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The Biostructural Features of Additional Jasplakinolide (Jasпамide) Analogues[†]

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Abstract

The cyclodepsipeptide jasplakinolide (**1**) (a.k.a. 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 re-investigate 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**) (a.k.a. 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 investigations⁵⁻¹⁰ 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 bio-geographical 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

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

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Supporting Information Available: ¹H, ¹³C, 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>.

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 vs. 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 - I)¹⁵ families, and the 18-membered macrolides of the chondramide^{18,19} / seragamide¹⁷ / 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**, C₃₆H₄₅BrN₄O₆) and chondramide D (**2d**, C₃₅H₄₃ClN₄O₆).

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 anthelmintic,¹ insecticidal,² fungicidal,² and fish toxicity.²⁰ Also noteworthy was an early observation of nM GI₅₀s 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 micro-filament (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 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 - 5).¹⁰ A list of structural features that must be present in the jasplakinolide skeleton to impart activity at a nM level can be enumerated as follows: (a) the PKS region of the macrolide must be maintained in a semi-rigid 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} Based on the current literature, we estimate that 45 synthetic congeners have been tested based on the jasplakinolide³⁵⁻⁴¹ 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 therapeutics 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. Secondly, 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 vs. 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 semi-pure 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 semi-pure fractions indicated the presence of a series of minor bromine-containing constituents with molecular ion peaks in the range of 700-800 amu. Establishing the identity of these as new or known jasplakinolide congeners proceeded by re-applying our dereplication scheme¹⁰ analyzing: (a) LC-MS profiles; (b) ¹H NMR signature δs; (c) the pattern of heavy atom formulas for all reported jasplakinolide analogues (C₃₅₋₃₇N₄O_{6/7/9}, C₃₅₋₃₇BrN₄O₆₋₇, C₃₆Br₂N₄O₆); and (d) the ¹H NMR δs 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 CH₂Cl₂-soluble preparative RP-HPLC fractions further separated by semi-preparative chromatography (individually coded in the experimental as H7, H8, and H10). The first three were bromine-containing and consisted of a C₃₅ isomeric pair (C₃₅H₄₃BrN₄O₆: [M+H]⁺ *m/z* 695.2/697.2) plus a C₃₇ homolog (C₃₇H₄₇BrN₄O₆: [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 ¹H 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**) $R_1=\text{Br}$, $R_2=\text{H}$, $R_3=\text{CH}_3$, $R_4=\text{CH}_2\text{CH}_3$, $R_5=\text{CH}_3$
 jasplakinolide J (**5**) $R_1=\text{Br}$, $R_2=\text{H}$, $R_3=\text{CH}_3$, $R_4=\text{CH}_3$, $R_5=\text{H}$
 jasplakinolide M (**6**) $R_1=\text{Br}$, $R_2=\text{H}$, $R_3=\text{H}$, $R_4=\text{CH}_3$, $R_5=\text{CH}_3$
 jasplakinolide Q (**7**) $R_1=\text{H}$, $R_2=\text{H}$, $R_3=\text{CH}_3$, $R_4=\text{CH}_3$, $R_5=\text{CH}_3$
 jasplakinolide **R₁** (**8**) $R_1=\text{Br}$, $R_2=\text{Br}$, $R_3=\text{CH}_3$, $R_4=\text{CH}_3$, $R_5=\text{CH}_3$

An additional compound was isolated from the previously described semi-pure fractions (the one noted above as H10). The properties of this dibromo analogue ($\text{C}_{36}\text{H}_{44}\text{Br}_2\text{N}_4\text{O}_6$: $[\text{M} + \text{H}]^+$ m/z 787.2/789.2/791.2, 1:2:1) of **1** were in agreement with its assignment as jasplakinolide **R₁**⁹ (see regioisomer B in Figure 1), though the literature for this compound included only an ^1H NMR data set in CDCl_3 . While the ^1H 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 ^1H data in two additional solvents. One powerful result included the $^3J_{\text{H-C}}$ gHMBC⁴⁶ from H-23 (δ_{H} 7.48, d, $^3J_{\text{H}}=8.5$, 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, $J_{\text{H}}=8.5$) (this work) vs. δ_{H} 7.42 (d, $J_{\text{H}}=8.2$)⁹) and H-26 (δ_{H} 7.42 (d, $J_{\text{H}}=1.2$) (this work) vs. δ_{H} 7.41 (bs)⁹) led to an unclear interpretation of the NOE correlations shown for the putative regioisomer B in Figure 1. However in $\text{DMSO}-d_6$ these signals were well resolved. Irradiation of the NH-Tyr (δ_{H} 8.23 (d, $J_{\text{H}}=8.7$)), revealed a clear correlation to H-23 (δ_{H} 7.59 (d, $J_{\text{H}}=8.4$): Figure S1, Supporting Information) and provided the final basis for our assignment of the revised structure, named (+)-jasplakinolide **R₁** (**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 sub-fraction. One of the most polar compounds, the free acid form of jasplakinolide named (+)-jasplakinolide **Z₁** (**9**, $\text{C}_{36}\text{H}_{47}\text{BrN}_4\text{O}_7$: $[\text{M} + \text{H}]^+$ m/z 727.3/729.3), was obtained from the butanol solvent partition fraction. 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 ^1H NMR data.¹⁷ Although the careful storage conditions of semi-pure 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 work-up were also obtained (from CH_2Cl_2 solvent partition fractions). These consisted of (+)-jasplakinolide **Z₂** (**10**, $\text{C}_{37}\text{H}_{49}\text{BrN}_4\text{O}_7$), with the $-\text{OCH}_3$ group identified by a methyl (H_3) singlet at δ_{H} 3.65, and (+)-jasplakinolide **Z₃** (**11**, $\text{C}_{38}\text{H}_{51}\text{BrN}_4\text{O}_7$), with the $-\text{OCH}_2\text{CH}_3$ group identified by two ^1H signals, δ_{H} 4.11 (H_2 , q, $J_{\text{H}}=7.2$) / 1.22 (H_3 , t, $J_{\text{H}}=7.0$). Another truncated acyclic analogue, (-)-jasplakinolide **Z₄** (**12**, $\text{C}_{27}\text{H}_{39}\text{BrN}_4\text{O}_4$: $[\text{M} + \text{H}]^+$ 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 (+)-jaspalakinolide V (**13**, $C_{36}H_{45}BrN_4O_7$) and (+)-jaspalakinolide 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 $^1H/^13C$ 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 jaspalakinolide B possessing a hydroquinone,²¹ described by our laboratory in 1995 (based on unreported 1D NMR data, and not identical to jaspalakinolide B / jaspamide B (**3**), reported in 1999⁵). 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'-OH, δ_H =8.84, br s; 19-OH δ_H =8.81, br s) further confirmed the placement of these substituents (18'-OH to C-17', C-19 and 19-OH to C-18, C-18'). Although an authentic sample of the proposed hydroquinone compound²¹ 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 tri-substituted 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 geodiamolides¹⁶ and seragamides.¹⁷

The last open-chain congener (+)-jaspalakinolide Z₅ (**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 vs. that of **1** were rationalized by proposing the R₁ 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 R₁ as follows: (a) correlations from H-17, H-17' and H₂-2 to the low-field carbon (δ_C =191.6, C-3), and (b) from H₂-2 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 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 re-open 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 sub-fraction. Subsequently, we successfully used ¹H NMR data to track the conversion of **14** to **15**. Compound **14** was treated with 1% TFA-*d* in CDCl₃ and after six days diagnostic proton shifts for **15** were visible (H₂-2 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 work-up 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 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 analogues¹⁰ 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 screen⁴⁹ vs. MF-disruption properties provided by the UCSC Chemical Screening Center¹⁰ for compounds **1**, **4** - **15**. A comprehensive array of GI₅₀ 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 are shown in Tables 4 and 5 (the expanded data set from the NCI-DTP screen, including GI₅₀, TGI, and LC₅₀ 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** vs. for Ala in **1** is well tolerated⁶ and enhances potency 10-fold vs. **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 nM 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 Δ^{2,3} double bond (**13** vs. **14**) obliterates nM activity. (5) Our new data suggest a refined view of the SAR at the Trp-*N*-methyl group (see **6**) and C-9 methyl group (see **5**), displaying equally potent nM 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) vs. **1**.^{7,8,40} (7) Inexplicably, the acyclic analogue jasplakinolide Z₃ **11** exhibits similar potency to jasplakinolide (**1**) in colon, CNS, and lung 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 GI₅₀ 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 μ M. Among the four compounds displaying nM 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 cytotoxic effect at varying concentrations of the compound for exposure times of two hours, 24 hours and seven 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 A⁵⁰ 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 two hour and 24 hour dosing schedules of **1**, however the desired therapeutic effect was achieved at 0.004 μ M with continuous exposure for seven 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 un-scrutinized 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 nM cytotoxicity and potent microfilament disruption activity of macrolides **4**, **7**, **8** and **13** vs. the diminished cytotoxicity ($\approx$ 1 μ M) 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 sub-micromolar 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** underlines 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 GI₅₀ results in the matched cell line. The new evidence that jasplakinolide B (**3**) operates by interaction with MF counters our previous hypothesis¹⁰ 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 A⁵² 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 CD₃OD, CDCl₃, or DMSO-d₆ with a 5 mm triple resonance (HCN) probe. Chemical shifts are reported in ppm relative to CD₃OD (δ_H 3.31 and δ_C 49.0), CDCl₃ (δ_H 7.27 and δ_C 77.4), or DMSO-d₆ (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 μ m) cartridges. Both ELSD and UV (254 nm) were used for peak detection. Semipreparative RP HPLC used a Phenomenex Synergi C18 4 μ m column, 10 × 250 mm, and UV peak detection (230 nm). Compound purity (>95%) was confirmed using both ¹H and ¹³C 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') February 2000. Taxonomic identification of *Jaspis 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 CH₃OH : H₂O, decanted, and shipped back to UC Santa Cruz where they were then submersed in 100% CH₃OH and stored at 4 °C. For extraction, the CH₃OH was decanted and the sponge was soaked in 1L CH₃OH four more times to obtain the crude CH₃OH extract. The extract was partitioned between 1:1 hexanes and CH₃OH-H₂O (9:1). After separation from the hexane layer, H₂O was added to the CH₃OH-H₂O layer adjusting the solution to 1:1. The aqueous layer was then extracted with CH₂Cl₂ to give two crude extracts called FM (CH₃OH-H₂O soluble, 1.6 grams) and FD (CH₂Cl₂-soluble, 2.1 g). The FM extract was then partitioned between H₂O and butanol to provide the WB extract (butanol-soluble, 150.1 mg).

Next, 00101 FD was subjected to preparative RP HPLC (10 – 65% CH₃CN : H₂O, 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 semi-preparative HPLC (70% CH₃OH : H₂O). Fraction H7 was treated in a similar manner (50% CH₃CN : H₂O, 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% CH₃OH : H₂O) 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% CH₃CN : H₂O) RP HPLC resulting in 10 fractions, of which fractions H10 H4 (7.8 mg) and H10 H6 (3.9 mg) were further purified by semi-preparative RP HPLC (80% CH₃OH : H₂O) 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% CH₃CN : H₂O) RP HPLC and two rounds of semi-preparative isocratic (85% CH₃OH : H₂O, 60% CH₃CN : H₂O) 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% CH₃CN : H₂O, 60 min) giving 11 fractions (H1 – H11). Fraction H9 (37.8 mg) was then subjected to isocratic (44% CH₃CN : H₂O) semi-preparative HPLC to yield **9** (4.0 mg, H9 H2).

(+)-Jasplakinolide R₁ (**8**)

White solid (1.5 mg). $[\alpha]_D^{24} +48$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 203 (1.43), 227 (1.24), 281 (0.27) nm; ¹H and ¹³C NMR (see Tables 1 and 2); HRESITOFMS [M+Na]⁺ *m/z* 809.1482 (calcd for C₃₆H₄₄Br₂N₄O₆Na, 809.1520).

(+)-Jasplakinolide Z₁ (**9**)

White solid (4.0 mg). $[\alpha]_D^{23} +37$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 202 (0.76), 222 (0.59), 280 (0.12) nm; ¹H and ¹³C NMR (see Tables 1 and 2); HRESITOFMS [M+Na]⁺ *m/z* 749.2590 (calcd for C₃₆H₄₇BrN₄O₇Na, 749.2520).

(+)-Jasplakinolide Z₂ (**10**)

White solid (13.6 mg). $[\alpha]_D^{23} +46$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 218 (1.30), 283 (0.38), 290 (0.33) nm; ¹H and ¹³C NMR (see Tables 1 and 2); HRESITOFMS [M+H]⁺ *m/z* 741.2866 (calcd for C₃₇H₅₀BrN₄O₇, 741.2857).

(+)-Jasplakinolide Z₃ (**11**)

White solid (2.8 mg). $[\alpha]_D^{23} +64$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 220 (0.41), 283 (0.15), 290 (0.16); ¹H and ¹³C NMR (see Tables 1 and 2); HRESITOFMS [M+Na]⁺ *m/z* 777.2885 (calcd for C₃₈H₅₁BrN₄O₇Na, 777.2833).

(-)-Jasplakinolide Z₄ (**12**)

White solid (2.1 mg). $[\alpha]_D^{22} -32$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 204 (1.48), 220 (1.37), 282 (0.32); ¹H and ¹³C NMR (see Tables 1 and 2); HRESITOFMS [M+H]⁺ *m/z* 563.2230 (calcd for C₂₇H₄₀BrN₄O₄, 563.2227).

(+)-Jasplakinolide V (**13**)

White solid (16.6 mg). $[\alpha]_D^{23} +120$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 203 (0.95), 220 (0.60), 282 (0.16); ¹H and ¹³C NMR (see Table 3); HRESITOFMS [M+Na]⁺ *m/z* 747.2319 (calcd for C₃₆H₄₅BrN₄O₇Na, 747.2364).

(+)-Jasplakinolide W (14)

White solid (13.0 mg). $[\alpha]_D^{23} +60$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 204 (0.85), 218 (0.75), 281 (0.29); ^1H and ^{13}C NMR (see Table 3); HRESITOFMS $[\text{M}+\text{H}]^+ m/z$ 723.2397 (calcd for $\text{C}_{36}\text{H}_{44}\text{BrN}_4\text{O}_7$, 723.2388).

(+)-Jasplakinolide Z₅ (15)

White solid (3.5 mg). $[\alpha]_D^{23} +15^\circ$ (c, 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 203 (0.55), 222 (0.45), 280 (0.18); ^1H and ^{13}C NMR (see Table 3); HRESITOFMS $[\text{M}+\text{Na}]^+ m/z$ 763.2322 (calcd for $\text{C}_{36}\text{H}_{45}\text{BrN}_4\text{O}_8\text{Na}$, 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 gas-tight 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 six 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 $\times$ 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 h 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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

Figure 2. Choosing between catechol and hydroquinone substructures of **13** and **14** using ¹H shifts (**14**. CD₃OD, 600 MHz) and selected gHMBC correlations (CD₃OD, DMSO-*d*₆).

Figure 3.
Conversion of **14** to **15** with 1% TFA-*d*.

Figure 4.

Anti-microfilament 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 hrs, 24 hrs, or 7 days (continuous exposure).

Table 1

¹³C NMR Data for Compounds **7** - **12** in CD₃OD at 150 MHz.

compd.	7	8	9	10	11	12
pos #	δ _C , type ^d	δ _C , type ^d	δ _C , type ^d	δ _C , type ^d	δ _C , type ^d	δ _C , type ^d
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

compd.	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
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	

^aCarbon type determined from gHMQC data.

Table 2

¹H NMR Data for Compounds 7 - 12 in CD₃OD at 600 MHz

compd.	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) 2.63, dd (15.2, 9.0)	2.72, dd (15.4, 4.0) 2.69, dd (15.4, 8.6)	3.01, dd (15.6, 9.0) 2.78, dd (15.6, 6.0)	3.05, dd (15.8, 9.4) 2.83, dd (15.8, 5.8)	3.03, dd (15.8, 9.0) 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) 1.97, d (15.4)	2.21, dd (15.4, 11.6) 1.95, br d (15.4)	2.26, dd (13.4, 5.6) 1.89, dd (13.4, 9.6)	2.25, dd (13.4, 5.4) 1.86, dd (13.4, 9.4)	2.26, dd (13.4, 5.2) 1.88, dd (13.4, 9.6)	2.24, dd (13.5, 6.0) 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) 1.23, ddd (13.2, 8.2, 5.4)	1.52, ddd (13.2, 9.6, 6.0) 1.22, ddd (13.2, 8.4, 5.4)	1.44, ddd (13.4, 8.4, 6.6) 1.29, dd (13.4, 6.8)	1.44, ddd (13.2, 8.4, 6.4) 1.28, ddd (13.2, 6.6, 6.6)	1.45, ddd (13.2, 8.4, 6.6) 1.29, ddd (13.2, 6.6, 6.6)	1.42, ddd (13.4, 8.2, 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, dddq (6.8, 6.6, 6.2)	3.68, dddq (6.6, 6.4, 6.2)	3.64, dddq (6.6, 6.6, 6.2)	3.68, dddq (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)
23	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)
24	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, dt (8.0)	7.53, dt (8.0, 0.8)
25	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)
26	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)
28	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, dt (8.0)	7.24, dt (8.0, 0.8)
29	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)

compd.	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])
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 based on gHMQC and gHMBC data.

Table 3
¹H and ¹³C NMR Data for Compounds **13** - **15** in CD₃OD at 600 and 150 MHz, respectively.

compd.		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		131.4, 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)	

compd.		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])	
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 CDCl₃

^b Carbon type determined from gHMQC data

^c Shift assigned based on gHMQC and gHMBC data

Bioassay Data for Compounds in this Study.

Table 4

cmpd.	NSC #	GI ₅₀ (μM)			Cell-based microfilament assay ^b						
		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	<i>_d</i>	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	-	-	-	-	-	-		

^aGI₅₀ 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/>.

^bThe microfilament-disrupting effects were evaluated based on image analysis of the actin cytoskeleton in fixed HCT-116 cells; (+) Loss of microfilament; (++) Additional loss of microfilament; (+++) Total loss of microfilament network; (-) inactive.

^cIC₅₀ determined by the Valeriot 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.

Table 5Selected 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
compd.	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

^aNT=not tested