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New Structures and Bioactivity Properties of Jasplakinolide (Jaspamide) Analogues from Marine Sponges

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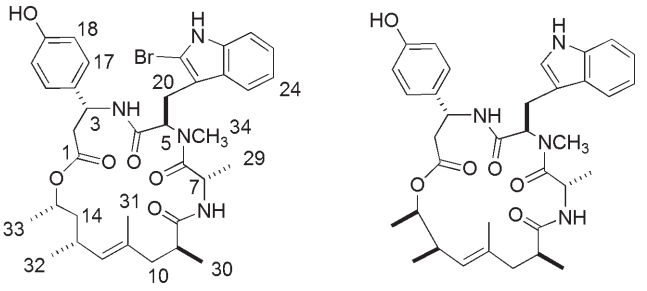
The goal of this study was to isolate and study additional jasplakinolide analogues from two taxonomically distinct marine sponges including two *Auletta* spp. and one *Jaspis splendens*. This led to the isolation of jasplakinolide (**1**) and eleven jasplakinolide analogues (**3**−**13**) including seven new analogues (**6**−**10**, **12**, and **13**). Structure elucidation of the new compounds was based on a combination of 1D and 2D NMR analysis, optical rotation, circular dichroism, and preparation of Mosher's esters. Five of the new compounds are oxidized tryptophan derivatives of **1**, including a unique quinazoline derivative (**9**). Compounds **1**, **3**, **5**−**8**, and **11** were evaluated in the NCI 60 cell line screen, and all compounds were tested in a microfilament disruption assay. Jasplakinolide B (**11**) exhibited potent cytotoxicity (GI_{50} < 1 nM vs human colorectal adenocarcinoma (HCT-116) cells) but did not exhibit microfilament-disrupting activity at 80 nM.

Introduction

In 1986, two publications appeared describing the structure of an unusual depsipeptide−polyketide compound named jasplakinolide (**1**; Chart 1),[1] by our lab, or jaspamide, by the Ireland-Faulkner-Clardy consortium.[2] The scope of this initial discovery has been greatly extended by the more than 400 papers published to date referencing **1** or its analogues, with the greatest number regarding cellular biology studies utilizing **1** (see Table S1, Supporting Information).[3] An initial stimulus for further research was the impressive data from the U.S. National Cancer Institute 60 cell screen data showing a significant cytotoxicity pattern for **1** (NSC: 613009 and P3933). The results indicated selectivity against renal, prostate, and CNS tumor cell lines. Subsequent biological evaluation revealed a mechanism of action involving microfilament disruption in vitro and an ability to permeate cells and alter the actin skeleton organization in vivo. This effect has also been exhibited by the close analogue chondramide C (**2**; Chart 1) isolated from the myxobacterium *Chondromyces crocatus*.[4] Sustained interest in **1** can also be attributed to its ability to induce apoptosis,[5−11] along with its additional actions as an anthelmintic,[1] insecticide,[2] and fish toxin.[12]

Development of **1** as a biological probe has transpired owing to its commercial availability based on natural sources.[13] An evaluation of 20 marine sponges in the NCI repository identified samples in the *Jaspis* or *Doryplores* genera from seven Indo-Pacific regions as sources of **1**.[14] Similarly, the Crews and Zampella groups have isolated **1** from sponge collections spanning two different orders as summarized in Table 1.[13,16,17] The Vanuatu collections of

Chart 1



Jaspis splendens studied by Zampella from 1999 to 2009 afforded **1** plus 14 analogues (renamed here using our favorite synonym):[19] jasplakinolides B−H and J−P.[16,20−22] Analogous results obtained by Schmitz in 1998, remaining yet unpublished, involved the examination of sponges, *Jaspis* or *Doryplores*, one each from Truk lagoon and Yap Micronesia, which contained **1**, debromo **1**, 13-demethyl **1**, plus jasplakinolides B, C, E, and three other Trp modified analogues.[23] Most recently, Proksch reported jasplakinolides Q and R, debromo and dibromo **1** respectively, from Kalimantan, Indonesia.[24]

There have been two attempts to assess the structure activity pattern of the jasplakinolide framework based on natural material. Primarily through semisynthesis, we were able to study **1** plus six analogues as inhibitors of human prostate adenocarcinoma (PC-3) cell proliferation and observed that changes to the polyketide moiety involving transformation of the double bond into an epoxide or diol formation were detrimental to biological activity.[25] The Zampella lab evaluated their collection of fifteen compounds for cytotoxicity effects against human breast adenocarcinoma

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Table 1. Marine Sponges Containing the Jasplakinolide (**1**) Family

order	family	genus	species	collection site	ref
Astrophorida	Ancorinidae	*Jaspis*	*splendens*	Fiji	1, this work
		Jaspis	sp.	Palau	2
		Jaspis	*splendens*	Vanuatu	16, 20−22
		Jaspis	*splendens*	Kalimantan, Indonesia	24
Halichondrida	Axinellidae	*Auletta*	*cf. constricta*	Milne Bay, Papua New Guinea	17
		Auletta	sp.	Papua New Guinea	this work

(MCF-7) and colon adenocarcinoma (HT-29) cells and found that the levels of activity tracked with the antimicrofilament activity. Changes not well-tolerated included the following: (a) replacement of the polyketide framework methyl groups, (b) migration of the polyketide double bond to an exocyclic position, and (c) modification of the Trp residue. Alternatively, replacement of the Ala side chain methyl did not substantially diminish the activity.

The eight total syntheses of **1**, with the first appearing in 1988, have reaffirmed its absolute configuration, originally established by X-ray,[2] as 3*R*, 5*S*, 7*S*, 9*S*, 13*R*, and 15*S*.[26−32] These works also provide insight into routes for the preparation of **1** in small amounts (usually less than 10 mg). Some of these schemes have been used to prepare small sets of analogues for structure−activity relationship studies, but none have shown biological activity on par with that of **1**. Highlights of the deleterious changes include inserting amino acids into the polyketide synthase (PKS[a]) segment and replacing the methyl groups at C-13/C-15.[33,34] Similarly, total synthesis of **2** and three non-natural diastereomers revealed the sensitivity of cytotoxic action against solid tumors[35] and relative ability to induce actin polymerization to changes in configurations of substituents on the polyketide portion of the molecular framework. These results mesh well with the structure−activity relationship (SAR) patterns described above for **1**.

The most recent report by Zampella[22] described research similar to that ongoing in our lab for the past decade on Indo-Pacific sponges rich in jasplakinolide and analogues. At one point, we designated these sponges as *Jaspis johnstoni*,[1] but revised their identification to *Jaspis splendens*. It is important to underscore that the synonym, *Dorypleres splendens*, can be found in the literature for this organism.[36,37] The research reported below is based on three collections: two from Papua New Guinea (*Auletta* spp. coll. nos. 02137 and 02118) and one from Fiji (*Jaspis splendens* coll. no. 00101). As anticipated, LCMS profiling of each extract showed that **1** was present, and further research was begun to identify the several minor components present that appeared to be jasplakinolide analogues. A total of 11 jasplakinolide analogues were isolated and evaluated; these results are reported below.

Results and Discussion

The molecular formula and structural characteristics of **1** ($C_{36}H_{45}BrN_4O_6$) provided the benchmark to evaluate the three extracts and their chromatographic fractions. While the isolation steps, outlined in the experimental section, were straightforward, focusing on the intense LCMS molecular ion cluster for **1** ($[M+H]^+$ m/z 709.2/711.2) provided an unambiguous handle to pinpoint fractions rich in this compound. Further evaluation of fractions containing known and/or new analogues of **1** was accomplished using a table of molecular formulas for 17 known jasplakinolides (see Table S10, Supporting Information). Strikingly, all congeners of **1** could be subdivided into three classes based on heavy atom formulas as follows: $C_{35-37}N_4O_{6/7/9}$ (single MS peak), $C_{35-37}BrN_4O_{6-7}$ (doubled MS peak cluster), or $C_{36}Br_2N_6O_6$ (tripled MS peak cluster). This realization provided a rapid approach to dereplicate compounds suspected of being known, and also pinpointed compounds that appeared to be unknown. In addition, all previously described jasplakinolide analogues were divided into two groups as shown in Figure 1. Establishing the hybridization of C-31 by ^{1}H NMR provided the basis to discern between these groups: Group 1 = δ_H 1.5−1.6 (3H) and Group 2 = δ_H 5−6 (2H).

Both known and new compounds, possessing either Group 1 or Group 2 frameworks, were isolated from the extracts of the three sponges used in this study. A total of twelve compounds were obtained consisting of eight (four new) from Group 1 and four (three new) from Group 2. Overall, each metabolite was completely characterized and the absolute configuration shown here was largely assigned on the basis of analogy to that unequivocally established (as described above) for **1**. The constituents of the *Auletta* sp. coll. no. 02137 proved to be the most diverse. The known compounds included the following: Group 1, **1** ($C_{36}H_{45}BrN_4O_6$),[1,2] jasplakinolide E (**3**, $C_{36}H_{45}BrN_4O_7$),[20] jasplakinolide F (**4**, $C_{35}H_{43}BrN_4O_6$),[20] jasplakinolide P (**5**, $C_{37}H_{48}N_4O_9$);[22] and Group 2, jasplakinolide B (**11**, $C_{36}H_{43}BrN_4O_7$).[16] The new analogues from this sponge consisted of 21-epi-jasplakinolide P (**6**, $C_{37}H_{48}N_4O_9$), jasplakinolide S (**7**, $C_{36}H_{46}N_4O_8$), 21-epi-jasplakinolide S (**8**, $C_{36}H_{46}N_4O_8$), jasplakinolide C_a (**12**, $C_{37}H_{46}N_4O_{10}$), and jasplakinolide C_b (**13**, $C_{37}H_{46}N_4O_{10}$). Less complex mixtures were present in the other two sponges. The *Auletta* sp. coll. no. 02118 was a source of the new compound jasplakinolide U (**10**, $C_{37}H_{46}N_4O_{10}$) and *Jaspis splendens*, coll. no. 00101, provided the new compound jasplakinolide T (**9**, $C_{36}H_{45}N_5O_6$). The stereostructures of the known compounds were set by thorough examination of their NMR and MS physical properties compared to those in literature, and structure elucidation of the new compounds proceeded as described below.

Two compounds possessing the molecular formula and overall structure of jasplakinolide P ($C_{37}H_{48}N_4O_9$)[22] were isolated and designated as diastereomers **5** and **6**. The unique heavy atom formulas of these compounds matched that of known jasplakinolide P (see Table S10, Supporting Information), and these data plus the aliphatic NMR resonance positions of C-31 for **5** ($\delta_{H/C}$ 1.59/18.9) and **6** ($\delta_{H/C}$ 1.58/18.8) facilitated completing the first steps in the de-replication process. The extensive NMR data in Tables 2 and 3 alongside the selected 2D NMR correlations shown in Figure 2 confirmed that all structural elements previously shown in jasplakinolide P,[22] including the unusual benzoxazinone moiety, were present in **5** and **6**. We eventually decided that the former compound was identical to that previously described and that

[a] Abbreviations: PKS, polyketide synthase; NRPS, nonribosomal peptide synthetase; CD, circular dichroism.

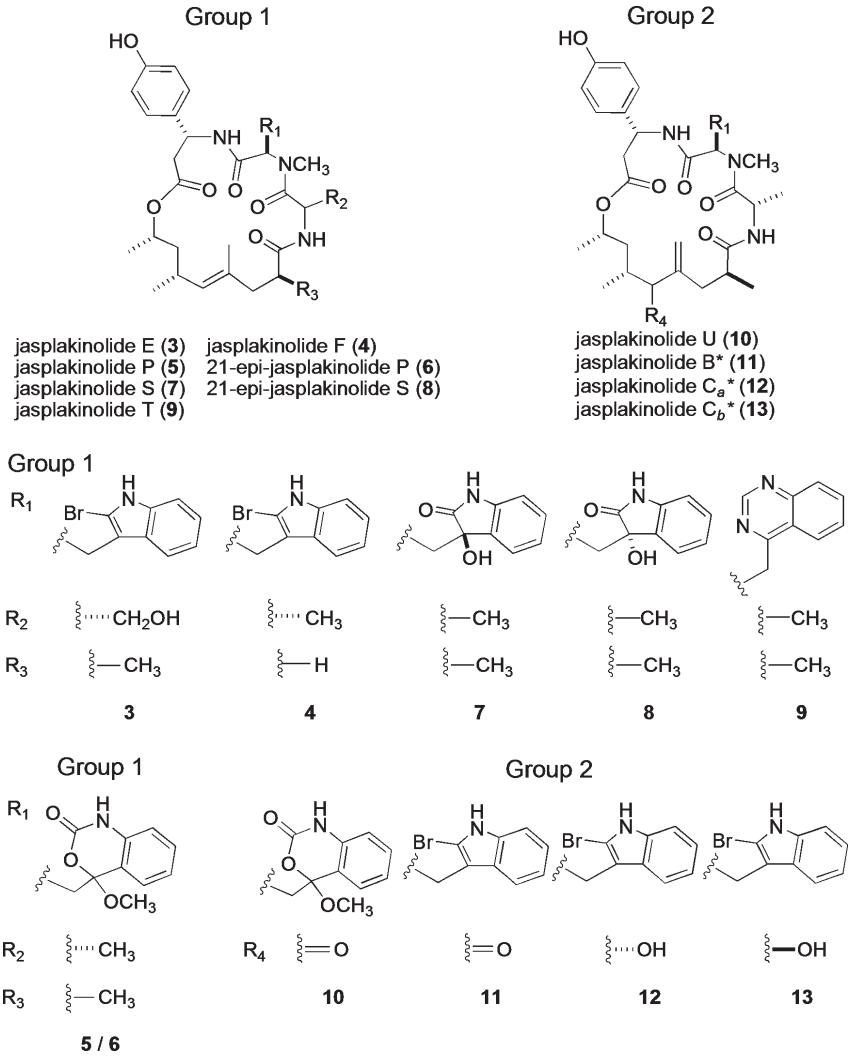

Figure 1. Two groups of jasplakinolide frameworks (*structures of jasplakinolide B and C have been switched in various publications[18,20−22]).

the latter was a new diastereomer. Part of the decision network involved assessing differences in ^{13}C chemical shifts ($\Delta\delta_C$) between all three data sets as shown in Figure 3. Overall, we observed very similar δ_C values for **5** and **6**, with the greatest differences occurring at C-2 ($\Delta\delta_C$ 3.3 ppm) and C-20 ($\Delta\delta_C$ 1.6). This implied epimerization at adjacent chiral centers such as C-3, C-5, or C-21. Then, comparison of the $\Delta\delta_C$ data for **5**, **6**, and known jasplakinolide P provided the basis for the conclusion noted above that jasplakinolide P[22] and **5** were identical, because (see Figure 3) this pair provided the best match at key sites including C-20, C-21, and C-2. While the original publication was silent on the assignment of the configuration at C-21 for jasplakinolide P,[22] all other chiral centers were assigned in this compound, based on semirigorous analysis, as being identical to that of **1**. Our final configurational assignment at C-21 was based on analogy to the structures of two new jasplakinolide diastereomers described next.

During LCMS screening, a second set of isomers was observed displaying an $[M+H]^+$ at m/z 663 and molecular formula of $C_{36}H_{46}N_4O_8$. These new compounds (**7** and **8**) possessed the same carbon count as **1** and were initially designated as jasplakinolides S, and their heavy atom formulas were closest to that of jasplakinolide P ($C_{37}N_4O_9$) and jasplakinolide O ($C_{35}N_4O_7$).[22] Similar to the analysis above, the aliphatic resonance positions of C-31 for **7** ($\delta_{H/C}$ 1.58/17.3) and **8** ($\delta_{H/C}$ 1.57/17.3) indicated the presence of the Group 1 macrolide. Somewhat problematic for assigning the site(s) of configurational differences between **7** and **8** was that evaluation of their $\Delta\delta_C$ chemical shifts revealed no difference greater than δ 0.9 ppm. The major difference between **1** and **7**/**8** was quickly recognized as due to the bromo-indole moiety being replaced by a 3-hydroxyindolin-2-one group. The 2D NMR correlations seen in Figure 4 were key to establishing the connectivity network between the non-benzenoid ring atoms consisting of $C_2H_2NO_2$. The most significant $\Delta\delta_C$'s between **1** and **7**/**8** were the shifts of C-28 (**1**, δ_C 109.2; **7**, δ_C 180.2; and **8**, δ_C 179.3) and C-21 (**1**, δ_C 110.4; **7**, δ_C 75.0; and **8**, δ_C 74.8), both consistent with the substructure shown in Figure 4. At this point, though none of the data collected provided the basis for a definitive conclusion, it was assumed that a configurational change at C-21 was the sole source of difference between **7** and **8**.

A rapid way to interrogate the hypothesis advanced above involved collecting circular dichroism (CD) data for **7** and **8**. We believed that this information could be used in a direct comparison to literature CD traces for a pair of 3-hydroxy-2-oxytryptophan derivatives.[38] Listed in Figure 5 are the published CD data for two key models, (−)-3(*S*)-hydroxy-2-oxo-*S*-tryptophan and (+)-3(*R*)-hydroxy-2-oxo-*S*-tryptophan,

Table 2. ^{13}C NMR Data of Selected Compounds Including **5**−**10**, **12**, and **13** in CD_3OD at 150 MHz

compound	**5**	**6**	**7**	**8**	**9**	**10**[a]	**12**	**13**
pos#	δ_C, type[b]	δ_C, type[b]	δ_C, type[b]	δ_C, type[b]	δ_C, type[b]	δ_C, type[b]	δ_C, type[b]	δ_C, type[b]
1	172.4, qC	172.5, qC	170.8, qC	171.1, qC	169.7, qC	172.0, qC	170.7, qC	170.5, qC
2	41.9, CH_2	38.6, CH_2	40.3, CH_2	39.8, CH_2	40.2, CH_2	41.7, CH_2	40.7, CH_2	39.7, CH_2
3	50.8, CH	50.9, CH	49.2, CH	49.2, CH	48.5, CH	50.9, CH	49.5, CH	49.4, CH
4	170.6, qC	170.3, qC	169.1, qC	168.6, qC	167.9, qC	171.1, qC	169.8, qC	169.8, qC
5	53.6, CH	54.4, CH	52.6, CH	52.8, CH	54.1, CH	54.1, CH	56.0, CH	56.3, CH
6	174.7, qC	175.1, qC	173.3, qC	173.7, qC	172.2, qC	175.1, qC	174.9, qC	174.9, qC
7	47.6, CH	47.7, CH	45.9, CH	46.4, CH	44.3, CH	46.7, CH	45.4, CH	45.6, CH
8	178.3, qC	178.2, qC	176.6, qC	176.6, qC	174.2, qC	177.7, qC	177.3, qC	177.1, qC
9	40.8, CH	40.4, CH	39.2, CH	38.4, CH	37.8, CH	40.4, CH	39.8, CH	38.2, CH
10	42.2, CH_2	42.2, CH_2	40.6, CH_2	40.3, CH_2	41.6, CH_2	37.6, CH	38.8, CH	36.3, CH
11	134.3, qC	134.1, qC	132.6, qC	132.5, qC	131.2, qC	146.6, qC	148.5, qC	147.8, qC
12	130.3, CH	130.0, CH	128.7, CH	127.9, CH	129.2, CH	207.2, CH	77.4, CH	78.2, CH
13	31.1, CH	31.1, CH	29.5, CH	29.4, CH	28.8, CH	37.5, CH	30.8, CH	31.1, CH
14	44.6, CH_2	44.7, CH_2	43.2, CH_2	43.0, CH_2	42.4, CH_2	42.5, CH_2	35.7, CH2	36.3, CH_2
15	72.0, CH	72.0, CH	70.4, CH	70.4, CH	70.3, CH	71.5, CH	69.8, CH	68.7, CH
16	132.8, qC	132.4, qC	130.9, qC	130.6, qC	131.4, qC	133.7, qC	131.5, qC	132.0, qC
17	128.7, CH	128.5, CH	126.8, CH	126.9, CH	126.9, CH	128.8, CH	127.2, CH	127.2, CH
18	116.4, CH	116.4, CH	114.8, CH	114.7, CH	114.8, CH	116.5, CH	114.7, CH	114.7, CH
19	158.1, qC	158.1, qC	156.5, qC	156.5, qC	156.4, qC	158.1, qC	156.3, qC	156.3, qC
20	39.9, CH_2	41.5, CH_2	34.1, CH_2	34.2, CH_2	31.7, CH_2	40.4, CH_2	23.0, CH_2	22.9, CH_2
21	108.6, qC	109.2, qC	75.0, qC	74.8, qC	167.6, qC	108.6, qC	109.1, qC	108.9, qC
22	119.0, qC	118.5, qC	130.9, qC	130.1, qC	123.4, qC	118.9, qC	127.2, qC	127.3, qC
23	127.2, CH	127.0, CH	124.0, CH	123.8, CH	125.1, CH	127.3, CH	117.6, CH	117.5, CH
24	124.7, CH	124.9, CH	122.2, CH	122.4, CH	127.7, CH	124.7, CH	119.1, CH	119.0, CH
25	132.1, CH	132.1, CH	129.3, CH	129.6, CH	133.9, CH	132.1, CH	121.3, CH	121.3, CH
26	115.7, CH	115.7, CH	110.1, CH	110.0, CH	128.2, CH	115.8, CH	110.1, CH	110.1, CH
27	137.5, qC	137.7, qC	141.2, qC	142.0, qC	148.8, qC	137.6, qC	136.5, qC	136.5, qC
28	152.2, qC	152.0, qC	180.2, qC	179.3, qC	153.4, CH	152.2, qC	108.9, qC	109.1, qC
29	18.3, CH_3	18.2, CH_3	16.5, CH_3	16.4, CH_3	17.5, CH_3	18.6, CH_3	12.9, CH_3	14.6, CH_3
30	20.6, CH_3	20.6, CH_3	19.0, CH_3	19.3, CH_3	19.6, CH_3	18.9, CH_3	14.8, CH_3	15.1, CH_3
31	18.9, CH_3	18.8, CH_3	17.3, CH_3	17.3, CH_3	17.6, CH_3	129.1, CH_2	111.7 CH_2	111.2, CH_2
32	22.5, CH_3	22.5, CH_3	20.9, CH_3	21.0, CH_3	21.6, CH_3	17.8, CH_3	16.8, CH_3	16.8, CH_3
33	19.8, CH_3	19.8, CH_3	18.2, CH_3	18.1, CH_3	19.2, CH_3	20.6, CH_3	19.5, CH_3	19.7, CH_3
34	31.6, CH_3	31.8, CH_3	30.0, CH_3	30.3, CH_3	30.6, CH3	32.4, CH_3	31.7, CH_3	31.2, CH_3
O−CH_3	51.2, CH_3	51.1, CH_3				51.1, CH_3		

[a] Measured at 125.7 MHz. [b] Carbon type established from ^{13}C DEPT and/or gHMQC experiments.

each prepared from *S*-tryptophan. These compounds display antipodal-like Cotton effect curves, which dramatically shift from positive to negative as a function of the chirality at C-3. The CD response of **7** exhibited a negative curve at 242 nm and positive curves at 264 and 292 nm, which was analogous to that of the second tryptophan model indicating 21*R* assignment. Opposite CD results were obtained for **8** indicating 21*S* designation. As a next consideration, it seemed useful to exploit a parallel pattern in the chiroptical data we observed between the jasplakinolide S (**7** and **8**) and P (**5** and **6**) diastereomers. These observations concerning optical rotation values and the conclusions drawn are as follows: (a) One isomer of each pair exhibited a relatively small optical rotation (**7**, $[\alpha]_D$ = +36.8, MeOH; **6**, $[\alpha]_D$ = +39.2, MeOH); (b) one isomer of each pair exhibited a relatively large optical rotation (**8**, ($[\alpha]_D$ = +62.6, MeOH; **5**, $[\alpha]_D$ = +61.6, MeOH); (c) the configurations established above for **7** and **8** appeared to be reasonable models to provisionally define the benzoxazinone ring configurations of **5** and **6**, respectively, as 21*S* and 21*R*; (d) lastly, the configurational information shown at all other sites for **6**−**8** were based on biosynthetic analogy to **1**.

An especially unique analogue, jasplakinolide T (**9**), was obtained through LCESIMS-guided isolation by focusing on $[M+H]^+$ peaks of *m*/*z* 644.3. Its molecular formula was established as $C_{36}H_{45}N_5O_6$, which differed from **1** by the absence of a Br and the presence of an additional N atom. Existence of the Group 1 framework was clear because of the diagnostic shift for C-31 ($\delta_{H/C}$ 1.51/17.6) and $\Delta\delta_C$'s < 1 ppm vs those of **1** were observed for all of the remaining resonances of the core carbons. Defining the constitution of the R_1 appendage of the Group 1 structure, whose formula consisted of $C_9H_7N_2$ (7 sites of unsaturation), was addressed next. The $^1H/^{13}C$ NMR data readily indicated this to be a 4-alkylquinazoline group fully consistent with the $^1J_{CH}$ = 201 Hz at C-28 (δ_C 153.4) as observed by 2D NMR (see Figure S1, Supporting Information). Additional sets of 2D gHMBC and gCOSY NMR data represented in Figure 6 further reaffirmed this proposal. One pattern involved gHMBC correlations from H-20 (δ_H 3.92) to C-21 (δ_C 167.6) and to C-22 (δ_C 123.4). Another consisted of $^3J_{H-C}$ gHMBC correlations from H-28 (δ_H 9.08) to C-21 (δ_C 167.6) and to C-27 (δ_C 148.8). Also shown in Figure 6 are supporting data for the close model, 6,7-dimethoxy-4-propylquinazoline (**14**, also see Figure S2, Supporting Information). The stereostructure of **9** shown here is based on analogy to that of **1**.

The isolation of jasplakinolide U (**10**, $C_{38}H_{48}N_4O_{10}$) was prioritized once it was recognized that there was no Br atom present. An initial inference based on this single observation was that a modified indole array was present, similar to that seen in **5**, **6**, **7**, and **8**. The isolation of **10** was completed by tracking the LCMS peak having $[M+H]^+$ *m*/*z* = 707. The incorporation of a Group 2 core was set from the diagnostic

Table 3. ^{1}H NMR Data of Selected Compounds Including **5**−**10**, **12**, and **13** in CD_3OD at 600 MHz

Compound	**5**	**6**	**7**	**8**	**9**	**10**[a]	**12**	**13**
pos#	δ_H, (int., m., *J* [Hz])	δ_H, (int., m., *J* [Hz])	δ_H, (int., m., *J* [Hz])	δ_H, (int., m., *J* [Hz])	δ_H, (int., m., *J* [Hz])	δ_H, (int., m., *J* [Hz])	δ_H, (int., m., *J* [Hz])[c]	δ_H, (int., m., *J* [Hz])[c]
2	2.65 (1H, dd, 15, 7.7) 2.73 (1H, dd, 15.3, 4.8)	2.61 (1H, dd, 14.5, 7) 2.69 (1H, dd, 15, 4.5)	2.63 (1H, dd, 14.8, 7.5) 2.72 (1H, dd, 14.1, 4.0)	2.64 (1H, dd, 14.7, 3.2) 2.67 (1H, dd, 14.1, 4.7)	2.59 (1H, dd, 15.1, 6.5) 2.72 (1H, dd, 15.0, 4.5)	2.70 (1H, dd, 16.2, 4.2) 2.82 (1H, dd, 16, 7.0)	2.72 (1H, dd, 15.7, 5.0) 2.84 (1H, dd, 15.7, 7.0)	2.67 (1H, dd, 16.0, 4.8) 2.86 (1H, dd, 16.0, 7.2)
3	5.17 (1H, dd, 7.9, 4.7)	5.08 (1H, dd, 6.5, 4.8)	5.13 (1H, m)	5.04 (1H, m)	5.10 (1H, m)	5.19 (1H, dd, 9.5, 4)	5.20 (1H, bdd, 12.3, 7.0)	5.15 (1H, dd, 12.4, 6.6)
5	5.56 (1H, dd, 8.0, 5.2)	5.30 (1H, dd, 9.5, 3.5)	5.46 (1H, dd, 10.5, 4.9)	4.82[b]	5.85 (1H, bt, 7)	5.45 (1H, dd, 8.5, 4.5)	5.80 (1H, dd, 10.0, 6.5)	5.73 (1H, dd, 8.8, 7.5)
7	4.70 (1H, q, 6.7)	4.87[b]	4.65 (1H, m)	4.80 (1H, q, 7.0)	4.62 (1H, dd, 6.8, 7.2)	4.53 (1H, q, 6.5)	4.61 (1H p, 7.0)	4.58 (1H p, 6.6)
9	2.72 (1H, m)	2.71 (1H, m)	2.71 (1H, m)	2.71 (1H, m)	2.56 (1H, m)	2.61 (1H, m)	2.33 (1H, m)	2.51 (1H, ddd, 14.4, 11.8, 6.9)
10	1.94 (1H, d, 16.0) 2.27 (1H, dd, 15.5, 10.8)	1.91 (1H, d, 16.3) 2.30 (1H, dd, 15.9, 11.1)	1.92 (1H brd, 15.3) 2.29 (1H, dd, 15.7, 11)	1.89 (1H, brd, 16.3) 2.31 (1H, dd, 15.9, 11.5)	1.81 (1H, dd, 15.5, 4) 2.13 (1H, dd, 15.5, 9.5)	2.33 (1H, dd, 13.2, 11.4) 2.55 (1H, dd, 12.6, 3.6)	2.12 (1H, dd, 13.9, 8.8) 2.25 (1H, dd, 14.6, 4.7)	2.14 (1H, dd, 14.4, 3.8) 2.34 (1H, dd, 14.9, 7.1)
12	4.86[b]	4.85[b]	4.86[b]	4.82 (1H, d, 7.0)	4.82 (1H, d, 9.2)		3.91 (1H, 4.0)	3.72 (1H, d, 6.2)
13	2.32 (1H, m)	2.31 (1H, m)	2.33 (1H, m)	2.30 (1H, m)	2.27 (1H, m)	3.32 (1H, m)	1.63[b]	1.61[b]
14	1.22 (1H, m) 1.43 (1H, m)	1.14 (1H, m) 1.31 (1H, m)	1.20 (1H, m) 1.39 (1H, m)	1.10 (1H, m) 1.27 (1H, m)	1.27 (1H, ddd, 13.8, 6, 4.5) 1.48 (1H, m)	1.40 (1H, ddd, 13.8, 6, 4.8) 2.12 (1H, ddd, 15.6, 14.4, 7.8)	1.17 (1H, ddd, 14.4, 6.2, 5.5) 1.56 (ovl)	1.05 (1H, ddd, 14.4, 8.4, 3.5) 1.80 (1H, ddd, 14.6, 10.2, 2.2)
15	4.70 (1H, m)	4.66 (1H, m)	4.65 (1H, m)	4.62 (1H, m)	4.66 (1H, dd, 13.0, 6.5)	4.79 (1H, m)	4.82 (1H, bp, 6.3)	4.87 (1H, m)
17	7.11 (2H,d, 8.7)	7.01 (2H, d, 8.5)	7.08 (2H, d, 8.5)	6.96 (2H, d, 8.8)	7.08 (2H, d, 8.5)	7.12 (2H,d, 8.4)	6.92 (2H, d, 8.5)	6.77 (2H, d, 8.5)
18	6.72 (2H, d, 8.8)	6.70 (2H, d, 8.5)	6.71 (2H, d, 8.8)	6.68 (2H, d, 8.6)	6.68 (2H, d, 8.5)	6.74 (2H, d, 8.4)	6.61 (2H, d, 8.5)	6.55 (2H, d, 8.5)
20	2.44 (1H, dd, 13.3, 7.8) 2.72 (1H, dd, 15, 4.5)	2.56 (1H, dd, 15.5, 9.5) 2.78 (1H, dd, 15.5, 3.5)	2.35 (1H, dd, 14.8, 4.1) 2.44 (1H, dd, 14.9, 9.6)	2.51 (1H, dd, 14.7, 9.7) 2.58 (1H, dd, 14.9, 6.2)	3.40[b] 3.92 (1H, dd, 15.5, 7.5)	2.53 (1H, dd, 15.6, 8.4) 2.66 (1H, dd, 15.0, 4.2)	3.16 (1H, dd, 15.2, 10.0) 3.41 (1H, dd, 15.2, 6.6)	3.11 (1H, dd, 14.9, 9.0) 3.40 (1H, dd, 14.9, 7.3)
23	7.32 (1H, dd, 8.1, 1)	7.27 (1H, dd, 7.5, 1)	7.43 (1H, d, 7.5)	7.30 (1H, d, 8.0)	8.41 (1H, d, 8.4)	7.32 (1H, dd, 7.8, 1.2)	7.53 (1H, d, 7.8)	7.58 (1H, bd, 7.7)
24	7.11 (1H, dt, 7.8, 1.2)	7.10 (1H, dt, 8,1)	6.99 (1H, dt, 7.6, 0.8)	7.02 (1H, dt, 7.6, 0.8)	7.76 (1H, ddd, 8.4, 8.5, 1.2)	7.13 (1H, dt, 7.2, 1.2)	7.08 (1H, dt, 8.0, 1.0)	7.09 (1H, t, 8.4, 0.9)
25	7.34 (1H, dt, 7.6, 1.2)	7.35 (1H, dt, 7.7, 1.2)	7.22 (1H, dt, 7.8, 1.2)	7.24 (1H, dt, 7.7, 1.2)	7.99 (1H,bt, 8.5)	7.36 (1H, dt, 7.8, 1.2)	7.13 (1H, dt, 8.0, 1.0)	7.14 (1H, dt, 8.4, 1.2)
26 28	6.89 (1H, dd, 7.9, 0.7)	6.89 (1H, dd, 8.0, 1.0)	6.84 (1H, d, 7.7)	6.84 (1H, d, 7.7)	7.98 (1H, dd, 8.5, 1.2) 9.08 (1H, s)	6.89 (1H, dd, 8.4, 1.2)	7.25[b]	7.24[b]
29	1.16 (3H,d, 6.7)	1.31 (3H, d, 7.0)	1.05 (3H, d, 6.5)	1.35 (3H, d, 6.8)	0.86 (3H d, 6.8)	1.12 (3H, d, 6.6)	0.75 (3H, d, 7.1)	0.84 (3H, d, 6.9)
30	1.13 (3H,d, 7.0)	1.14 (3H, d, 7.0)	1.12 (3H, d, 6.9)	1.14 (3H, d, 7.2)	0.97 (3H, d, 6.6)	1.15 (3H, d, 7.2)	1.13 (3H, d, 6.7)	1.15 (3H, d, 6.8)
31	1.59 (3H, s)	1.58 (3H, s)	1.58 (3H, s)	1.57 (3H, s)	1.51 (3H, s)	5.82 (1H, s) 6.15 (1H, s)	5.07 (1H, bs) 4.95 (1H, bs)	5.06 (1H, bs) 4.94 (1H, bs)
32	0.86 (3H, d, 6.6)	0.83 (3H, d, 7.0)	0.86 (3H, d, 6.6)	0.81 (3H, d, 6.7)	0.83 (3H, d, 6.6)	1.03 (3H, d, 6.6)	0.79 (3H, d, 6.8)	0.87 (3H, d, 6.9)
33	1.06 (3H, d, 6.3)	1.02 (3H, d, 6.0)	1.07 (3H, d, 6.5)	1.01 (3H, d, 6.4)	1.04 (3H, d, 6.6)	1.14 (3H, d, 7.2)	1.04 (3H, d, 6.1)	0.95 (3H, d, 6.2)
34	2.91 (3H, s)	3.06 (3H, s)	2.89 (3H, s)	2.97 (3H, s)	3.00 (3H, s)	2.87 (3H, s)	3.08 (3H, s)	3.13 (3H, s)
O−CH_3	3.01 (3H, s)	3.11 (3H, s)				3.04 (3H, s)		

[a] Measured at 500 MHz. [b] Shift assigned based on gHMQC and gHMBC data. [c] Spectra recorded in $CDCl_3$.

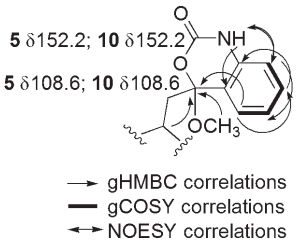

Figure 2. Selected 2D NMR (600/150 MHz, CD_3OD) correlations of the benzoxazinone substructure of jasplakinolide P (**5**), 21-epi-jasplakinolide P (**6**), and jasplakinolide U (**10**).

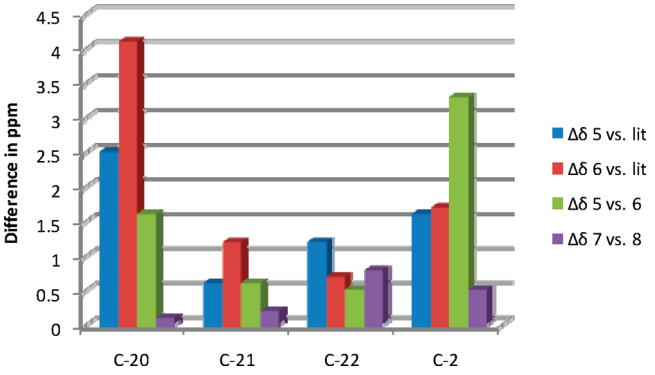

Figure 3. NMR $\Delta\delta_C$ shift differences (ppm) between (a) literature values for jasplakinolide P[22] vs experimental values of jasplakinolide P (**5**) or 21-epi-jasplakinolide P (**6**) and (b) shift differences between jasplakinolide S (**7**) and 21-epi-jasplakinolide S (**8**).

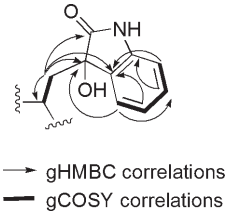

Figure 4. Selected 2D NMR (600/150 MHz, CD_3OD) correlations for the 3-alkyl-3-hydroxyindolin-2-one substructure of jasplakinolide S epimers **7** and **8**.

shift for C-31 ($\delta_{H/C}$ 5.82 and 6.15/129.1) and $\Delta\delta_C$'s < 1 ppm vs those of jasplakinolide B (**11**)[16] were observed for all of the remaining resonances of the core carbons including that of the enone: C-12 (**10**, δ_C 207.2; **11**, δ_C 207.2).[16] The final structural element R_1 was determined to be a benzoxazinone appendage, analogous to that in **5** and **6**: ($\delta_{C\text{-}OCH3}$ **5**, 51.2; **6**, 51.1; **10**, 51.1; and $\delta_{C\text{-}28}$ **5**, 152.2; **6**, 152.0; **10**, 152.2). As expected, a set of 2D NMR correlations parallel to those shown in Figure 2 were observed for **10**. The pattern of optical rotations discussed above for **7** ($[\alpha]_D$ = +36.8°, MeOH) and **6** ($[\alpha]_D$ = +39.2°, MeOH) vs that for **10** ($[\alpha]_D$ = +39.2°, MeOH) provided a putative *R* configuration at C-21, and as above, the chirality shown at all other sites is based on biosynthetic analogy to **1**.

The final pair of diastereomers isolated (**12** and **13**) possessed the same molecular formula as jasplakinolide C ($C_{36}H_{45}BrN_4O_7$), first described by Zampella in 1999.[16] Challenges faced when comparing our material to this compound included the lack of a defined configuration at C-12 in the original description and the inadvertent switch of structures between jasplakinolides B and C in the last three reports

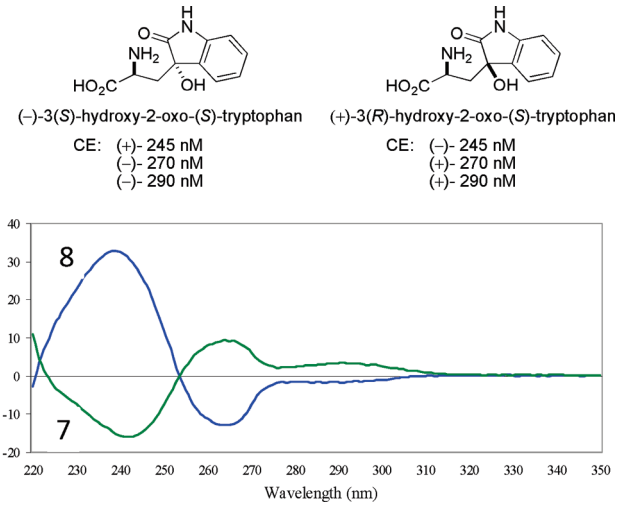

Figure 5. Cotton effects (CE) exhibited by synthetic 3-hydroxy-*S*-tryptophan derivatives (top)[38] and CD spectra of jasplakinolide S epimers **7** (green) and **8** (blue).

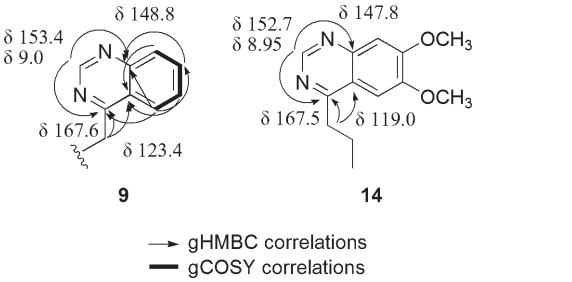

Figure 6. Selected 2D NMR (600/150 MHz, DMSO-d_6) correlations and $\delta_{H/C}$ values of the 4-alkyquinazoline substructure of **9** and of the standard 6,7-dimethoxy-4-propylquinazoline (**14**).

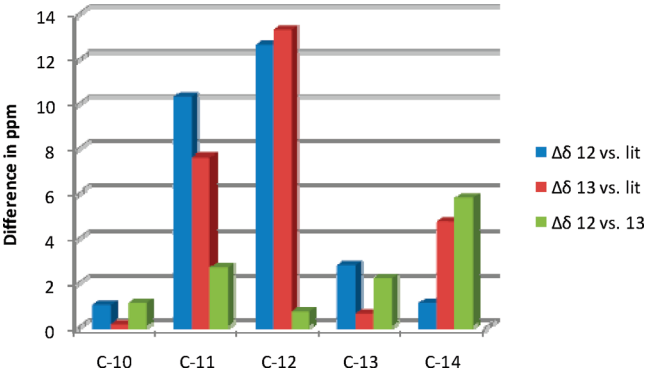

Figure 7. NMR $\Delta\delta_C$ shift differences (ppm) between literature values of jasplakinolide C[16] vs experimental values of jasplakinolide C_a (**12**) and jasplakinolide C_b (**13**).

of jasplakinolide congeners.[20–22] Clearly, both **12** and **13** possessed the Group 2 framework (**12**: $\delta_{H\text{-}31/C\text{-}31}$ 4.95, 5.04/113.1; **13**, $\delta_{H\text{-}31/C\text{-}31}$ 4.97, 5.12/111.2), and they differed from **11** by an additional degree of saturation consistent with the presence of an allylic alcohol constellation at C-11—C-12—C-31 vs the enone of **11**. Further, the NMR shift positions for protons and carbons at these sites had the expected δ values in **12** and **13**. Resolving the configuration at C-12 was not straightforward, because most of the $\Delta\delta_C$ data between this pair was less than 1.6 ppm. Also, as shown in Figure 7, the

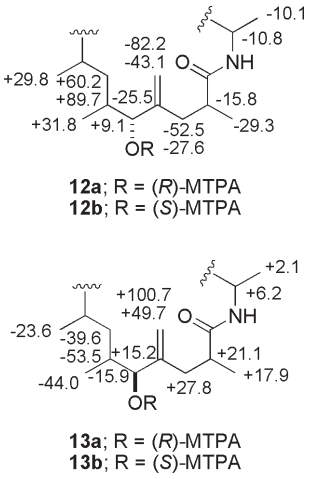

Figure 8. Modified Mosher analysis, $\Delta\delta^{SR}$ (Hz), of **12a**, **12b**, **13a**, and **13b** prepared from jasplakinolide C_a (**12**) and jasplakinolide C_b (**13**), respectively (for framework numbering, see **1**).

$\Delta\delta_C$'s for atoms in or near the allylic alcohol moiety (C-10 to C-14) were small, with the largest at C-14 ($\Delta\delta_C$ = 5.8), two bonds away from the chiral center. In spite of this circumstance, the acquisition of NOESY data provided useful correlations such as **12**, H-12 (δ 3.81) to H-13 (δ 1.65), and **13**, H-12 (δ 3.72) to CH_3-32 (δ 0.93). These data showed that, for jasplakinolide C_a (**12**), H-12 and H-13 were on a common face indicating that C-12 OH was α, and for jasplakinolide C_b (**13**), H-12 and CH_3-32 were on a common face indicating that C-12 OH was β. Defining the absolute chirality at this position for both compounds could now be addressed by derivatization using modified Mosher's method to form **12a**, **12b**, **13a**, and **13b** as seen in Figure 8.[39,40] The straightforward analysis of $\Delta\delta^{SR}$ concluded that **12** possessed 12*R* configuration and **13** was the 12*S* diastereomer. At this point, the CH_3-32 absolute configuration could be assigned as *R*, and the configurations at the remaining sites were concluded to be unchanged relative to **1**. Still unresolved was the relationship between **12** and **13** vs the previously described jasplakinolide C. No headway could be made on this issue, as a comparison of δ_C's between **12** and **13** vs literature (see Table S2, Supporting Information) indicated the substantial differences shown in Figure 7 and at other sites ($\Delta\delta_C$) including C-4 (5), C-29 (3), C-30 (5), C-31 (8), and C-32 (7).

At completion of isolation and identification of compounds **1** and **3**−**13**, a two pronged approach was used to evaluate their biological properties. This consisted of the following assays: (1) cytotoxicity assessment in the National Cancer Institute-Developmental Therapeutics Program (NCI-DTP) 60 cell line screen and (2) evaluation in the microfilament (MF) disruption assay in the UCSC-Chemical Screening Center (UCSC−CSC). We have demonstrated the value of such a parallel approach with 18-epi-latrunculol which exhibited cytotoxicity (IC_{50} = 2.1 μM against HCT-116 cells) without MF disruption at 5 μM.[41] NCI evaluation of seven compounds (**1**, **3**, **5**−**8**, and **11**) against the NCI-DTP 60 cell line panel resulted in GI_{50}, TGI, and LC_{50} data (see Tables S3−S9, Supporting Information)[42] with selected GI_{50} values from this assessment shown in Table 4. On the basis of results reported in Tables 4 and 5, two compounds (**3** and **11**) were selected by the NCI Biological Evaluation Committee for further study in the hollow fiber assay, pending availability of more material. Further, as seen in Tables 4 and 5, compound **3** exhibited slightly enhanced inhibition of leukemia cells (RPMI-8226), while compound **11** exhibited significant inhibition of both RPMI-8226 and colon adenocarcinoma (HCT-116) cells. Overall, these results shown in Tables 4 and 5 counter the previously reported[20] cytotoxicity findings demonstrating greatest human breast adenocarcinoma (MCF-7) cell inhibition by **1** (IC_{50} = 0.019 μM) and **3** (IC_{50} = 0.02 μM) with modest inhibition by compound **11** (IC_{50} = 3.4 μM).

Table 4. Bioassay Data for **1** and **3**−**13**

		GI_{50} (μM)		
cmpd	NSC#	HCT-116[a]	MCF-7[a]	MF disruption assay[b]
1	613009	0.1	Not Tested	+
3	731257	0.14	0.18	-
4		Not Tested		+
5	731256	0.35	4.9	-
6	731253	0.38	2.4	-
7	731254	0.81	2.3	-
8	731255	> 10	> 10	-
9		Not Tested		-
10		Not Tested		-
11	731258	< 0.001	0.13	-
12		Not Tested		-
13		Not Tested		-

[a] GI_{50} data provided by the National Cancer Institute-Developmental Therapeutics Program (NCI-DTP). For the comprehensive data set against 60 cell lines use the NSCs above at http://dtp.nci.nih.gov/. [b] The microfilament-disrupting effects were evaluated in HeLa cells: (+) active at 80 nM; (−) inactive at 80 nM.

Table 5. Selected NCI 60 Cell Screen Testing Results for Jasplakinolide (**1**), Jasplakinolide E (**3**), and Jasplakinolide B (**11**)

	1	**3**	**11**
cells	GI_{50} (μM)	GI_{50} (μM)	GI_{50} (μM)
RPMI-8226	0.031	0.022	0.0019
HOP-62	0.15	0.14	0.14
HCT-15	0.69	0.97	6.6
SF-539	0.30	0.17	0.064
M14	0.056	0.078	0.053
OVCAR-3	0.040	0.53	0.11
786−0	0.020	0.18	0.51

As stated above, all compounds (**1**, **3**−**13**) were screened in the UCSC−CSC HeLa (human cervical cancer) cell MF disruption assay. Table 4 shows the results of the phenotypic assay, with the last column indicating changes in actin structural organization in the presence of 80 nM of each compound (see Figure S13, Supporting Information). Two compounds (**1** and **4**) exhibited microfilament disruption at 80 nM as seen in Figure 9. Jasplakinolides A−P have been previously tested for microfilament disruption, and it was shown that antimicrofilament activity parallels cytotoxicity.[22] Alternatively, the NCI-DTP 60 cell screen data in combination with the UCSC−CSC microfilament disruption assay data showed that **11** exhibits significant cytotoxicity against HCT-116 colon adenocarcinoma cells (< 1 nM) without microfilament disruption at 80 nM (Figure 8). Both **1** and **11** possess identical scaffolds in the nonribosmal peptide synthetase (NRPS) region, but they differ by the enone group in the PKS region. Overall, these results are analogous to the above-mentioned reports that oxidation or derivatization of the brominated tryptophan or β-tyrosine groups decrease potency,[22,25,43−46] replacement of Ala for Ser does not significantly decreases potency,[20] and limited modifications in the PKS region are tolerated.[22,44,45] Complementary conclusions were gained

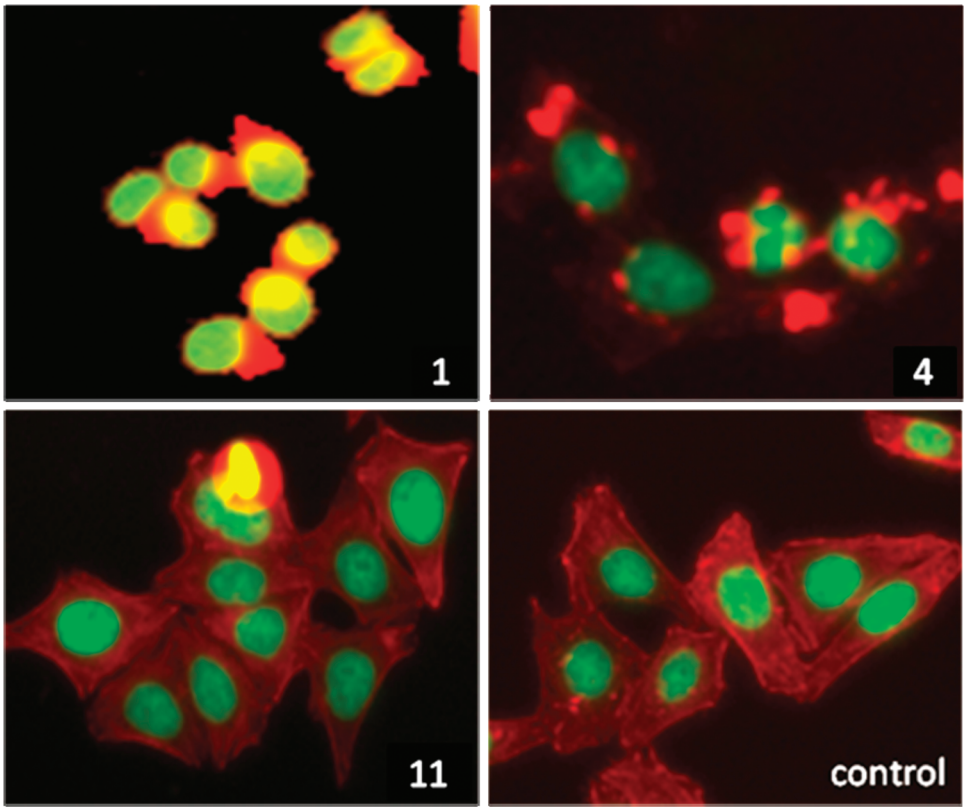

Figure 9. Influence of jasplakinolide (**1**), jasplakinolide F (**4**), jasplakinolide B (**11**), and DMSO (negative control) at 80 nM on the actin cytoskeleton of HeLa cells after 24 h incubation. The F-actin was labeled red (rhodamine-phalloidin) and nuclei and chromosomes labeled green (hoechst 33342, AnaSpec Inc.).

from SAR data published by two different groups on four synthetic chondramide C diastereomers obtained through total synthesis. A binding site analysis of synthetic **2** identified the presence of the double bond and the 15*R* configuration as two key elements required for optimal activity.[47] Alternatively, the **2** diastereomer with 13*S*, 15*S* configurations also exhibited similar conformation and bioactivity to natural **2**.[48] In conclusion, further biological evaluation of jasplakinolide diastereomers having modified configurations at each chiral center is warranted.

The results reported above, including seven new compounds (**6**−**10**, **12**, and **13**), have significant structural and biological impact. First, the unique quinazoline substructure in **9** is the first reported sponge-derived natural product containing quinazoline functionality.[49] The benzoxazinone substructure of **5** and **6** and the 3-hydroxyindolin-2-one of epimers **7** and **8** represent rare tryptophan functionalization. Counter to previous findings,[22] this is the first account where discrepancy between MF disruption and cytotoxicity has been exhibited by a jasplakinolide congener (**11**). Compound **11** exhibited considerable cytotoxicity (GI_{50} < 1 nM vs HCT-116 cells) without MF disruption at 80 nM, which has prompted both hollow fiber assessment by the NCI-DTP and further microfilament investigation at the UCSC−CSC. At this point, it is difficult to explain the pattern of activity or inactivity pertaining to MF disruption results of Table 4. The presence of an *exo* double bond may be a factor toward contributing to the inactivity of compounds **11**−**13**. Extensive tryptophan modifications were exhibited in compounds **5**−**10**, and these changes greatly diminish the MF binding effects. Also of high impact is the loss of activity through the simple replacement of Ala (in **1**) by Ser (in **3**). Finally, changing R_3 from CH_3 (in **1**) to H (in **4**) has no impact on the MF disruption activity. It is tantalizing to ponder the results from a recent molecular genetics study of the pathways responsible for producing **2** by *C. crocatus*.[4,50] An interesting point to resolve in regard to this study pertains to the different mechanisms at work to produce PKS domains differing in geometry and constitution in the C-13 to C-15 region.

Experimental Section

General Experimental Procedures. The optical rotations were determined on a Jasco DIP 370 digital polarimeter, and UV data were obtained on an Agilent 8453 UV/vis spectrophotometer. All NMR spectra were recorded in CD_3OD, $CDCl_3$, or DMSO-d_6 with a 5 mm triple resonance (HCN) probe. Chemical shifts are reported in ppm relative to CD_3OD (δ_H 3.31 and δ_C 49.0), $CDCl_3$ (δ_H 7.27), or DMSO-d_6 (δ_H 2.50 and δ_C 39.5). A Mariner ESITOF mass spectrometer was used for low- and high-resolution mass measurements. Preparative reversed-phase (RP) separation was carried out utilizing a Waters 600E system controller and pumps with a Prep LC 25 mm radial compression column using 25 × 100 mm C18 Nova-Pak HR16 (6 μm) cartridges. Both ELSD and UV (254 nm) were used for peak detection. Semipreparative RP HPLC used a Phenomenex Synergi C18 4 μm column, 10 × 250 mm and UV peak detection (254 nm). Compound purity (>95%) was confirmed using both ^1H and ^{13}C NMR and LCMS (UV and ELSD detection) experiments.

Animal Material. Samples of *Auletta* sp. sponges (coll. nos. 02137, 1.9 kg wet weight; and 02118, 2.0 kg wet weight) were collected in Papua New Guinea (S 9°43.969′, E 150°44.412′) June 2002. Samples of *Jaspis splendens* (coll. no. 00101, 1.9 kg wet weight) were collected in Fiji (S 18°22.28′, E 177°58.87′)

February 2000. Taxonomic identification *Auletta* sp. and *Jaspis splendens* were performed by Dr. Rob van Soest of the Zoological Museum of Amsterdam. Underwater pictures are in Figure S12, Supporting Information, and voucher specimens are available from the corresponding author (P.C.).

Extraction and Isolation. All three sponge materials were preserved in the field in 1:1 CH_3OH-H_2O, decanted, and shipped back to UC Santa Cruz where they were then submersed in 100% CH_3OH and stored at 4 °C. For extraction, the CH_3OH was decanted and the sponge was soaked in 1 L four more times to obtain the crude methanol extract. The extract was partitioned between hexanes and CH_3OH-H_2O (9:1). After separation from the hexane layer, H_2O was added to the CH_3OH-H_2O layer, adjusting the solution to 1:1. The CH_3OH-H_2O layer was then extracted with CH_2Cl_2 to give the crude (FD) extract (2.8 g for sponge coll. no. 02137, 1.6 g for sponge coll. no. 02118, and 2.1 g for 00101 for sponge coll. no. 00101).

The FD extract of 02137 was subjected to preparative reversed-phase (RP) HPLC (10−70% CH_3CN-H_2O, 60 min) yielding eight fractions (H1−H8). Fractions H4 (79.4 mg) and H5 (98.9 mg) were combined and subjected to semiprepartive RP HPLC using isocratic conditions (19:31, CH_3CN-H_2O) to afford **7** (4.1 mg, H4 H8 H2), **8** (2.5 mg, 02137 FD H45 H4 H3), **12** (5.6 mg, H45 H10 H3), and **13** (2.6 mg, H45 H11 H3). Fraction H6 (83.9 mg) was subject to multiple rounds of RP HPLC utilizing isocratic conditions (21:29, CH_3CN-H_2O) to afford **3** (1.5 mg, H6 HW H2 H2 H3 H2 H2), **4** (1.1 mg, H6 H2 H3), **5** (2.9 mg, H6 H3 H2), **6** (2.5 mg, H6 H2 H2), and **11** (4.0 mg, H6 H5 H3). Fraction H7 (221.1 mg) was purified by repetitive RP HPLC over isocratic conditions (11:13, CH_3CN-H_2O) to yield **1** (70.1 mg, H7 H1 H2).

The FD extract for 02118 was subjected to preparative RP HPLC (10−65% CH_3CN-H_2O, 60 min) yielding eight fractions (H1−H8). Fraction H2 (22.9 mg) was subjected to three rounds of RP HPLC employing isocratic conditions (9:16, CH_3CN-H_2O) to yield compound **10** (4.3 mg, H2 H6 H2 H2).

Similarly, 00101 FD was subjected to preparative RP HPLC (10−65% CH_3CN-H_2O, 50 min) yielding 11 fractions (H1−H11). Fraction H6 (133.2 mg) was subjected to multiple rounds of semipreparative RP HPLC using isocratic conditions (43% CH_3CN-H_2O, 40 min) to yield **9** (2.3 mg, H6 H11 H3 H3 H3). Fraction H8 (159.2 mg) was separated via multiple rounds of isocratic (77% CH_3OH-H_2O, 45 min) RP HPLC to yield **4** (1.9 mg, H8 H5 H5).

HeLa Cell Microfilament Disruption Assay. HeLa Cells were plated in 384 well tissue culture treated plates (Corning) at a density of 1500 cells per well. After incubating at 37 °C with 5% CO_2 overnight, compounds were pinned into plates using a Janus MDT (PerkinElmer) automated liquid handler. After 24 h, cells were fixed in 4% formaldehyde for 20 min then washed with PBS using an automated plate washer (BioTek). The cells were then treated with 0.5% Triton X-100 in PBS for 10 min, washed, and then blocked with a 2% BSA PBS solution for 20 min. Actin was stained with rhodamine−phalloidin for 20 min and then washed. Lastly, the DNA was stained with Hoechst 33342 (AnaSpec Inc.) followed by a wash with the automated plate washer. Images were taken using an automated fluorescence microscope (ImageXpress, MDS) at 10× magnification.

Jasplakinolide P (5). Colorless glass (2.9 mg). $[\alpha]^{27}_D = +61.6°$ (*c*, 0.062, MeOH); UV (MeOH) λ_{max} (log ε): 227 nm (3.19), 244 nm (2.87), 277 nm (2.50); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+H]^+$ *m*/*z* 693.3491 (calculated for $C_{37}H_{49}N_4O_9$, 715.3314).

21-Epi-Jasplakinolide P (6). Colorless glass (2.5 mg). $[\alpha]^{27}_D = +39.2°$ (*c*, 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 228 nm (3.07), 244 nm (2.77), 278 nm (2.23); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+Na]^+$ *m*/*z* 715.3321 (calculated for $C_{37}H_{48}N_4O_9Na$, 715.3314).

Jasplakinolide S (7). Colorless glass (4.1 mg). $[\alpha]^{27}_D = +36.8°$ (*c*, 0.056, MeOH); UV (MeOH) λ_{max} (log ε): 230 nm (2.70), 253 nm (2.33); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESI-TOFMS $[M+H]^+$ *m*/*z* 663.3392 (calculated for $C_{36}H_{47}N_4O_8$, 663.3388); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 242 nm (−16), 264 nm (+10), 292 nm (+3).

21-Epi-Jasplakinolide S (8). Colorless glass (2.5 mg). $[\alpha]^{27}_D = +62.6°$ (*c*, 0.054, MeOH); UV (MeOH) λ_{max} (log ε) 230 nm (2.75), 253 nm (2.43); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+Na]^+$ *m*/*z* 685.3209 (calculated for $C_{36}H_{46}N_4O_8Na$, 685.3208); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 239 nm (+32), 264 nm (−12), 293 nm (−1).

Jasplakinolide T (9). White powder (2.3 mg). $[\alpha]^{27}_D = +26°$ (*c*, 0.056 MeOH); UV (MeOH) λ_{max} (log ε) 202 nm (4.10), 223 nm (3.98) 274 nm (3.07); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+H]^+$ *m*/*z* 644.3495 (calculated for $C_{36}H_{46}N_5O_6$, 644.3442).

Jasplakinolide U (10). Colorless glass (4.3 mg). $[\alpha]^{27}_D = +39.4°$ (*c*, 0.054, MeOH); UV (MeOH) λ_{max} (log ε): 228 nm (3.07), 244 nm (2.77), 278 nm (2.23); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+H]^+$ *m*/*z* 707.3315 (calculated for $C_{37}H_{47}N_4O_{10}$, 707.3287).

Jasplakinolide C_a (12). Colorless glass (4.3 mg). $[\alpha]^{27}_D = +37.0°$ (*c*, 0.054, MeOH); λ_{max} (log ε) 220 nm (3.41), 281 nm (2.61); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+H]^+$ *m*/*z* 707.3315 (calculated for $C_{37}H_{47}N_4O_{10}$, 707.3287).

Jasplakinolide C_b (13). Colorless glass (2.6 mg). $[\alpha]^{27}_D = +62.3°$ (*c*, 0.058 MeOH); λ_{max} (log ε) 222 nm (3.32), 280 nm (2.70); ^1H and ^{13}C NMR (see Tables 2 and 3); HRESITOFMS $[M+Na]^+$ *m*/*z* 747.2373 (calculated for $C_{36}H_{45}N_4O_7NaBr$, 747.2364).

Reaction of 12 to Form 12a and 12b. To a solution of 1.8 mg of jasplakinolide C_a (**12**) 20 μL of (+)-α-methoxy-α-trifluoromethylphenylacetic chloride (MTPA-Cl) was added, followed by 100 μL of pyridine. The reaction mixture was stirred at room temperature for 30 min and dried under N_2. The residue was then purified by RP HPLC using CH_3CN-H_2O (22:3; isocratic) yielding 1.1 mg of the (*R*)-MTPA ester **12a**. The same procedure was used with 2.0 mg of **12** to furnish 1.3 mg of product for the (*S*)-MTPA ester **12b**. In both cases, the secondary as well as phenolic hydroxyl groups reacted with the MTPA-Cl.

(*R*)-MTPA Ester 12a. Obtained as a colorless glass. ^1H NMR (CD_3OD) δ_H 0.76 (3H, d, *J* = 6.5 Hz, CH_3-32), 0.84 (3H, d, *J* = 7 Hz, CH_3-29), 1.05 (3H, d, *J* = 6.5 Hz, CH_3-33), 1.13 (3H, d, *J* = 7 Hz, CH_3-30), (1H, m, H14a), 1.49 (1H, m, H14b), 1.87 (1H, m, H13), 2.17 (1H, dd, *J* = 15.5, 6.5 Hz, 10a), 2.41 (1H, dd, *J* = 16.5, 9 Hz, H10b), 2.61 (1H, dd, *J* = 14.8, 6.8 Hz, H9), 2.77 (1H, dd, *J* = 17.3, 5.3 Hz, H2a), 2.99 (1H, dd, *J* = 16.8, 8.8 Hz, H2b), 3.07 (1H, dd, *J* = 15, 10 Hz, H20a), 3.12 (3H, s, CH_3-34), 3.38 (1H, dd, *J* = 14.5, 6 Hz, H20b), 3.47 (3H, s, OMe), 3.68 (3H, s, OMe), 4.41 (1H, q, *J* = 7 Hz, H7), 4.96 (1H, s, H31a), 5.08 (1H, s, H31b), 5.16 (1H, dd, *J* = 6.5, 5 Hz, H3), 5.23 (1H, d, *J* = 4.5 Hz, H12), 5.59 (1H, dd, *J* = 10, 6.5 Hz, H5), 6.96−7.63 (9H, Ar).

(*S*)-MTPA Ester 12b. Obtained as a colorless glass. ^1H NMR (CD_3OD) δ_H 0.82 (3H, d, *J* = 7 Hz, CH_3-29), 0.82 (3H, d, *J* = 7 Hz, CH_3-32), 1.07 (3H, d, *J* = 7 Hz, CH_3-30), 1.11 (3H, d, *J* = 6 Hz, CH_3-33), 1.23 (1H, m, H14a), 1.62 (1H, m, H14b), 1.89 (1H, m, H13), 2.06 (1H, dd, *J* = 14.5, 5 Hz, H10a), 2.36 (1H, dd, *J* = 15, 8 Hz, H10b), 2.58 (1H, dq, *J* = 13.5, 6.5 Hz, H9), 2.74 (1H, dd, *J* = 16.8, 5 Hz, H2a), 3.00 (1H, dd, *J* = 17, 9 Hz, H2b), 3.07 (1H, dd, *J* = 15, 10 Hz, H20a), 3.12 (3H, s, CH_3-34), 3.38 (1H, dd, *J* = 15, 6 Hz, H20b), 3.49 (3H, s, OMe) 3.68 (3H, s, OMe), 4.39 (1H, q, *J* = 6.5 Hz, H7), 4.80 (1H, s, H31a), 4.84 (1H, H15), 5.00 (1H, s, H31b), 5.15 (1H, dd, *J* = 9, 5 Hz, H3), 5.18 (1H, d, *J* = 4.5 Hz, H12), 5.61 (1H, dd, *J* = 9.8, 5.8 Hz, H5), 6.97−7.63 (9H, Ar).

Reaction of 13 to Form 13a and 13b. To a solution of 1.1 mg of jasplakinolide C_b (**13**) 20 μL of (+)-α-methoxy-α-trifluoromethylphenylacetic chloride (MTPA-Cl) was added, followed by 100 μL of pyridine. The reaction mixture was stirred at room temperature for 30 min and dried under N_2. The residue was

then purified by RP HPLC using CH_3CN-H_2O (22:3; isocratic) yielding 0.7 mg of the (*R*)-MTPA ester **13a**. The same procedure was used with 1.0 mg of **13** with (−)-MTPA-Cl to furnish 0.5 mg of the (*S*)-MTPA ester **13b**. In both cases, the secondary as well as phenolic hydroxyl groups reacted with the MTPA-Cl.

(*R*)-MTPA Ester 13a. Obtained as a colorless glass. ^{1}H NMR (CD_3OD) δ_H 0.80 (3H, d, J = 7.0 Hz, CH_3-29), 0.91 (3H, d, J = 6.5 Hz, CH_3-32), 1.02 (1H, m, H14a), 1.09 (3H, d, J = 7.0 Hz, CH_3-30), 1.11 (3H, d, J = 6.0 Hz, CH_3-33), 1.69 (1H, m, H14b), 1.99 (1H, m, H13), 2.31 (2H, d, J = 6.0 Hz, CH_2-10), 2.57 (1H, dd, J = 13.5, 6.5 Hz, H9), 2.74 (1H, dd, J = 17.5, 4.5 Hz, H2a), 2.97 (1H, dd, J = 17.5, 9.5 Hz, H2b), 3.08 (1H, dd, J = 14.8,10.3 Hz, H20a), 3.13 (3H, s, CH_3-34), 3.52 (3H, s, OMe), 3.68 (3H, s, OMe), 4.38 (1H, q, J = 7 Hz, H7), 4.73 (1H, s, H31a), 4.96 (1H, s, H31b), 5.00 (1H, H15), 5.10 (1H, d, J = 4.5 Hz, H12), 6.97−7.63 (9H, Ar).

(*S*)-MTPA Ester 13b. Obtained as a colorless glass. ^{1}H NMR (CD_3OD) δ_H 0.80 (3H, d, J = 7 Hz, CH_3-29), 0.82 (3H, d, J = 6.5 Hz, CH_3-32), 0.91 (1H, m, H14a), 1.06 (3H, d, J = 6 Hz, CH_3-33), 1.12 (3H, d, J = 7 Hz, CH_3-30), 1.61 (1H, dd, J = 11.8 Hz, H14b), 1.96 (1H, m, H13), 2.36 (2H, d, J = 8 Hz, CH_2-10), 2.61 (1H, dd, J = 12.8, 6.8 Hz, H9), 2.73 (1H, dd, J = 17.8, 4.8 Hz, H2a), 2.96 (1H, dd, J = 17.5, 9.5 Hz, H2b), 3.08 (1H, dd, J = 15, 10 Hz, H20a), 3.13 (3H, s, CH_3-34), 3.35 (1H, dd, J = 15.3, 5.8 Hz, H20b), 3.47 (3H, s, OMe), 3.68 (3H, s, OMe), 4.39 (1H, q, J = 7 Hz, H7), 4.93 (1H, s, H31a), 4.97 (1H, H15), 5.05 (1H, s, H31b), 5.13 (1H, d, J = 4.5 Hz, H12), 5.19 (1H, dd, J = 9.5, 4.5 Hz, H3), 5.58 (1H, dd, J = 10, 6 Hz, H5), 6.98−7.63 (9H, Ar).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1**, **5**−**10**, **12**, and **13** and sponge pictures for 02137, 02118, and 00101, selected 2D NMR spectra, HeLa cell MF disruption pictures, and NCI 60 cell line results. This material is available free of charge via the Internet at http://pubs.acs.org.

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