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Biostructural Features of Additional Jasplakinolide (Jaspamide) Analogues

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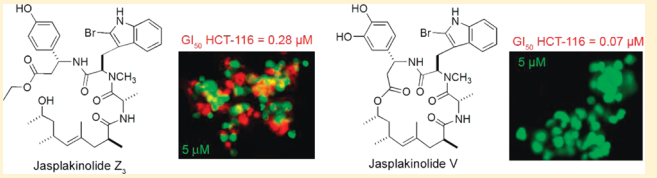
Supporting Information

ABSTRACT: The cyclodepsipeptide jasplakinolide (**1**) (aka jaspamide), isolated previously from the marine sponge *Jaspis splendens*, is a unique cytotoxin and molecular probe that operates through stabilization of filamentous actin (F-actin). We have recently disclosed that two analogues of **1**, jasplakinolides B (**3**) and E, were referred to the National Cancer Institute's (NCI) Biological Evaluation Committee, and the objective of this study was to reinvestigate a Fijian collection of *J. splendens* in an effort to find jasplakinolide congeners with similar biological properties. The current efforts have afforded six known jasplakinolide analogues (**4**−**7**, **9**, **10**), two structures requiring revision (**8** and **14**), and four new congeners of **1** (**11**−**13**, **15**) including open-chain derivatives and structures with modified β-tyrosine residues. Compounds were evaluated for biological activity in the NCI's 60 cell line screen and in a microfilament disruption assay in both HCT-116 and HeLa cells. These two phenotypic screens provide evidence that each cytotoxic analogue, including jasplakinolide B (**3**), operates by modification of microfilaments. The new structure jasplakinolide V (**13**) has also been selected for study by the NCI's Biological Evaluation Committee. In addition, the results of a clonogenic dose−response study on jasplakinolide are presented.



The unique cyclodepsipeptide jasplakinolide (**1**) (aka jaspamide) has been a seed for continuous chemical and biological investigations since its discovery in 1986.[1,2] This mixed polyketide−peptide compound (often referred to as a PKS−NRPS hybrid)[3] was first isolated from *Jaspis* (syn: *Doryplores*) *splendens*[4] (order Astrophorida, family Ancorinidae) sponges collected in Fiji and Palau, and subsequent investigations[5−10] have shown that **1** can be obtained from *Jaspis* sponges collected throughout the Indo-Pacific. We recently disclosed seven new analogues of **1**[10] and included an account of the 15 additional natural derivatives divided into two frameworks along with a biogeographical outline of the two sponge orders that are the source of these metabolites. Other marine sponge genera are also the source of related cyclodepsipeptides named the geodiamolides (*Geodia* and *Cymbastela*)[11−16] and the seragamides (*Suberites*).[17] Similar to the jasplakinolide family, the members of these two classes occur in taxonomically distant groups of sponges. The discovery of yet another related PKS−NRPS cyclodepsipeptide ring system represented in chondramides A−D (**2a**−**2d**) isolated from *Chondromyces crocatus* (a terrestrial myxobacterium)[18,19] adds additional insights to a structure versus bioactivity pattern still being developed. Strikingly, each of the five framework types in this assemblage including the 19-membered macrolides of the jasplakinolide/geodiamolide (i.e., H and I)[15] families and the 18-membered macrolides of the chondramide[18,19]/seragamide[17]/geodiamolide (i.e., A−G, J−R, TA, and neosiphoniamolide)[11−14,16] families share an identical *N*-methyl and *N*-hydrogen pattern. All of these compounds also possess virtually identical absolute configurations at the sites of aryl and alkyl ring substituents. Further, there is remarkable halogenation parallelism between jasplakinolide (**1**, $C_{36}H_{45}BrN_4O_6$) and chondramide D (**2d**, $C_{35}H_{43}ClN_4O_6$).

The push to understand the biological properties of jasplakinolide (**1**) has been continuous and can be traced back to the initial remarkable spectrum of activities including anthelmenthic,[1] insecticidal,[2] fungicidal,[2] and fish toxicity.[20] Also noteworthy was an early observation of nanomolar GI_{50}'s for **1** against human prostate carcinoma cell lines (PC-3 = 65, LnCap = 41, and TSU-Pr1 = 170) and that these effects were due to disruption of the actin cytoskeleton through the specific stabilizing interaction with polymerized F-actin (filamentous-actin) and not G-actin (globular-actin).[21] Although attempts to prepare **1** tagged with fluorescent probes to facilitate fundamental microfilament (MF)-based studies never matured, subsequent results showed that **1** exhibits an in vivo stabilizing effect against F-actin.[22] The current understanding of the cytotoxicity profile of this scaffold is based on studies of natural jasplakinolide and its analogues conducted against colon (HT-29 or HCT-116) and breast (MCF-7) cancer cells,[6−8,10] and complementary results were obtained against renal, prostate, and CNS

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tumor cell lines in the National Cancer Institute's (NCI) 60 cell line screen (see later discussion in connection with the data of Tables 4 and 5).[10] A list of structural features that must be present in the jasplakinolide skeleton to impart activity at a nanomolar level can be enumerated as follows: (a) the PKS region of the macrolide must be maintained in a semirigid form, and the double bond can be modified to exist as an enone; and (b) there can be no oxidation or derivatization of the aromatic amino acid residues, but the aliphatic amino acids can be altered. In addition, it has also become evident that **1** and **2c** have a shared cytotoxicity mechanism via F-actin binding.[23]

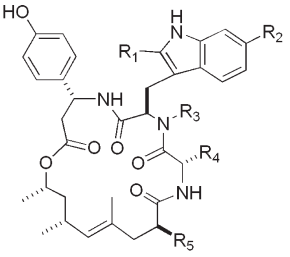
Recently published findings provide further insights on the jasplakinolide−chondramide pharmacophore with regards to actin-mediated cytotoxicity. These have been obtained through the examination of compounds obtained through total synthesis, and 10 such pathways exist to create analogues of **1**,[24−31] **2a**,[32] or **2c**.[23] Some additional confirmatory results have been obtained through the screening of geodiamolides.[33,34] On the basis of the current literature, we estimate that 45 synthetic congeners have been tested based on the jasplakinolide[35−41] and chondramide[40,42] scaffolds. Adding to the SAR pattern outlined above are the following: (1) no compound has been obtained with greater potency than **1**; (2) C-9 *S*-configuration is required (in the **1** or **2** framework) for optimal activity;[40] (3) modifying the *E*/*Z* geometry about the PKS double bond (C-11 and C-12, in the **1** or **2** framework) has a small effect;[23,40] (4) inverting the methyl group configuration at C-13/C-15 (in **1**) or at C-13/C-14 (in **2**) results in only a 10-fold loss in activity;[40,42] (5) removal of the *N*-methyl moiety decreases potency;[8,40] and (6) the β-tyrosine unit is essential for binding to the actin target.[40]

Although a wealth of research has been invested in exploring the experimental therapeutic potential of actin-targeting compounds, the NCI has not identified any leads with a realistic in vivo therapeutic index.[43] However, a new theme in the biological profiles for the jasplakinolide class appears to be on the horizon. The selection by the NCI's Biological Evaluation Committee of two analogues, jasplakinolide B (**3**) and jasplakinolide E, for follow-up in vivo assessment is the first encouraging outcome. Second, jasplakinolide (**1**) has been identified as an antimalarial lead, effecting the growth, invasion, and actin cytoskeleton of *Plasmodium falciparum*.[44] The goal of the current study was to further examine *J. splendens* extracts and compounds in our repository to uncover additional congeners with significant bioactivities, specifically, those with comparable responses in the NCI 60 cell line screen and the microfilament disruption assay versus that of jasplakinolides B (**3**) and E. Our new results achieved this intent and involved a study of 12 additional analogues.

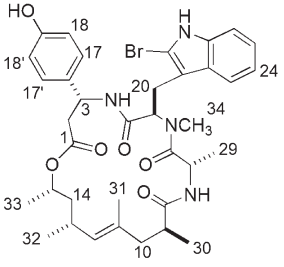
RESULTS AND DISCUSSION

The decision to continue isolation efforts on the Fijian *J. splendens* coll. no. 00101 proved to be worthwhile and led to several new outcomes. Previously, we analyzed two of its 11 semipure extract fractions,[10] which afforded only two compounds, the unusual quinazoline analogue jasplakinolide T and the known compound jasplakinolide F.[10,45] Further LC-MS scrutiny of the unstudied semipure fractions indicated the presence of a series of minor bromine-containing constituents with molecular ion peaks in the range 700−800 amu. Establishing the identity of these as new or known jasplakinolide congeners proceeded by reapplying our dereplication scheme[10] analyzing (a) LC-MS profiles; (b) ^{1}H NMR signature δ's; (c) the pattern of heavy atom formulas for all reported jasplakinolide analogues ($C_{35-37}N_4O_{6/7/9}$, $C_{35-37}BrN_4O_{6-7}$, $C_{36}Br_2N_4O_6$); and (d) the ^{1}H NMR δ's of the C-11 PKS carbon substituents to assign the macrolides as belonging to group 1 (see **1**) or group 2 (see **3**).

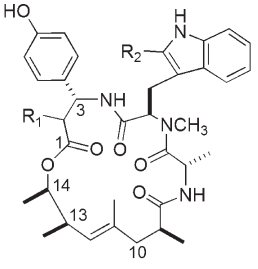
All 12 of the compounds isolated herein were either members of group 1 as macrocycles or their acyclic analogues. A set of four known compounds (**4**−**7**) was isolated from three different CH_2Cl_2-soluble preparative RP-HPLC fractions further separated by semipreparative chromatography (individually coded in the experimental as H7, H8, and H10). The first three were bromine-containing and consisted of a C_{35} isomeric pair ($C_{35}H_{43}BrN_4O_6$: $[M + H]^+$ *m*/*z* 695.2/697.2) plus a C_{37} homologue ($C_{37}H_{47}BrN_4O_6$: $[M + H]^+$ *m*/*z* 723.2/725.2). These were identified as jasplakinolide D (**4**),[6] jasplakinolide J (**5**),[7] and jasplakinolide M (**6**)[8] by using the ^{1}H NMR patterns for the five methyl groups of jasplakinolide (**1**) along with the 2D NMR correlations to these methyls as a benchmark for comparison to the data sets for this trio. The fourth compound was assigned as the known debromo analogue of **1**, named jasplakinolide Q (**7**),[9] based on comparison to the ESI $[M + H]^+$ peak at *m*/*z* 631.3 and the aromatic NMR fingerprint shown in Tables 1 and 2.



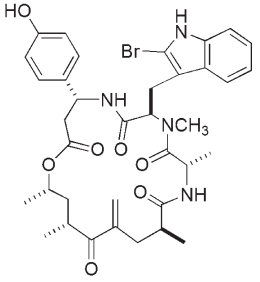
jasplakinolide D (**4**) R_1=Br, R_2=H, R_3=CH_3, R_4=CH_2CH_3, R_5=CH_3
jasplakinolide J (**5**) R_1=Br, R_2=H, R_3=CH_3, R_4=CH_3, R_5=H
jasplakinolide M (**6**) R_1=Br, R_2=H, R_3=H, R_4=CH_3, R_5=CH_3
jasplakinolide Q (**7**) R_1=H, R_2=H, R_3=CH_3, R_4=CH_3, R_5=CH_3
jasplakinolide R_1 (**8**) R_1=Br, R_2=Br, R_3=CH_3, R_4=CH_3, R_5=CH_3



jasplakinolide (**1**)



chondramide A (**2a**) R_1= OCH_3 R_2 = H
chondramide B (**2b**) R_1= OCH_3 R_2 = Cl
chondramide C (**2c**) R_1= H R_2 = H
chondramide D (**2d**) R_1= H R_2 = Cl



jasplakinolide B (**3**)

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Table 1. ^{13}C NMR Data for Compounds 7−12 in CD_3OD at 150 MHz

	7	8	9	10	11	12
pos.	δ_C type[a]	δ_C type[a]	δ_C type[a]	δ_C type[a]	δ_C type[a]	δ_C type[a]
1	172.1, C	172.3, C	174.8, C	173.2, C	172.6, C	
2	42.1, CH_2	42.1, CH_2	41.9, CH_2	41.6, CH_2	41.9, CH_2	
3	50.5, CH	50.6, CH	51.3, CH	51.3, CH	51.3, CH	
4	171.3, C	170.9, C	171.1,C	171.3, C	171.2, C	175.1, C
5	57.9, CH	56.9, CH	58.0, CH	58.1, CH	58.0, CH	57.7, CH
6	174.9, C	175.0, C	176.1, C	176.3, C	176.2, C	175.7, C
7	47.1, CH	47.0, CH	46.9, CH	47.0, CH	47.0, CH	46.9, CH
8	177.9, C	177.9, C	179.1, C	179.3, C	179.2, C	179.3, C
9	40.8, CH	40.6, CH	39.0, CH	39.0, CH	39.1, CH	39.4, CH
10	42.6, CH_2	42.8, CH_2	44.9, CH_2	45.1, CH_2	45.1, CH_2	44.8, CH_2
11	134.1, C	134.1, C	131.9, C	132.0, C	131.9, C	132.0, C
12	130.6, CH	130.7, CH	134.8, CH	135.0, CH	134.9, CH	134.8, CH
13	30.5, CH	30.6, CH	30.5, CH	30.6, CH	30.6, CH	30.5, CH
14	44.1, CH_2	44.2, CH_2	48.1, CH_2	48.2, CH_2	48.2, CH_2	48.2, CH_2
15	71.7, CH	71.7, CH	66.7, CH	66.9, CH	66.9, CH	66.8, CH
16	132.7, C	132.9, C	133.7, C	133.4, C	133.4, C	
17	128.2, CH	128.3, CH	128.9, CH	129.0, CH	129.0, CH	
18	116.2, CH	116.4, CH	116.2, CH	116.4, CH	116.3, CH	
19	157.7, C	157.9, C	157.8, C	158.0, C	158.0, C	
20	25.2, CH_2	25.2, CH_2	24.5, CH_2	24.5, CH_2	26.6, CH_2	24.8, CH_2
21	110.6, C	110.8, C	110.8, C	110.9, C	110.8, C	110.8, C
22	128.5, C	127.6, C	128.8, C	128.9, C	128.8, C	128.9, C
23	119.3, CH	120.9, CH	119.0, CH	119.2, CH	119.1, CH	119.2, CH
24	119.8, CH	123.8, CH	120.4, CH	120.6, CH	120.5, CH	120.6, CH
25	122.4, CH	116.5, C	122.7, CH	122.9, CH	122.8, CH	122.9, CH
26	112.3, CH	114.6, CH	111.5, CH	111.7, CH	111.6, CH	111.7, CH
27	138.1, C	138.7, C	137.9, C	138.0, C	138.0, C	138.1, C
28	124.2, CH	111.6, C	110.3, C	110.4, C	110.4, C	110.5, C
29	18.5, CH_3	18.4, CH_3	16.0, CH_3	15.7, CH_3	15.8, CH_3	16.3, CH_3
30	20.0, CH_3	20.1, CH_3	17.0, CH_3	17.1, CH_3	17.1, CH_3	17.5, CH_3
31	18.2, CH_3	18.3, CH_3	15.7, CH_3	16.1, CH_3	16.1, CH_3	16.2, CH_3
32	22.0, CH_3	22.1, CH_3	21.4, CH_3	21.5, CH_3	21.5, CH_3	21.5, CH_3
33	19.6, CH_3	19.8, CH_3	23.3, CH_3	23.5, CH_3	23.5, CH_3	23.5, CH_3
34	31.1, CH_3	31.8, CH_3	32.8, CH_3	33.0, CH_3	33.0, CH_3	32.7, CH_3
35				52.4, CH_3	61.8, CH_2	
36					14.7, CH_3	

[a] Carbon type determined from gHMQC data.

An additional compound was isolated from the previously described semipure fractions (the one noted above as H10). The properties of this dibromo analogue ($C_{36}H_{44}Br_2N_4O_6$: $[M + H]^+$ m/z 787.2/789.2/791.2, 1:2:1) of **1** were in agreement with its assignment as jasplakinolide R[9] (see regioisomer B in Figure 1), although the literature for this compound included only an ^{1}H NMR data set in $CDCl_3$. While the ^{1}H shifts in $CDCl_3$ of our material were superimposable with those previously published, on completion of our 2D NMR analysis, it was clear that this structure was assigned in error. The process we used to clarify the ambiguity in the bromo-indole region chemistry is shown in Figure 1, and it required collecting ^{1}H data in two additional solvents. One powerful result included the $^3J_{H-C}$ gHMBC[46] from H-23 (δ_H 7.48, d, ^3J_H = 8.5, CD_3OD) to C-21 (δ_C 110.8), which required the arrangement of regioisomer A. Apparently spectra obtained in $CDCl_3$ where there is close overlap of H-23 (δ_H 7.43 (d, J_H = 8.5) (this work) vs δ_H 7.42 (d, J_H = 8.2)[9]) and H-26 (δ_H 7.42 (d, J_H = 1.2) (this work) vs δ_H 7.41 (br s)[9]) led to an unclear interpretation of the NOE correlations shown for the putative regioisomer B in Figure 1. However in DMSO-d_6 these signals were well resolved. Irradiation of the *N*H-Tyr (δ_H 8.23 (d, J_H = 8.7)) revealed a clear correlation to H-23 (δ_H 7.59 (d, J_H= 8.4): Figure S1, Supporting Information) and provided the final basis for our assignment of the revised structure, named (+)-jasplakinolide R_1 (**8**). The absolute configuration shown for this compound and those following below are based on the obvious biosynthetic analogy to **1**.

The next phase of this work focused on two macrocyclic and five acyclic analogues isolated from three different CH_2Cl_2-derived chromatographic fractions and one butanol subfraction. One of the most polar compounds, the free acid form of jasplakinolide named (+)-jasplakinolide Z_1 (**9**, $C_{36}H_{47}BrN_4O_7$: $[M + H]^+$ m/z 727.3/729.3), was obtained from the butanol solvent partition fraction.

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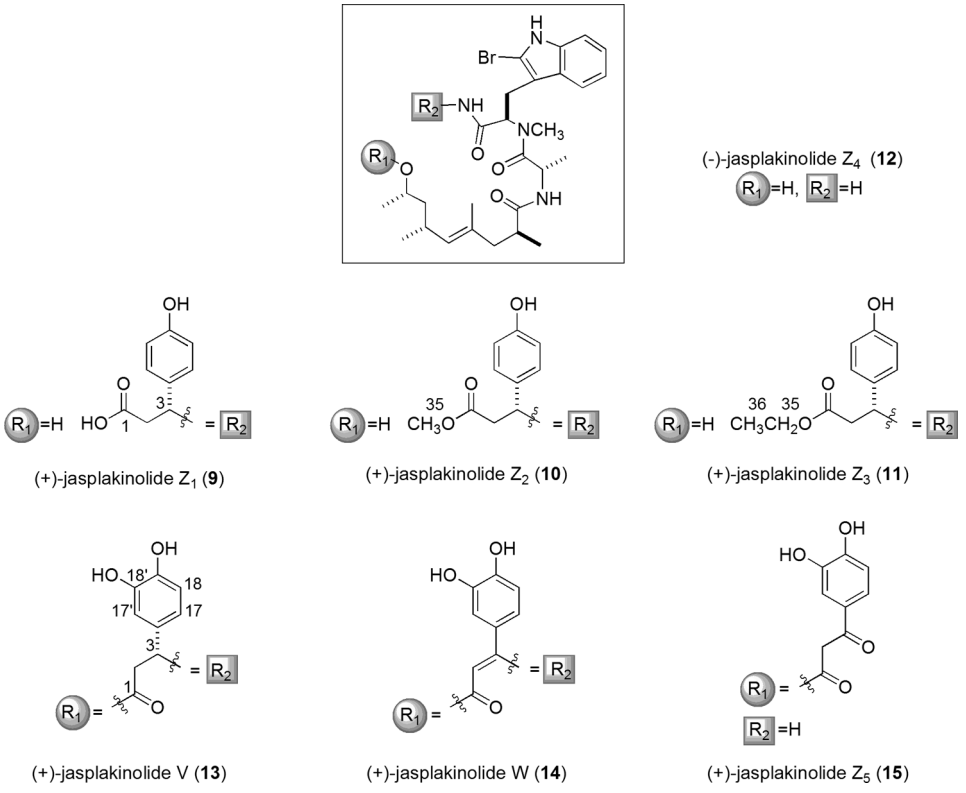
This compound, without accompanying physical properties, was first described in 1986,[2] as the saponification product of **1**, but it was reported again in 2006 accompanied by detailed ^{1}H NMR data.[17] Although the careful storage conditions of semipure extracts (4 °C) containing **9** suggest that it could have been present in the sponge tissue, it is also likely that **9** is a hydrolysis product of **1**. Two esters undoubtedly created from **9** during storage or workup were also obtained (from CH_2Cl_2 solvent partition fractions). These consisted of (+)-jasplakinolide Z_2 (**10**, $C_{37}H_{49}BrN_4O_7$), with the $-OCH_3$ group identified by a methyl (H_3) singlet at δ_H 3.65, and (+)-jasplakinolide Z_3 (**11**, $C_{38}H_{51}BrN_4O_7$), with the $-OCH_2CH_3$ group identified by two ^{1}H signals, δ_H 4.11 (H_2, q, J_H = 7.2)/1.22 (H_3, t, J_H = 7.0). Another truncated acyclic analogue, (−)-jasplakinolide Z_4 (**12**, $C_{27}H_{39}BrN_4O_4$: $[M + H]^+$ m/z 563.2/565.2), lacking the β-tyrosine moiety, but with the 2-bromoabrine retained, was straightforwardly characterized. None of the NMR signals for the R_2 group of **9** were present in the data for **12**, as shown in Tables 1 and 2.



Our characterization of two unique and closely related macrolides, (+)-jasplakinolide V (**13**, $C_{36}H_{45}BrN_4O_7$) and (+)-jasplakinolide W (**14**, $C_{36}H_{43}BrN_4O_7$), was streamlined by scrutiny of their MS-derived molecular formulas accompanied by NMR-derived count (Table 3) of the benzenoid ring oxygens. The low-field ^{1}H/^{13}C NMR δ's of **13** and **14** clearly showed that the β-tyrosine of **1** was further functionalized with an extra oxygen atom. The molecular formula of the latter was identical to that of jasplakinolide B possessing a hydroquinone,[21] described by our laboratory in 1995 (based on unreported 1D NMR data and not identical to jasplakinolide B/jaspamide B (**3**), reported in 1999[5]). Assigning the oxy-aryl ring in **13** and **14** as a catechol rather than the hydroquinone was based on the gHMBC correlations from H-17 to C-3 and H-17′ to C-3 shown in Figure 2. Additional three-bond gHMBC correlations from the hydroxy protons observed in DMSO-d_6 (18′-O*H*, δ_H = 8.84, br s; 19-O*H* δ_H = 8.81, br s) further confirmed the placement of these substituents (18′-O*H* to C-17′, C-19 and 19-O*H* to C-18, C-18′). Although an authentic sample of the proposed hydroquinone compound[21] was not available from our repository to examine by 2D NMR, we believe that the structure was mis-assigned and must be revised to **14**. While the aromatic ^{13}C chemical shifts (C-16 through C-19) matched closely for compounds **13** and **14**, there was great disparity for positions C-2 and C-3, as shown in Table 3 (also see Figure S2, Supporting Information). Compound **13** contained methylene and methine carbons (C-2 δ_C = 41.9; C-3 δ_C = 50.7), whereas the shifts of **14** were indicative of a trisubstituted alkene (C-2 δ_C = 101.8; C-3 δ_C = 155.1), allowing the structural assignment shown here. Interestingly, multiple examples of halogenation exist at the same site (C-18′) in other marine sponge metabolites such as the geodiamolides[16] and seragamides.[17]

The last open-chain congener, (+)-jasplakinolide Z_5 (**15**, $C_{36}H_{45}BrN_4O_8$), was immediately seen as being different than the other acyclics, **9**−**12**, described above. The two extra oxygens versus that of **1** were rationalized by proposing the R_1 substructure of 3-(3,4-dihydroxyphenyl)-3-oxopropanoate. The parallel ^{13}C NMR shifts (Table 3 and Figure S2, Supporting Information) at C-16 to C-19 among **13**−**15** supported the catechol (see Figure 2) ring assignment. A series of 2D gHMBC NMR data sets were used to interconnect additional key proximal residues of functional group R_1 as follows: (a) correlations from H-17, H-17′, and H_2-2 to the low-field carbon (δ_C = 191.6, C-3) and (b) those from H_2-2 to the ester carbonyl (δ_C = 167.7, C-1). The 2D gHMBC correlation from H-15 to C-1 was also observed, and remaining NMR shifts of **15** overlapped with those of **1**. We next considered the plausible source of this compound and deemed it unlikely that **15** was a true natural product. Insights from the biosynthetic gene cluster defined for chondramide C (**2c**)[47]

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Table 2. ^{1}H NMR Data for Compounds 7−12 in CD_3OD at 600 MHz

	7	8	9	10	11	12
pos.	δ_H (J[Hz])	δ_H (J[Hz])	δ_H (J[Hz])	δ_H (J[Hz])	δ_H (J[Hz])	δ_H (J[Hz])
2	2.74, dd (15.2, 4.0)	2.72, dd (15.4, 4.0)	3.01, dd (15.6, 9.0)	3.05, dd (15.8, 9.4)	3.03, dd (15.8, 9.0)	
	2.63, dd (15.2, 9.0)	2.69, dd (15.4, 8.6)	2.78, dd (15.6, 6.0)	2.83, dd (15.8, 5.8)	2.83, dd (15.8, 5.6)	
3	5.26, dd (9.0, 4.0)	5.25, dd (8.6, 4.0)	5.37, dd (9.0, 6.0)	5.36, dd (9.4, 5.8)	5.36, dd (9.0, 5.6)	
5	5.55, dd (9.0, 7.2)	5.63, dd (9.5, 7.0)	5.65, dd (11.4, 4.8)	5.64, dd (11.4, 4.8)	5.65, dd (11.4, 4.8)	5.67, dd (11.6, 4.8)
7	4.76, q (6.6)	4.72, q (6.6)	4.37, q (7.0)	4.35, q (7.0)	4.37, q (6.8)	4.45, q (7.0)
9	2.70, m	2.69, m	2.46, m	2.46, m	2.47, m	2.46, m
10	2.22, dd (15.4, 11.4)	2.21, dd (15.4, 11.6)	2.26, dd (13.4, 5.6)	2.25, dd (13.4, 5.4)	2.26, dd (13.4, 5.2)	2.24, dd (13.5, 6.0)
	1.97, d (15.4)	1.95, br d (15.4)	1.89, dd (13.4, 9.6)	1.86, dd (13.4, 9.4)	1.88, dd (13.4, 9.6)	1.90, dd (13.5, 8.0)
12	4.90[a]	4.88[a]	4.91, d (9.6)	4.89[a]	4.91[a]	4.93[a]
13	2.34, dddq (9.6, 9.6, 8.4, 5.4)	2.32, dddq (9.0, 9.0, 8.4, 5.4)	2.46, m	2.46, m	2.47, m	2.46, m
14	1.54, ddd (13.2, 9.8, 5.8)	1.52, ddd (13.2, 9.6, 6.0)	1.44, ddd (13.4, 8.4, 6.6)	1.44, ddd (13.2, 8.4, 6.4)	1.45, ddd (13.2, 8,4, 6.6)	1.42, ddd (13.4, 8.2, 6.6)
	1.23, ddd (13.2, 8.2, 5.4)	1.22, ddd (13.2, 8.4, 5.4)	1.29, dd (13.4, 6.8)	1.28, ddd (13.2, 6.6, 6.6)	1.29, ddd (13.2, 6.6, 6.6)	1.28, ddd (13.4, 6.6, 6.6)
15	4.71, qdd (6.2, 6.0, 6.0)	4.72, m	3.69, ddq (6.8, 6.6, 6.2)	3.68, ddq (6.6, 6.4, 6.2)	3.64, ddq (6.6, 6.6, 6.2)	3.68, ddq (6.6, 6.6, 6.0)
17	6.91, d (8.5)	6.95, d (8.5)	7.18, d (8.5)	7.16, d (8.5)	7.16, d (8.5)	
18	6.66, d (8.5)	6.68, d (8.5)	6.71, d (8.5)	6.71, d (8.5)	6.72, d (8.5)	
20	3.28[a]	3.25, dd (15.0, 7.0)	3.43, dd (15.2, 4.8)	3.43, dd (15.2, 4.8)	3.43, dd (15.0, 4.8)	3.39, dd (15.2, 4.8)
	3.15, dd (15.0, 7.2)	3.16, dd (15.0, 9.5)	3.13, dd (15.2, 11.4)	3.12, dd (15.2, 11.4)	3.13, dd (15.0, 11.4)	3.18, dd (11.6, 15.2)
23	7.59, dt (8.0, 1.0)	7.48, d (8.5)	7.50, dt (8.0, 1.0)	7.49, dt (8.0, 0.6)	7.50, d (8.0)	7.53, dt (8.0, 0.8)
24	7.01, td (8.0, 1.0)	7.12, dd (8.5, 1.5)	6.99, td (8.0, 1.0)	6.99, td (8.0, 1.0)	7.01, td (8.0, 0.8)	7.01, td (8.0, 0.8)
25	7.09, td (8.0, 1.0)		7.06, td (8.0, 1.0)	7.05, td (8.0, 1.0)	7.07, td (8.0, 0.8)	7.07, td (8.0, 0.8)
26	7.32, dt (8.0, 1.0)	7.42, d (1.5)	7.23, dt (8.0, 1.0)	7.23, dt (8.0, 0.6)	7.23, d (8.0)	7.24, dt (8.0, 0.8)
28	7.00, s					
29	0.92, d (6.6)	0.79, d (6.6)	0.65, d (7.0)	0.66, d (7.0)	0.66, d (6.8)	0.58, d (7.0)
30	1.11, d (6.8)	1.09, d (6.8)	0.98, d (7.0)	0.97, d (6.8)	0.98, d (6.8)	0.97, d (7.0)
31	1.61, s	1.60, s	1.57, d (1.0)	1.57, d (1.2)	1.58, s	1.57, d (1.2)
32	0.87, d (6.6)	0.86, d (6.6)	0.91, d (6.6)	0.90, d (6.6)	0.91, d (6.6)	0.89, d (6.6)
33	1.06, d (6.2)	1.08, d (6.6)	1.11, d (6.2)	1.11, d (6.2)	1.12, d (6.2)	1.11, d (6.0)
34	3.09, s	3.11, s	3.08, s	3.06, s	3.07, s	3.06, s
35				3.65, s	4.11, q (7.2)	
36					1.22, t (7.0)	

[a] Shift assigned on the basis of gHMQC and gHMBC data.

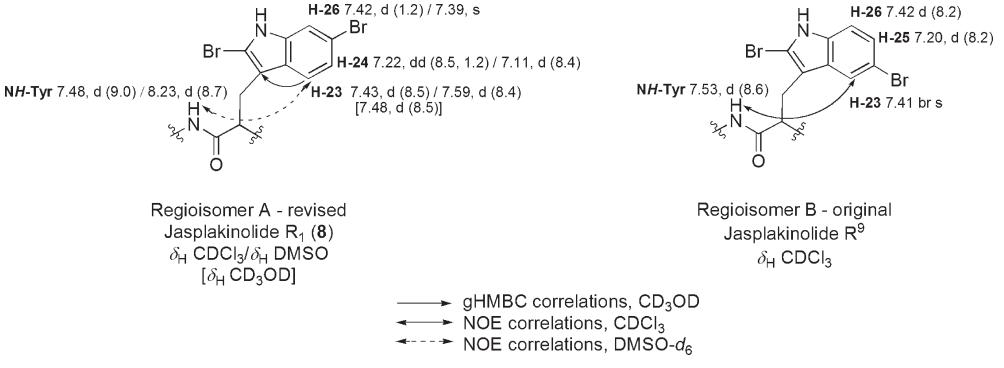

Figure 1. Clarifying the bromination regiochemistry for **8** (this work) vs literature.[9]

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Table 3. ^{1}H and ^{13}C NMR Data for Compounds 13−15 in CD_3OD at 600 and 150 MHz, Respectively

	13		14		15[a]	
pos.	δ_C, type[b]	δ_H (J[Hz])	δ_C, type[b]	δ_H (J[Hz])	δ_C, type[b]	δ_H (J[Hz])
1	172.5, C		169.5, C		167.7, C	
2	41.9, CH_2	2.73, dd (15.2, 4.2)	101.8, CH	5.22, s	46.8, CH_2	3.90, d (14.8)
		2.67, dd (15.2, 8.0)				3.78, d (14.8)
3	50.7, CH	5.21, dd (8.0, 4.2)	155.1, C		191.6, C	
4	171.2, C		170.6, C		172.1, C	
5	57.0, CH	5.71, dd (9.8, 6.4)	57.9, CH	6.07, dd (11.4, 4.8)	56.4, CH	5.74, dd (11.2, 4.8)
6	175.2, C		175.6, C		174.2, C	
7	47.2, CH	4.68, m	47.3, CH	4.77, m	45.7, CH	4.40, q (6.6)
8	177.9, C		177.8, C		177.3, C	
9	40.4, CH	2.65, m	38.7, CH	2.71, dqd (11.8, 7.2, 1.2)	39.7, CH	2.33, m
10	42.6, CH_2	2.21, dd (15.2, 11.4)	41.3, CH_2	2.35, dd (17.0, 11.8)	43.5, CH_2	2.19, dd (13.8, 9.2)
		1.93, d (15.2)		1.85, d (17.0)		1.82, dd (13.8, 4.8)
11	134.1, C		134.7, C		131.7, C	
12	130.4, CH	4.83[c]	127.7, CH	4.80[c]	132.5, CH	4.74, d (8.8)
13	30.6, CH	2.30, m	30.7, CH	2.41, m	28.9, CH	2.03, m
14	44.2, CH_2	1.50, m	44.8, CH_2	1.55, ddd (12.2, 12.0, 4.0)	43.2, CH_2	1.39, ddd (13.8, 9.0, 6.6)
		1.18, m		1.33, ddd (12.0, 10.8, 3.6)		1.20, ddd (13.8, 7.8, 4.2)
15	71.8, CH	4.68, m	70.7, CH	4.81, m	71.3, CH	4.85, dqd (9.0, 6.2, 4.2)
16	133.6, C		127.8, C		129.3, C	
17	118.5, CH	6.49, dd (8.2, 2.0)	121.1, CH	6.86, dd (8.2, 2.0)	123.4, CH	7.41, d (8.2, 1.2)
17′	114.5, CH	6.70, d (2.0)	116.0, CH	6.91, d (2.0)	115.0, C	
18	116.5, CH	6.67, d (8.2)	116.0, CH	6.79, d (8.2)	115.3, CH	7.52, d (1.2)
18′	146.5, C		146.1, C		144.5, C	
19	145.8, C		149.0, C		150.4, CH	6.84, d (8.2)
20	25.1, CH_2	3.22, m	24.0, CH_2	3.26, dd (15.4, 4.8)	23.5, CH_2	3.48, dd (15.4, 4.8)
				3.18, dd (15.4, 11.6)		3.17, dd (15.4, 11.2)
21	110.3, C		110.3, C		110.8, C	
22	128.7, C		128.9, C		127.6, C	
23	119.3, CH	7.54, d (8.0)	119.2, CH	7.55, dt (8.0, 0.6)	118.3, CH	7.53, d (8.0)
24	120.6, CH	7.00, td (8.0, 1.0)	120.6, CH	7.03, td (8.0, 0.8)	120.5, CH	7.09, td (8.0, 0.8)
25	123.0, CH	7.07, td (8.0, 1.0)	123.0, CH	7.09, td (8.0, 0.8)	122.7, CH	7.14, td (8.0, 0.8)
26	111.8, CH	7.25, d (8.0)	111.7, CH	7.25, dt (8.0, 0.6)	110.7, CH	7.25[c]
27	138.1, C		138.1, C		136.2, C	
28	110.6, C		110.5, C		109.1, C	
29	18.1, CH_3	0.66, d (6.6)	17.9, CH_3	0.49, d (6.6)	16.2, CH_3	0.72, d (6.6)
30	20.2, CH_3	1.08, d (6.8)	21.3, CH_3	1.06, d (7.2)	18.4, CH_3	1.04, d (6.8)
31	18.5, CH_3	1.59, d (0.8)	18.9, CH_3	1.62, s	16.2, CH_3	1.29, s
32	22.2, CH_3	0.85, d (6.6)	22.7, CH_3	0.87, d (6.6)	20.5, CH_3	0.72, d (6.6)
33	19.7, CH_3	1.07, d (6.2)	19.3, CH_3	1.14, d (6.2)	20.6, CH_3	1.15, d (6.2)
34	31.8, CH_3	3.08, s	32.1, CH_3	2.98, s	32.1, CH_3	3.10, s

[a] Spectra recorded in $CDCl_3$. [b] Carbon type determined from gHMQC data. [c] Shift assigned on the basis of gHMQC and gHMBC data.

suggest the PKS portion of the molecule is assembled first, followed by the final incorporation of the (*R*)-β-Tyr unit, biosynthesized from L-Tyr.[48] Tailoring enzymes subsequently carry out ring closure to create the macrocycle; thus it would be unexpected for a second group of tailoring enzymes to reopen the 19-membered ring at the C3−N bond.

Experiments were launched to pinpoint the pathway responsible for the production of **15**. A plausible important clue was provided by the observation that this open-chain congener was co-isolated with **14** from the same HPLC subfraction. Subsequently, we successfully used ^{1}H NMR data to track the conversion of **14** to **15**. Compound **14** was treated with 1% TFA-*d* in $CDCl_3$, and after 6 days diagnostic proton shifts for **15** were visible (H_2-2 δ_H = 3.78, 3.90; H-5 δ_H = 5.74) as seen in Figure 3, and these two were present in a ratio of **14**:**15** = 87:13. We conclude that the enamine functionality of **14** is labile and can be slowly hydrolyzed to yield **15**.

The combination of prolonged storage for several years at 4 °C with our standard workup procedures was the serendipitous recipe for expanding the set of compounds we were able to isolate. An overview of these events is shown in Figure S3, Supporting Information. Jasplakinolide (**1**) served as the precursor to the acyclic

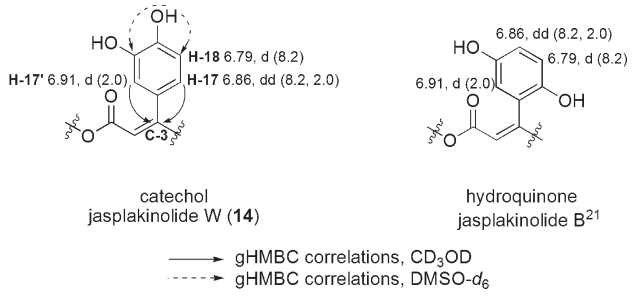

Figure 2. Choosing between catechol and hydroquinone substructures of **13** and **14** using ^{1}H shifts (**14**, CD_3OD, 600 MHz) and selected gHMBC correlations (CD_3OD, DMSO-d_6).

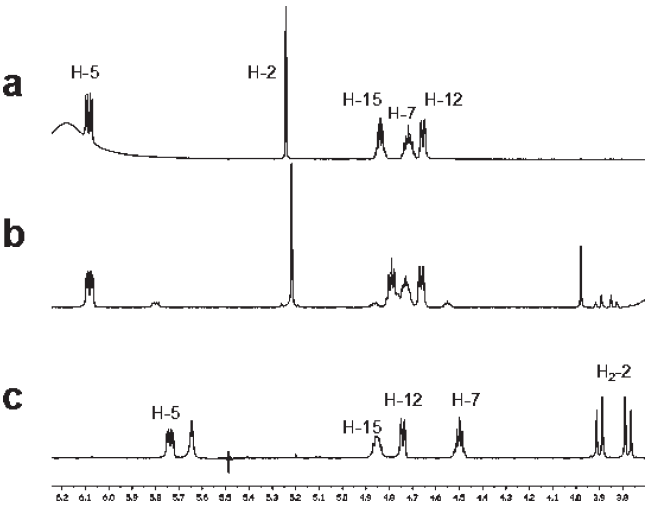

Figure 3. Conversion of **14** to **15** with 1% TFA-*d*. a) **14**, ^{1}H NMR, $CDCl_3$ and 1% TFA-*d*, day 1; b) **14**, 1H NMR, $CDCl_3$ and 1% TFA-*d*, day 6; c) **15**, ^{1}H NMR, $CDCl_3$.

acid **9**, which underwent further esterification to produce **10** and **11**. As demonstrated above, **14** is the precursor for **15**, which must be the starting point for further solvolysis providing the dipeptide−PKS analogue **12**.

The bilateral approach we employed to probe the bioactivity properties of our first library of jasplakinolide analogues[10] was again used to scan for cytotoxic and MF effects of the six known and six new compounds described above. This involved side-by-side tabulation of cytotoxicity data obtained from the National Cancer Institute-Developmental Therapeutic Program (NCI-DTP) 60 cell line screen[49] versus MF-disruption properties provided by the UCSC Chemical Screening Center[10] for compounds **1** and **4**−**15**. A comprehensive array of GI_{50} parameters was obtained, and compounds were also tested at six concentrations in the microfilament assay, in an effort to gain a broad view of their biological responses in this arm of the screening.

A subset of these data is shown in Tables 4 and 5 (the expanded data set from the NCI-DTP screen, including GI_{50}, TGI, and LC_{50} values for all cell lines, can be viewed in the Supporting Information). Our results provide a format to re-evaluate the SAR patterns summarized above and provide some important new insights. In regards to cytotoxicity the following trends merit further discussion. (1) Consistent with the observation that the aliphatic peptide residue can be modified and still retain potency is that the substitution of a 2-Aba (aminobutyric acid) unit in **4** versus for Ala in **1** is well tolerated[6] and enhances potency 10-fold versus **1** in some human cell lines (Table 5: ovarian adenocarcinoma (IGROV-1), renal carcinoma (A498), melanoma (LOX-IMVI)). (2) While oxidation of the Trp is ruinous to nanomolar activity,[8,10] this does not apply to the β-Tyr residue, as activity is retained in **13** in spite of further aryl hydroxylation. (3) Removal or addition of halogens to the Trp is also tolerated, as shown by the data of **7** and **8** in comparison to that of **1**.[9,40] (4) A conformation change in the macrolide ring induced by the $\Delta^{2,3}$ double bond (**13** vs **14**) obliterates nanomolar activity. (5) Our new data suggest a refined view of the SAR at the Trp-*N*-methyl group (see **6**) and C-9 methyl

Table 4. Bioassay Data for Compounds in This Study

		GI_{50} (μM)		cell-based microfilament assay[b]					
cmpd.	NSC no.	HCT-116[a]	MDA-MB-231[a]	0.01 μM	0.05 μM	0.1 μM	0.5 μM	1.0 μM	5.0 μM
1	613009	0.04	0.01	−	−	−	+++	+++	+++
3	731258	0.14[c]	0.04	−	+	+	++	++	++
4	751841	0.02	0.02	NT					
5	751842	0.11	0.26	NT					
6	751843	0.13	0.21	NT					
7	751844	0.05	0.07	NT					
8	751837	0.06	0.09	−	−	−	+++	+++	+++
9	666654	13.7[c]	2.50	−	−	−	−	−	−
10	—[d]	0.27[c]	NT	−	−	−	+	++	++
11	751840	0.28[c]	0.74	−	−	−	+	++	++
12	751838	11.1	22.3	−	−	−	−	−	−
13	751839	0.07	0.09	−	+	++	+++	+++	+++
14	662466	3.84	2.32	−	−	−	−	−	−
15	752380	NA[e]	NA	−	−	−	−	−	−

[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 NSC's above at http://dtp.nci.nih.gov/. [b] The microfilament disrupting effects were evaluated on the basis of image analysis of the actin cytoskeleton in fixed HCT-116 cells: (+) loss of microfilament; (++) additional loss of microfilament; (+++) total loss of microfilament network; (−) inactive. [c] IC_{50} determined by the Valeriote group at Henry Ford Hospital. [d] **10** was not selected for testing by the NCI-DTP. [e] **15** displayed <25% growth inhibition at 10 μM and was not selected for five-dose testing. NT = not tested, NA = not active.

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Table 5. Selected NCI 60 Cell Line Screening Results for Compounds 1, 4−9, 11, and 13

cell line	IGROV-1	U25-1	NCI-H522	DU-145	A498	LOX-IMVI
cell type	ovarian	CNS	lung	prostate	renal	melanoma
cmpd	GI_{50} (μM)					
1	0.02	0.07	0.03	0.03	0.03	0.01
4	0.008	0.01	0.03	0.02	0.002	0.003
5	0.02	0.05	0.61	0.29	0.21	0.03
6	0.03	0.06	0.20	0.25	0.17	0.02
7	0.01	0.03	0.16	0.10	0.08	0.02
8	0.03	0.03	0.003	0.31	0.04	0.04
9	1.76	0.60	0.12	18.8	NT[a]	0.28
11	0.17	0.05	0.04	3.72	0.44	0.16
13	0.03	0.04	0.06	0.08	0.01	0.007

[a] NT = not tested.

group (see **5**), displaying equally potent nanomolar activity in ovarian, CNS, and melanoma cell lines (Table 5: IGROV-1, glioma (U25-1), LOX-IMVI), but diminished activity in other cell lines (Tables 4, 5) versus **1**.[7,8,40] (7) Inexplicably, the acyclic analogue jasplakinolide Z_3 (**11**) exhibits similar potency to jasplakinolide (**1**) in colon, CNS, and lung cell lines (HCT-116, U25-1, NCI-H522), and another acyclic analogue, **9**, also showed selectivity for colon, CNS, lung, and melanoma cells.

The microfilament disruption effects of analogues **8**−**15** were first tested in HeLa cells, at concentrations ranging from 10 nM to 5 μM, with the goal of discovering another compound with muted MF properties similar to those of jasplakinolide B (**3**).[10] This first data set in HeLa cells indicated that the acyclic analogue **11** possibly operated through a mechanism not explained through MF association, as a 100-fold divergence between cytotoxicity (HCT-116) and MF activity (HeLa) was observed. However, a second screen in HCT-116 cells with the same concentration gradient provided important additional insights, and selected results of both MF screens can be viewed in Figure 4. Compounds **3** and **11** interact with microfilaments at their GI_{50} values in HCT-116 cells, but the phenotype differs from that of **1**, as a complete loss of the microfilament network is not detected as high as 5 μM. Among the four compounds displaying nanomolar cytotoxicity (**8**, **10**, **11**, **13** (HCT-116)) only analogues **8** and **13** caused complete loss of the microfilament network, similar to jasplakinolide (**1**). These MF responses for macrolides **8** and **13** may be attributed to retaining a conformation similar to **1**, as the point substitutions in these molecules should not affect the geometry of the Ala-*N*-Me-2BrTrp-β-Tyr segment. This hypothesis corresponds well with the binding model describing the actin association with chondramide C to F-actin.[23,40] Key points in this active site interaction model involve the Trp ring system of chondramide C with aromatic amino acids of F-actin and contact of the Tyr-OH with an actin threonine residue. Thus, it is noteworthy that acyclic compounds **10** and **11** interact with the MF network, while the slightly modified macrocycle **14** loses all binding activity.

We have just begun to consider secondary screening work on the jasplakinolide scaffold in a complementary approach designed to develop solid-tumor-selective anticancer agents from natural products.[50] The first step in assembling a relevant therapeutics data set is to interrogate a compound's solid tumor selectivity beyond that observed in the disk diffusion assay. This assessment involves a clonogenic dose−response study to determine the

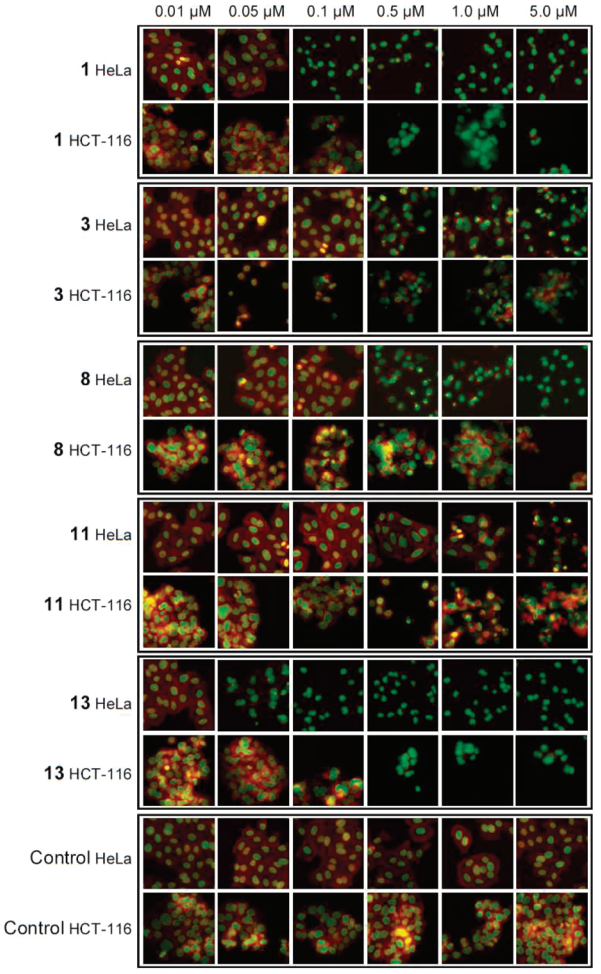

Figure 4. Antimicrofilament effects of compounds **1**, **3**, **8**, **11**, and **13** in HCT-116 and HeLa cells. F-actin is colored red, and nuclei and chromosomes are colored green. Jasplakinolide (**1**) is used as a positive control,[10,22] and negative controls are treated with DMSO. Screens were performed in duplicate, and representative images are shown. Multiple images of control wells are included to demonstrate experiment variability.

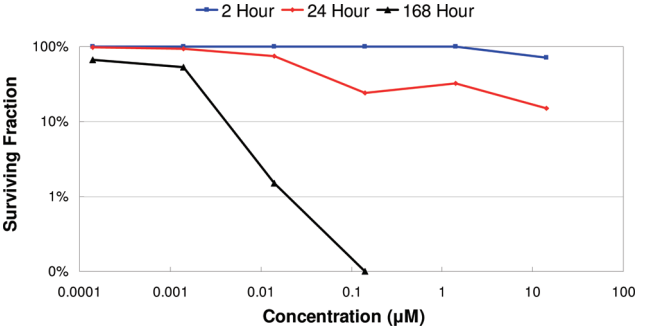

Figure 5. Results of a clonogenic dose−response study with jasplakinolide (**1**) in HCT-116 cells. Cells were exposed to different concentrations of **1** for either 2 h, 24 h, or 7 days (continuous exposure).

cytotoxic effect at varying concentrations of the compound for exposure times of 2 h, 24 h, and 7 days. Finally, a maximum tolerated dose (MTD) is measured, and the pharmacokinetic behavior of the compound is measured in both plasma and tumor

at the MTD. The combination of these results determines whether a compound shows therapeutic efficacy and if a mechanism of action study should be initiated. Molecules from our laboratory that have shown a favorable profile in this evaluation are fascaplysin A[50] and fijianolide B.[51]

Our results from the clonogenic assay for jasplakinolide (**1**) are shown in Figure 5. This study determines the required time−concentration profile to obtain a 90% kill of tumor cells. Little toxicity to HCT-116 cells was shown at 2 and 24 h dosing schedules of **1**; however the desired therapeutic effect was achieved at 0.004 μM with continuous exposure for 7 days. This outcome indicates that jasplakinolide would require a chronic dosing schedule for maximum therapeutic effectiveness in vivo. The MTD of **1** has been determined as 0.625 mg/kg, and the subsequent pharmacokinetic studies are in progress. In the future similar follow-up work will be launched on jasplakinolides B (**3**), E, and V (**13**).

CONCLUSIONS

In summary, we have extended the structure−activity understanding for the jasplakinolide family of compounds through a side-by-side study of seven macrolides and five closely related acyclic analogues. It is important to reiterate that in addition to jasplakinolide, the solid-tumor-selective jasplakinolide B (**3**), jasplakinolide E, and jasplakinolide V (**13**), pinpointed by the NCI Biological Evaluation Committee,[10] constitute targets for further therapeutic evaluation. In this work, previously unscrutinized biological functionality was represented in the structures of the known macrolides **4**−**7**, the unique macrolide **13**, and compounds **8** and **14**, whose structures have been revised. Further, access to the five acyclics **9**−**12** and **15**, named as the jasplakinolide Z family, also provided useful data. The nanomolar cytotoxicity and potent microfilament disruption activity of macrolides **4**, **7**, **8**, and **13** versus the diminished cytotoxicity (≈1 uM) for **5** and **6** extend the known structure−activity requirements and actin-binding models based on jasplakinolide (**1**) and chondramide C (**2c**). In addition the inactivity of **14** underscores the importance of the macrolide geometry for this binding model. It is significant that **3** and the acyclic analogues **10** and **11** demonstrated broad-spectrum submicromolar cytotoxicity and associate with microfilaments without complete loss of the actin network. By contrast, the diminished activity of other acyclics such as **12** and inactivity of **15** underline the importance in the connectivity sequence for the PKS and NPRS subunits. It is also important to emphasize the outcomes of the MF screening in HCT-116 cells. This information provided a realistic view of each compound's cellular actions when viewed in conjunction with the GI_{50} results in the matched cell line. The new evidence that jasplakinolide B (**3**) operates by interaction with MF counters our previous hypothesis[10] based on HeLa cell screening at 80 nM. It is possible that contradictory results could arise from similar testing of the latrunculin analogues 18-epi-latrunculol A[52] and oxalatrunculin B,[53] which were reported to have non-MF cytotoxic mechanisms. Overall, the results discussed above highlight that the frameworks of jasplakinolide B (**3**), jasplakinolide E, and jasplakinolide V (**13**) represent an ideal starting point for scaffold mining, and we believe that additional screening of natural and unnatural jasplakinolide compounds will provide a route for significant future innovation.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were determined on a Jasco P-2000 digital polarimeter, and UV data were obtained on an Agilent 8453 UV−vis photodiode array spectrophotometer. All NMR spectra were recorded on a Varian Unity Inova 600 spectrometer 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 and δ_C 77.4), 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 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 (230 nm). Compound purity (>95%) was confirmed using both ^{1}H and ^{13}C NMR and LC-MS (UV and ELSD detection) experiments.

Animal Material. Samples of *Jaspis splendens* (coll. no. 00101, 1.9 kg wet weight) were collected in Fiji (S 18°22.28′, E 177°58.87′) in February 2000. Taxonomic identification *of J. splendens* was performed by Dr. Rob van Soest of the Zoological Museum of Amsterdam. Underwater pictures are in Figure S11, Supporting Information, and voucher specimens are available from the corresponding author (P.C.).

Extraction and Isolation. 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 of CH_3OH four more times to obtain the crude CH_3OH extract. The extract was partitioned between 1:1 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 aqueous layer was then extracted with CH_2Cl_2 to give two crude extracts, called FM (CH_3OH-H_2O-soluble, 1.6 g) and FD (CH_2Cl_2-soluble, 2.1 g). The FM extract was then partitioned between H_2O and butanol to provide the WB extract (butanol-soluble, 150.1 mg).

Next, 00101 FD was subjected to preparative RP HPLC (10−65% CH_3CN-H_2O, 50 min), yielding 11 fractions (H1−H11). Fractions H4 (105.5 mg), H7 (145.3 mg), H8 (159.2 mg), H10 (198.4 mg), and H11 (192.5 mg) were purified further by RP HPLC utilizing isocratic conditions. Fraction H4 provided **12** (2.1 mg, H4 H4 H2) through repetitive semipreparative HPLC (70% CH_3OH-H_2O). Fraction H7 was treated in a similar manner (50% CH_3CN-H_2O, 2 iterations) to give **10** (13.6 mg, H7 H8 H2) and **6** (1.2 mg, H7 H10 H3 H4). Fraction H8 (159.2 mg) was also separated via multiple rounds of isocratic (77% CH_3OH-H_2O) RP HPLC to yield **11** (2.8 mg, H8 H4 H2), **13** (16.6 mg, H8 H4 H3), **7** (0.4 mg, H8 H5 H6 H3 H4 H2), and **5** (1.1 mg, H8 H5 H6 H3 H3 H4 H3). Fraction H10 was first subjected to preparative isocratic (57% CH_3CN-H_2O) RP HPLC, resulting in 10 fractions, of which fractions H10 and H4 (7.8 mg) and H10 H6 (3.9 mg) were further purified by semipreparative RP HPLC (80% CH_3OH-H_2O) to give **4** (0.6 mg, H10 H4 H6) and **8** (1.5 mg, H10 H6 H4). Fraction H11 was purified by one round of preparative isocratic (60% CH_3CN-H_2O) RP HPLC and two rounds of semipreparative isocratic (85% CH_3OH-H_2O, 60% CH_3CN-H_2O) RP HPLC, resulting in **15** (3.5 mg, H11 H4 H1) and **14** (13.0 mg, H11 H4 H3).

Similarly, the WB extract was separated by RP HPLC (10−65% CH_3CN-H_2O, 60 min), giving 11 fractions (H1−H11). Fraction H9 (37.8 mg) was then subjected to isocratic (44% CH_3CN-H_2O) semipreparative HPLC to yield **9** (4.0 mg, H9 H2).

(+)-Jasplakinolide R_1 (8):. white solid (1.5 mg); $[\alpha]^{24}_D$ +48 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (1.43), 227 (1.24), 281 (0.27) nm; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESITOFMS $[M + Na]^+$ *m*/*z* 809.1482 (calcd for $C_{36}H_{44}Br_2N_4O_6Na$, 809.1520).

(+)-Jasplakinolide Z_1 (9):. white solid (4.0 mg); $[\alpha]^{23}_D$ +37 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.76), 222 (0.59), 280 (0.12) nm; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESI-

TOFMS $[M + Na]^+$ m/z 749.2590 (calcd for $C_{36}H_{47}BrN_4O_7Na$, 749.2520).

(+)-Jasplakinolide Z_2 (10):. white solid (13.6 mg); $[\alpha]^{23}_D$ +46 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (1.30), 283 (0.38), 290 (0.33) nm; ^1H and ^{13}C NMR (see Tables 1 and 2); HRESITOFMS $[M + H]^+$ m/z 741.2866 (calcd for $C_{37}H_{50}BrN_4O_7$, 741.2857).

(+)-Jasplakinolide Z_3 (11):. white solid (2.8 mg); $[\alpha]^{23}_D$ +64 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (0.41), 283 (0.15), 290 (0.16); ^1H and ^{13}C NMR (see Tables 1 and 2); HRESITOFMS $[M + Na]^+$ m/z 777.2885 (calcd for $C_{38}H_{51}BrN_4O_7Na$, 777.2833).

(−)-Jasplakinolide Z_4 (12):. white solid (2.1 mg); $[\alpha]^{22}_D$ −32 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 204 (1.48), 220 (1.37), 282 (0.32); ^1H and ^{13}C NMR (see Tables 1 and 2); HRESITOFMS $[M + H]^+$ m/z 563.2230 (calcd for $C_{27}H_{40}BrN_4O_4$, 563.2227).

(+)-Jasplakinolide V (13):. white solid (16.6 mg); $[\alpha]^{23}_D$ +120 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.95), 220 (0.60), 282 (0.16); ^1H and ^{13}C NMR (see Table 3); HRESITOFMS $[M + Na]^+$ m/z 747.2319 (calcd for $C_{36}H_{45}BrN_4O_7Na$, 747.2364).

(+)-Jasplakinolide W (14):. white solid (13.0 mg); $[\alpha]^{23}_D$ +60 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 204 (0.85), 218 (0.75), 281 (0.29); ^1H and ^{13}C NMR (see Table 3); HRESITOFMS $[M + H]^+$ m/z 723.2397 (calcd for $C_{36}H_{44}BrN_4O_7$, 723.2388).

(+)-Jasplakinolide Z_5 (15):. white solid (3.5 mg); $[\alpha]^{23}_D$ +15 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.55), 222 (0.45), 280 (0.18); ^1H and ^{13}C NMR (see Table 3); HRESITOFMS $[M + Na]^+$ m/z 763.2322 (calcd for $C_{36}H_{45}BrN_4O_8Na$, 763.2313).

Conversion of 14 to 15. Compound **14** (2 mg) was dissolved in 1.4 mL of $CDCl_3$, and the solution was divided between two 5 mm NMR tubes. Using a gastight syringe, 7 μL of TFA-d was added to one tube. ^1H NMR spectra were acquired for both samples on the first day and again after 6 days. Spectra are displayed in Figure 3.

Microfilament Disruption Assay. HCT-116 or HeLa cells were plated in 384-well tissue culture treated plates at a density of 1500 cells per well. After incubating at 37 °C with 5% CO_2 overnight compounds were pinned into plates using an automated liquid handler. After 24 h cells were fixed in 4% formaldehyde for 20 min, then washed with PBS using an automated plate washer. The cells were then treated with 0.5% TritonX-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. The plates are then stored in a 0.1% azide PBS solution, and images were taken using an automated fluorescence microscope at 20× magnification after 24 h.

Jasplakinolide (1) Clonogenic Dose−Response Analysis. Concentration and time-survival studies of **1** were carried out against HCT-116 cells. HCT-116 cells were seeded at 200 to 20 000 cells in 60 mm dishes. Drug was added to the medium (RPMI + 10% FBS) at concentrations of 1 mg/mL and 10-fold dilutions thereof. At either 2 or 24 h, the drug-containing media was removed and fresh media without drug was added. For continuous exposure to the drug, it remained in contact with the cells for the entire incubation period. The dishes were incubated for 7 days, media was removed, and the colonies were stained with methylene blue. Colonies containing 50 cells or more were counted. The results were normalized to an untreated control. Plating efficiency for the untreated cells was about 90%. Repeat experiments were carried out to define the cell survival range between 100% and 0.1% survival. The results of this analysis are shown in Figure 5.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^1H, ^{13}C, gHMQC, and gHMBC NMR spectra for compounds **6**−**15**, a sponge picture of 00101, NCI 60 cell line results, and microfilament effects of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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