δ 6.80 (1H, dd, J = 14.4, 10.2 Hz, H-5), which in turn coupled with a vinylic proton at δ 6.26 (1H, d, J = 14.4 Hz, H-6) indicating the presence of eneamide system similar to that in bastadin-14 (**5**).[8] Further, the signals at δ 6.26 and 6.80 showed NOESY correlations with the aromatic signals at δ 7.01 (1H, d, J = 1.9 Hz, H-8) and 6.11 (1H, d, J = 1.9 Hz, H-12), respectively. In the NOESY spectrum, the aromatic two proton singlet at δ 7.60 (2H, s, H-17, H-19) showed correlations a methylene proton at δ 2.76 (2H, t, J = 6.5 Hz, H-20). The ^{1}H–^{1}H COSY spectrum, revealed that the signals at δ 2.76 coupled with a methylene group at δ 3.30 (2H, m, H-21) which in turn coupled with an amide proton at δ 8.08 (t, J = 5.7 Hz, NH-22). Finally the two isolated methylene signals at δ 3.62 (2H, s, H-25) and 3.76 (2H, s, H-1) showed NOESY correlations with δ 6.60 (1H, d, J = 1.9 Hz, H-31) and 7.24 (1H, d, J = 1.9 Hz, H-27) and δ 6.58 (1H, d, J = 1.9 Hz, H-38) and 7.05 (1H, dd, J = 8.3, 1.9 Hz, H-36), respectively. The foregoing spectral data established the structure of bastadin-23 as **2**, which is a new compound.

Bastadin-6[7a] (**3**) was previously isolated from *Ianthella basta* and the authors provided only physical and ^{1}H NMR data. Now we are furnishing for the first time the full data (^{1}H, ^{13}C NMR and HMBC) for the same. Previously bastadin-12 (**4**) (formerly bastadin-9)[7c] and bastadin-15[7e] (**6**) were characterized as tetramethyl derivatives and we are reporting them intact for the first time and furnishing their ^{1}H, ^{13}C NMR and HMBC data.

As it was well demonstrated that bastadin alkaloids exhibit anticancer activity, the isolates [bastadin-22 (**1**), bastadin-14 (**5**), bastadin-6 (**3**), bastadin-12 (**4**), bastadin-15 (**6**), bastadin-16 (**7**), and bastadin-19 (**8**)] were tested in vitro for their anticancer activity against Sup-T_1 cancer cells (T cell lymphoma) and the IC_{50} values are given in Table 2.

3. Conclusion

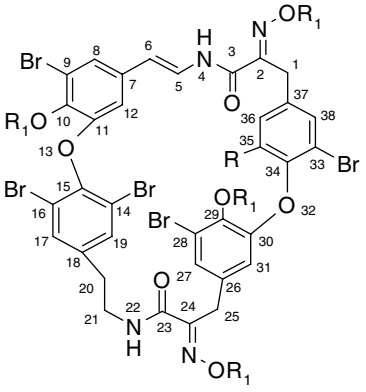
In summary, two new bastadins, bastadins-22 (**1**) and -23 (**2**), together with six known bastadins, bastadins-6 (**3**), -12 (**4**) (formerly basatadin-9), -14 (**5**), -15 (**6**), -16 (**7**), -19 (**8**), and common steroid cholesterol were isolated from the sponge *D. cactos*. Further, so far bastadins are reported from the sponges *Ianthella* sp. and *Psammaplysilla purpurea*. To date, 21 bastadin alkaloids have been described in the literature. Bastadin alkaloids are reported to possess anticancer activity and hence, the isolates [bastadin-22 (**1**), bastadin-14 (**5**), bastadin-6 (**3**), bastadin-12 (**4**), bastadin-15 (**6**), bastadin-16 (**7**), and bastadin-19 (**8**)] were tested in vitro for their anticancer activity against Sup-T_1 cancer cells (T cell lymphoma).

4. Experimental

4.1. General experimental procedures

Melting points were obtained on a Mel-Temp apparatus and are uncorrected, and IR spectra were recorded on Shimadzu-240 and Perkin-Elmer 240-C instruments, respectively. The ^{1}H and ^{13}C NMR was recorded on 600 MHz (Inova), 400 MHz (Bruker) instruments using TMS as internal standard. Chemical shifts are reported in δ (parts per million) and coupling constants (J) are expressed in hertz. The MS were recorded on a Quattro LC (Micro Mass, Manchester, UK) and HRMS were recorded on a Q STAR XL Mass spectrometer Applied biosystems, USA. Preparative scale HPLC was

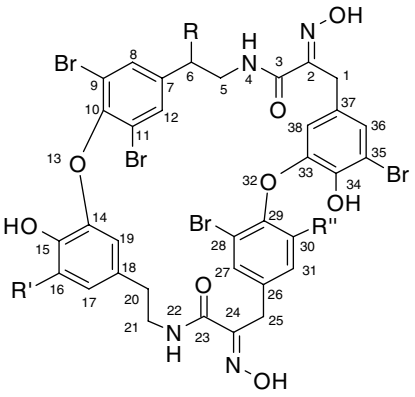
Table 2. IC_{50} values of bastadin alkaloids against Sup-T_1 cell lines



(**1**) R = Br, R_1= H

(**1a**) R = Br. R_1 = Me

(**2**) R = H

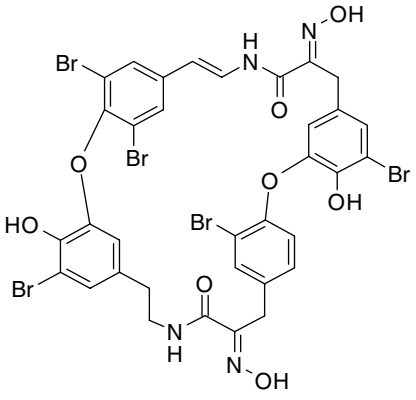


(**3**) R = H, R' = Br, R" = Br

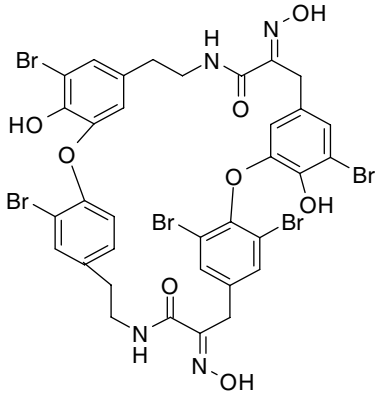
(**4**) R = OH, R' = Br R" = H

(**6**) R = H, R' = Br, R" = H

(**7**) R = H, R' = H, R" = Br



(**5**)



(**8**)

Compound	IC_{50} values
Bastadin-22 (**1**)	7.15×10^{-9}
Bastadin-14 (**5**)	1.4×10^{-7}
Bastadin-6 (**3**)	7.9×10^{-11}
Bastadin-12 (**4**)	8.01×10^{-9}
Bastadin-15 (**6**)	6.45×10^{-7}
Bastadin-16 (**7**)	1×10^{-12}
Bastadin-19 (**8**)	7.3×10^{-8}

performed using a Supelcosil C_{18} column (60 Å, 12 μm, and 25 cm × 21.2 mm).

4.2. Animal material

The sponge *D. cactos* was collected from the Mandapam coast in the Gulf of Mannar, Tamilnadu, India, during November, 2003. A voucher specimen (IIC-607) was deposited at the National Institute of Oceanography, Goa, India.

4.3. Extraction and isolation

The freshly collected sponge specimens (2.5 kg wet weight) were soaked in methanol at the site of collection until workup. The initial methanol extract was decanted and the sponge material was extracted with 1:1 dichloromethane/methanol (3× 3 l) at room temperature. The combined extract including initial methanol extract was filtered, and the solvent was removed under reduced pressure to give predominantly an aqueous suspension, and was extracted into ethyl acetate (3× 0.5 l). The organic layer was concentrated under reduced pressure to give a dark brown gummy mass (6.5 g) and was subjected to gel filtration chromatography (Sephadex LH-20, CH_2Cl_2/MeOH (1:1), 35 × 950 mm) by collecting a total of 20 continuous fractions (30 ml each). Following the TLC pattern and ^{1}H NMR spectra of crude fractions, the 20 fractions were pooled. The fractions containing bastadins were again purified on silica gel

column chromatography, followed by reversed-phase (C-18) HPLC column (Methanol/H_2O, 80:20) at a flow rate of 4 ml/min. to afford two new bastadin alkaloids, bastadin-22 (**1**) and bastadin-23 (**2**) along with 6 known bastadin alkaloids, bastadin-6[7a] (**3**), bastadin-12[7c] (formerly basatadin-9) (**4**), bastadin-14[8] (**5**), bastadin-15[7e] (**6**), bastadin-16[7d] (**7**), and bastadin-19[10a] (**8**).

4.4. Biological assay

Biological activity of the compounds was monitored in terms of inhibition of the proliferation of Sup-T_1 cells (A Non-Hodgkin's T cell lymphoma cell line obtained from NIH AIDS Research and Reference Reagent program, USA, the Reagent Contributor, Dr. James Hoxie). The ability of reduction of 3-(4,5-dimethylthiozol-3-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondria of the viable cells was used as a quantitative indicator for cell viability.[13] 0.2 Million cells in RPMI-1640 (Invitrogen Inc., USA) containing 10% fetal bovine serum (JRH Biosciences, USA) were seeded into a microwell plate. The cells were incubated in presence of increasing concentration of test compounds at 37 °C in a CO_2 incubator (SANYO, Japan) for 14 h. After 14 h, the cells were centrifuged at 800*g* for 10 min and the supernatant was discarded. The cells were re-suspended in 200 μl complete medium with 20 μl of 5 mg/ml MTT (Sigma, USA) and incubated for 4 h at 37 °C in a CO_2 incubator. The cells were centrifuged 800*g* for 10 min, 100 μl of supernatant was discarded. The insoluble crystal formed due to the reduction of MTT by viable cells was dissolved in 0.1 M acidic isopropanol and quantified in a microplate reader at 570 nm.[13] The percentage of inhibition of cell viability was computed with reference to the MTT reduction in control cells without test compound. The experimental measurements were made in triplicate and the average value was taken as percentage inhibition. The concentration of test compound required for 50% inhibition of cell viability (IC_{50}) was determined.

4.4.1. Bastadin-22 (1). White solid (10 mg), mp 198–202 °C, IR (KBr): ν_{max} 3600, 3410, 1990, 1515, 1410, 1225, 1090 and 1037 cm^{-1}. ESI/HRMS *m/z* $(M-H)^-$ 1088.6630 (Calcd for $C_{34}H_{23}N_4{}^{79}Br_6O_8$: 1088.6616); ^1H NMR (400 MHz, CD_3OD) δ 7.50 (1H, s, H-36), 7.50 (1H, s, H-38), 7.49 (1H, s, H-17), 7.49 (1H, s, H-19), 7.08 (1H, d, J = 2.6 Hz, H-27), 7.01 (1H, d, J = 2.6 Hz, H-8), 6.96 (1H, d, J = 14.4 Hz, H-5), 6.33 (1H, d, J = 2.6 Hz, H-12), 6.25 (1H, d, J = 14.4 Hz, H-6), 6.15 (1H, d, J = 2.6 Hz, H-31), 3.92 (2H, s, H-1), 3.68 (2H, s, H-25), 3.49 (2H, t, J = 6.5 Hz, H-21), 2.80 (2H, t, J = 6.5 Hz, H-20); ^{13}C NMR (75 MHz, CD_3OD) data: Table 1.

Bastadin-22 tetramethyl ether (**1a**): ^1H NMR (400 MHz, $CDCl_3$) δ 8.20 (1H, d, J = 8.0 Hz), 7.47 (1H, s, H-36), 7.47 (1H, s, H-38), 7.48 (1H, s, H-17), 7.48 (1H, s, H-19), 7.12 (1H, d, J = 2.0 Hz, H-27), 7.05 (1H, d, J = 2.0 Hz, H-8), 6.98 (1H, d, J = 14.5, 8.5 Hz, H-5), 6.80 (1H, t, J = 6.5 Hz), 6.32 (1H, d, J = 2.0 Hz, H-12), 6.10 (1H, d, J = 14.5 Hz, H-6), 6.30 (1H, d, J = 2.0 Hz, H-31), 3.85 (2H, s, H-1), 3.73 (2H, s, H-25), 3.35 (2H, t, J = 6.5 Hz, H-21), 2.75 (2H, t, J = 6.5 Hz, H-20), 3.85 (3H, s, 29-OMe), 3.87 (3H, s, 10-OMe), 3.98 (3H, s, NOMe), 4.05 (3H, s, NOMe).

4.4.2. Bastadin-23 (2). White solid (4 mg), mp 205–208 °C, IR (KBr): ν_{max} 3385–3219, 1651, 1586, 1495, 1452, 1427, 1282, 1189, 896, 745, and 714 cm^{-1}. ESI/HRMS *m/z* $(M^{+\cdot})$ 1012 (Calcd for $C_{34}H_{25}N_4{}^{79}Br_5O_8$: 1011.7589); ^1H NMR (600 MHz, DMSO-d_6) δ 10.18 (1H, d, J = 10.2 Hz, NH-4), 8.08 (1H, t, J = 5.7 Hz, NH-22), 7.60 (1H, s, H-17), 7.60 (1H, s, H-19), 7.38 (1H, d, J = 7.5 Hz, H-35), 7.24 (1H, d, J = 1.9 Hz, H-27), 7.05 (1H, dd, J = 7.5, 1.9 Hz, H-36), 7.01 (1H, d, J = 1.9 Hz, H-8), 6.80 (1H, dd, J = 14.4, 10.2 Hz, H-5), 6.60 (1H, d, J = 1.9 Hz, H-31), 6.57 (1H, d, J = 1.9 Hz, H-38), 6.26 (1H, d, J = 14.4 Hz, H-6), 6.11 (1H, d, J = 1.9 Hz, H-12), 3.76 (2H, s, H-1), 3.62 (2H, s, H-25), 3.30 (2H, m, H-21), 2.76 (2H, t, J = 6.5 Hz, H-20); NOESY (600 MHz, DMSO-d_6) data: Table 1.

4.4.3. Bastadin-6 (3). White solid (15 mg), mp 265–269 °C, IR (KBr): ν_{max} 3500–3289, 2927, 2854, 1646, 1620, 1549, 1496, 1453, 1425, 1280, 1246, 1181, 1032, 980, 737 and 707 cm^{-1}. ESI/MS *m/z* $(M^+-H)^-$ 1090.8; ^1H NMR (300 MHz, DMSO-d_6) δ 8.04 (1H, t, J = 6.2 Hz, NH-22), 7.91 (1H, t, J = 6.2 Hz, NH-4), 7.62 (1H, s, H-8), 7.62 (1H, s, H-12), 7.62 (1H, s, H-27), 7.62 (1H, s, H-31), 7.08 (1H, d, J = 1.9 Hz, H-36), 7.04 (1H, d, J = 1.7 Hz, H-17), 6.22 (1H, d, J = 1.9 Hz, H-19), 6.16 (1H, d, J = 1.9 Hz, H-38), 3.68 (2H, s, H-25), 3.57 (2H, s, H-1), 3.29 (2H, m, H-5), 3.29 (2H, m, H-21), 2.71 (2H, m, H-6), 2.71 (2H, m, H-20); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.0 (s, C-3), 163.31 (s, C-23), 151.5 (s, C-2), 150.4 (s, C-24), 146.1 (s, C-10), 146.1 (s, C-29), 144.8 (s, C-33), 144.6 (s, C-14), 141.8 (s, C-34), 141.6 (s, C-15), 140.1 (s, C-7), 137.6 (s, C-26), 133.6 (d, C-8), 133.6 (d, C-12), 133.2 (d, C-27), 133.2 (d, C-31), 130.7 (s, C-18), 128.1 (s, C-37), 126.8 (d, C-36), 126.2 (d, C-17), 111.7 (d, C-19), 117.6 (s, C-11), 117.4 (s, C-9), 117.1 (s, C-28), 117.1 (s, C-30), 112.6 (d, C-38), 110.2 (s, C-16), 109.8 (s, C-35), 40.4 (t, C-21), 38.39 (t, C-5), 33.9 (t, C-6), 32.7 (t, C-20), 28.71 (t, C-25), 27.34 (t, C-1); HMBC (300 MHz, DMSO-d_6) 1H (C_2, C_3, C_{36}, C_{37}, C_{38}), 5H (C_3), 6H (C_7, C_8, C_{12}), 8H (C_{10}, C_{12}, C_9, C_6), 12H (C_{10}, C_8, C_6, C_{11}), 17H (C_{15}, C_{19}, C_{20}), 19H (C_{15}, C_{17}, C_{20}), 20H (C_{18}, C_{17}, C_{19}), 21H (C_{18}, C_{20}), 25H (C_{23}, C_{24}, C_{26}, C_{27}, C_{31}), 27H (C_{29}, C_{31}, C_{25}), 31H (C_{29}, C_{27}, C_{25}), 36H (C_{34}, C_{38}), 38H (C_{34}, C_{36}, C_{33}).

4.4.4. Bastadin-12 (4). White solid (15 mg), mp 265–269 °C, IR (KBr): ν_{max} 3386–3180, 1660, 1534, 1492, 1426, 1282, 1243, 1191, 1040, 991, 741 cm^{-1}. ESI/MS (M^+) *m/z* 1030; ^1H NMR (400 MHz, DMSO-d_6) δ 12.15 (2H, br s, 2N–OH), 8.06 (1H, t, J = 6.1 Hz, NH-22), 7.96 (1H, t, J = 6.1 Hz, NH-4), 7.81 (1H, br s, H-8), 7.81 (1H, br s, H-12), 7.62 (1H, d, J = 2.0 Hz, H-27), 7.28 (1H, dd, J = 8.0, 2.0 Hz, H-31), 7.29 (1H, d, J = 2.0 Hz, H-36), 7.16 (1H, d, J = 2.0 Hz, H-17), 6.88 (1H, d, J = 8.0 Hz, H-30), 6.5 (1H, d, J = 2.0 Hz, H-38), 6.25 (1H, d, J = 2.0 Hz, H-19), 4.80 (1H, m, H-6), 3.81 (2H, d, J = 13.0 Hz, H-25), 3.73 (2H, d, J = 12.9 Hz, H-1), 3.50 (1H, m, Ha-5) and 3.20 (1H, m, Hb-5), 3.31

(2H, m, H-21), 2.74 (2H, m, H-20); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.5 (s, C-3), 163.4 (s, C-23), 151.2 (s, C-24), 151.1 (s, C-2), 150.9 (s, C-29), 146.6 (s, C-10), 145.1 (s, C-14), 144.7 (s, C-33), 144.1 (s, C-7), 143.7 (s, C-15), 141.7 (s, C-34), 134.4 (s, C-26), 133.5 (d, C-27), 130.7 (d, C-8), 130.7 (d, C-12), 129.7 (d, C-31), 128.8 (s, C-37), 127.9 (d, C-17), 126.1 (d, C-36), 119.8 (d, C-30), 117.4 (s, C-9), 117.4 (s, C-11), 113.2 (s, C-28), 112.0 (d, C-38), 112.1 (d, C-19), 110.6 (s, C-35), 110.3 (s, C-16), 70.3 (d, C-6), 66.9 (t, C-5), 46.7 (t, C-21), 27.4 (t, C-1), 33.20 (t, C-20), 28.48 (t, C-25).

4.4.5. Bastadin-15 (6). White solid (10 mg), mp 265–269 °C, IR (KBr): ν_{max} 3415, 1665, 1531, 1450, 1288, 1247, 1026, 997, 825, 764 cm^{-1}. ESI/MS $(M^+-H)^-$ *m*/*z* 1012.7; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.16 (1H, t, J = 6.2 Hz, H-22), 7.87 (1H, t, J = 6.2 Hz, NH-4), 7.52 (1H, s, H-8), 7.52 (1H, s, H-12), 7.35 (1H, d, J = 1.9 Hz, H-27), 7.04 (1H, d, J = 1.9 Hz, H-36), 7.01 (1H, dd, J = 7.8, 1.9 Hz, H-31), 6.98 (1H, d, J = 1.9 Hz, H-17), 6.75 (1H, d, J = 7.8 Hz, H-30), 6.23 (1H, d, J = 1.9 Hz, H-38), 6.06 (1H, d, J = 2.0 Hz, H-19), 3.60 (2H, s, H-25), 3.52 (2H, s, H-1), 3.40 (2H, m, H-5), 3.06 (2H, m, H-21), 2.71 (2H, m, H-6), 2.35 (2H, m, H-20); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.1 (s, C-23), 163.6 (s, C-3), 152.2 (s, C-2), 151.8 (s, C-24), 151.6 (s, C-14), 146.7 (s, C- 10), 146.0 (s, C-29), 145.3 (s, C-33), 144.0 (s, C-34), 142.6 (s, C-15), 141.3 (s, C-7), 135.2 (s, C-26), 135.2 (s, C-37), 134.1 (d, C-8), 134.1 (d, C-12), 133.8 (d, C-27), 131.7 (s, C-18), 129.8 (d, C-31), 128.2 (d, C-36), 126.6 (d, C-17), 121.0 (d, C-30), 117.7 (s, C-9), 117.7 (s, C-11), 117.3 (d, C-38), 114.0 (s, C-28), 113.7 (s, C-19), 111.3 (s, C-35), 111.0 (s, C-16), 41.3 (t, C-21), 40.6 (t, C-5), 34.8 (t, C-6), 34.3 (t, C-20), 28.9 (t, C-25), 28.2 (t, C-1); HMBC (600 Hz, DMSO-d_6) 1H (C_2, C_3, C_{36}, C_{38}), 4-NH (C_3), 5H (C_3, C_7), 6H (C_7, C_8, C_{12}), 8H (C_6, C_9, C_{10}, C_{12}), 12H (C_8, C_{10}, C_{11}), 17H (C_{15}, C_{19}, C_{20}), 19H (C_{15}, C_{17}, C_{18}, C_{21}), 21H (C_{20}), 22NH (C_{23}), 25H (C_{23}, C_{24}, C_{27}, C_{31}), 27H (C_{25}, C_{29}, C_{28}, C_{31}), 30H (C_{26}, C_{28}, C_{29}), 31H (C_{29}), 36H (C_1, C_{34}, C_{35}, C_{38}), 38H (C_1, C_{33}, C_{34}, C_{36}).

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