# Re-evaluation of the Fijianolide/Laulimalide Chemotype Suggests an Alternate Mechanism of Action for C-15/C-20 analogs

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Table S1. Structural Modifications of Analogs of 37 Fijianolide B/Laulimalide (1) with Selected Potencies Against Selected Cancer Cell Lines.

entry	R1	R2	R3	R4	R5	R6	MDA-MB- 435	NCI/ADR	MCF-7	HCT116	HT-29	A2780
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3	<b>٥</b>	H H	OMe 22 S	Joseph Contraction of the second seco	nn N	re re	$242^4$ $240^6$			590 <sup>4</sup> 470 <sup>6</sup>		
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9	p-NO <sub>2</sub> (C <sub>6</sub> H <sub>4</sub> ) CO <sub>2</sub>	Human Street	مربر مربر	HIM O	nr.	Nor St	374				
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13	08 ا	HIIIIII	× مرمر ملا	HIM O	* == *	2 cr	2,5006	13,7006			
14	۲ کرد کرد	anjur	of stand	HIII O	rr.	No No	$   \begin{array}{r}     289^4 \\     120^6 \\     120^8   \end{array} $		89 <sup>2</sup> 360 <sup>7</sup>	960 <sup>4</sup> 370 <sup>6</sup>	
15	OH	H HIMING	۲. مرکز مر		nn X	Nor St	2338	4338			
16	OH	H HIMME	۲. مرکز مر		nn X	Nor St	$1,170^9$ $1,200^8$	1,2008			
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18	OH	H HIMAN	۲. مرکز دیار		nr.	Nor St	1,3508	2,3808			

19	۳ ۲۰۰۰ کلیست کرک	H H	8- ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		nn Se	so so	7,3008	12,4008				
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27	OH Joy Joy Joy Joy Joy Joy Joy Joy Joy Joy	H H	OMe	HIM O	under and	Nor St	>1,000 <sup>4</sup>				>1,000 <sup>4</sup>	
28	OH SV/S		۲. مربع میر مربع	Harris Contraction		No st	2,7604				8,5004	
29	OH John Star		۲۹ می	Hard Contraction		Nor St	4,2954				9,6004	
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36	OH	and and a	۲. مربع میں	Harris Contraction	Nr.	2 A St				4305
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1) Johnson, et al., 2007, J. Med. Chem. 50, 3795-3803. 2) Mulzer et al., 2003, Chem. Rev. 103, 3753-3786. 3) Gollner et al., 2009, Chem. Eur. J. 15, 5979-5997. 4) Gallagher et al., 2004, Bioorg. Med. Chem. 14, 575-579. 5) Pateson et al., 2005, Bioorg. Med. Chem. 15, 2243-2247. 6) Wender et al., 2003, Org. Lett. 5, 3507-3509. 7) Pryor et al., 2002, Biochemistry, 41, 9109-9115. 8) Mooberry, et al., 2008, Mol. Pharmacol., 5, 829-838. 9) Wender, et. al., 2006, Org. Lett., 8, 1507-1510. 10) Quinoa et al., 1988, J. Org. Chem, 53, 3642-3644. 11) Trost, B.M. et al., 2012 Chem. Eur. J. 18, 2961-2971.

No.	Туре	$\delta_{ m H}{}^a$	mult.	$J(\mathrm{Hz})$
1	С			
2	СН	5.70	d	11.6
3	СН	6.01	ddd	11.4, 9.5, 3.6
4	$CH_2$	3.84	m	
		1.89	m	
5	CH	3.92	m	
6	CH	5.40	dq	10.2, 3.0
7	CH	5.61	ddd	10.1, 5.3, 2.5
8	$CH_2$	1.83	m	
		1.74	m	
9	CH	3.66	dd	9.0, 3.3
10	$CH_2$	1.52	m	
		1.15	ddd	14.3, 4.5, 3.1
11	СН	1.80	m	
12	$CH_2$	2.57	dd	13.1, 4.3
	_	1.85	m	
13	С			
14	CH <sub>2</sub>	2.11	m	
	-	2.09	m	
15	СН	3.96	m	
16	СН	2.71	dd	3.3, 2.5
17	СН	2.88	ddd	9.3, 3.6, 2.4
18	$CH_2$	2.15	ddd	13.8, 3.0, 1.5
	-	1.43	ddd	14.1, 8.4, 3.6
19	СН	5.09	ddd	10.0, 4.9, 1.6
20	СН	4.10	m	
21	СН	4.71	ddd	16.2, 5.4, 1.7
22	СН	5.79	ddd	16.1, 4.8, 1.2
23	СН	3.90	m	
24	$CH_2$	1.96	m	
	-	1.61	m	
25	С			
26	СН	5.11	bs	
27	CH <sub>2</sub>	4.07	bs	
-	- 2			
28	CH <sub>3</sub>	0.84	d	6.5
29	CH <sub>2</sub>	4.89	S	
-	- 2	4.87	S	
30	CH <sub>3</sub>	1.47	S	
	5			

Table S2. <sup>1</sup>H NMR Data of Fijianolide B/Laulimalide (3) in Benzene-d6

<sup>a</sup> Measured at 400 MHz (<sup>1</sup>H) in benzene-d6.

No.	Type	$\delta_{ m H}{}^a$	mult.	$J(\mathrm{Hz})$
1	С			
2	CH	5.77	d	10.8
3	CH	5.96	ddd	10.9, 10.9, 3.8
4	$CH_2$	3.96	m	
	-	2.06	m	
5	СН	4.13	bd	8.7
6	СН	5.43	bd	10.8
7	СН	5.64	m	
8	CH <sub>2</sub>	1.76	m	
9	CH	3.73	m	
10	CH <sub>2</sub>	1.53	m	
	2	1.16	ddd	14.3. 4.2. 3.0
11	СН	1.78	m	
12	CH <sub>2</sub>	2.54	dd	14.8.6.0
	2	1.84	m	,
13	С			
14	CH <sub>2</sub>	2.23	dd	15.9.9.6
	2	1 69	m	
15	СН	5.63	m	
16	СН	2.78	dd	32.28
17	СН	2.89	da	8920
18	CH2	2.13	m	0.9, 2.0
10	0112	2.15		
19	СН	5.35	ddd	11.5. 5.7. 1.6
20	СН	5.53	dd	,,
21	CH	5.75	dd	15.8.5.0
22	СН	5 88	ddd	159 47 06
23	СН	3.82	ddd	863333
24	CH2	1 94	m	0.0, 5.5, 5.5
2.	0112	1.50	m	
25	С	1.00		
26	СН	5 12	bs	
27	CH2	4 08	bs	
- /	0112	3.92	bs	
28	$CH_2$	0.82	d	6.0
29	CH <sub>2</sub>	4 97	bs	0.0
2)		4 86	bs	
30	$CH_2$		bs	
20	OAc	1 77	s	
	OAc	1.65	s	
<sup>a</sup> Meas	sured at 50	)0 MHz (	$(^{1}\text{H})$ in be	nzene-d6

Table S3. <sup>1</sup>H NMR Data of Fijianolide B Di-Acetate (4) in benzene-d6

No.	Type <sup>a</sup>	$\delta_{C}^{b}$	$\delta_{\rm H}{}^c$	mult.	J (Hz)	gHMBC <sup>c</sup>	gCOSY <sup>c</sup>
1	C	165.5	••			2,4	
2	СН	120.4	5.76	d	11.4	4	3, 4
3	СН	150.3	5.93	ddd	11.3, 10.2, 3.6	4	3, 4
4	$CH_2$	34.2	4.72	m	, ,	3,6	2, 3, 5
	2		2.04	m		,	, ,
5	СН	73.4	4.09	bd	10.2	3, 4	4, 6, 7
6	СН	129.1	5.36	bd	10.2	4	5,7
7	СН	125.2	5.57	m		8	5, 6, 8
8	$CH_2$	33.5*	1.73	m			9, 10
	-		1.55				
9	СН	68.2	3.54	m		8	8, 9, 10, 11
10	$CH_2$	44.0	1.46	m		8, 28	8, 9, 11
			1.10	ddd	14.3, 4.7, 2.4	-	
11	СН	30.0	1.86	m		10, 12, 28	10, 12
12	$CH_2$	46.7	2.53	dd	13.4, 5.1	11, 28, 29	10, 11, 29
			1.86	dd	13.9, 10.1		
13	С	145.5				11, 12	
14	$CH_2$	37.7	2.09	m		12, 29	15, 29
15	СН	66.4	3.97	m		14, 16	14, 16
16	СН	60.9	2.67	dd	3.0, 2.3	14, 18	15, 17
17	СН	51.4	2.87	ddd	8.8, 4.2, 2.2	16, 18, 19	16, 18
18	$CH_2$	33.5*	2.23	ddd	14.1, 4.2, 2.1	17	17, 19
			1.44	m			
19	СН	74.8	5.40	dd	11.6, 2.1	18	18
20	С	194.0				18, 19, 21, 22	
21	СН	122.5	6.65	dd	15.6, 2.0	22	22, 23
22	СН	147.9	6.96	dd	15.6, 3.5		21, 23
23	СН	72.4	3.68	m		21, 22	21, 22, 24
24	$CH_2$	34.8	1.63	m		22, 30	23
			1.28	m			
25	С	131.1				24, 30	
26	СН	120.1	5.02	bs			24, 27, 30
27	$CH_2$	65.8	4.07	bd	15.9		26
			3.85	bd	15.9	30	
28	$CH_3$	21.2	0.86	bd	6.7	10, 12	11
29	$CH_2$	112.0	4.93	S		12, 14, 15	12, 14
			4.89	S			
30	CH <sub>3</sub>	22.7	1.37	S			26, 27

Table S4. <sup>13</sup>C, <sup>1</sup>H, HMBC, and COSY NMR Data of Fijianolide J (5) in Benzene-d6.

<sup>*a*</sup> Carbon type determined by DEPT, HSQC, and HMBC experiments (see Figures S5, S7, and S8 in Supporting Information). <sup>*b*</sup>Measured at 175 MHz. <sup>*c*</sup>Measured at 700MHz. \* Interchangeable assignments.

No.	Type <sup>a</sup>	$\delta_{C}{}^{b}$	$\delta_{\mathrm{H}}{}^{c}$	mult.	$J(\mathrm{Hz})$	gHMBC <sup>c</sup>	gCOSY <sup>c</sup>
1	С	164.9					
2	СН	121.0	5.82	d	11.1	3	3, 4
3	СН	147.7	5.78	m			2, 4
4	$CH_2$	34.3	3.34	m		3, 5	3, 5
			1.78	m			
5	СН	72.7	4.23	dd	10.2, 6.3	4	6, 4
6	СН	128.7	5.38	bd	10.1		7, 8
7	СН	125.1	5.58	m			6, 8
8	$CH_2$	31.6	1.73	m		10	
			1.55				
9	СН	65.9	3.46	m			7, 8, 10
10	$CH_2$	44.9	1.44	m		11, 12, 28	11, 12
			0.84	ddd	14.1, 9.3, 2.0		-
11	СН	27.4	1.86	m		10, 12, 28	
12	$CH_2$	46.7	2.83	q	16.6		29
			1.73	dd	13.9, 10.1		
13	С	140.8					
14	$CH_2$	43.2	1.98	m		12	16, 17
15	С	202.2					-
16	СН	54.7	2.96	d	1.9	14, 17, 18, 19	14
17	СН	65.4	3.05	ddd	6.5, 6.0, 1.8	18	18
18	$CH_2$	33.2	2.08	m		17, 19	17, 19
	-		1.57	m		,	,
19	СН	74.6	5.41	dd	7.8, 5.7	16, 17, 18	18
20	С	193.4					
21	СН	122.2	6.60	dd	15.7, 1.9	18	22
22	СН	147.0	6.96	dd	15.7, 3.6		21
23	СН	72.0	3.66	m	,	22, 27	
24	$CH_2$	34.8	1.63	m			30
	-		1.27	m			
25	С	130.6					
26	СН	119.7	5.00	bs		24	27, 30
27	$CH_2$	59.7	3.99	bd	15.8		26, 30
	-		3.83	bd	15.8		,
28	CH <sub>3</sub>	19.2	0.74	bd	6.6	10, 11, 12	10, 12
29	CH <sub>2</sub>	116.0	4.88	S		12, 14	,
	-		4.80	S		,	
30	CH <sub>3</sub>	22.3	1.35	S		24	24, 26, 27
<sup>a</sup> Carbo	on type dete	rmined by F	ISOC an	d HMRC ex	neriments (see Figu	res S12 and S13 in	Supporting

Table S5. <sup>13</sup>C and <sup>1</sup>H NMR Data of Fijianolide L (6) in Benzene-d6.

<sup>*a*</sup> Carbon type determined by HSQC and HMBC experiments (see Figures S12 and S13 in Supporting Information). <sup>*b*</sup>Measured at 100 MHz. <sup>*c*</sup>Measured at 400MHz.

Figure S1. <sup>1</sup>H NMR spectrum of Fijianolide B/Laulimalide (3) in C<sub>6</sub>D<sub>6</sub> (400 MHz).







**Figure S3.** <sup>1</sup>H NMR spectrum of Fijianolide J (**5**) in  $C_6D_6$  (400 MHz).











## **Figure S6.** gCOSY spectrum of Fijianolide J (**5**) in C<sub>6</sub>D<sub>6</sub> (700 MHz)



f1 (ppm)





**Figure S10.** <sup>13</sup>C NMR spectrum of Fijianolide L (6) in  $C_6D_6$  (100 MHz)













Figure S13. HMBC spectrum of Fijianolide L (6) in C<sub>6</sub>D<sub>6</sub> (800 MHz)



#### Figure S14. ESI-HAMS spectrum of Fijianolide B/Laulimalide (3)

#### Figure S15. ESI-HAMS spectrum of Fijianolide J (5)





#### Figure S16. ESI-HAMS spectrum of Fijianolide L (6)



Figure S17. Cosedimentation of antiproliferative activity with purified tubulin. Compounds 3, 4, or 5 were incubated with purified tubulin protein at a 5-fold molar excess of drug (100 µM) to protein (20 µM) in GPEM buffer for one hour at 37°C. Control reactions lacking tubulin protein were performed side-by-side. Microtubules were then pelleted from each reaction along with any microtubule-associated compound by centrifugation at 21,000g for 30 min at room temperature to avoid any microtubule depolymerization. The supernatant was removed and pellets resuspended in DMSO. To take into account the difference in potency of the original compounds, the pellets containing the less potent compounds 4 and 5 were resuspended in 10  $\mu$ L DMSO while reactions with the most potent compound **3** were resuspended in 100 uL DMSO. Two-fold serial dilutions of these pellet fractions were prepared and 1 µL of each was added to MDA-MB-231 cells for 48 h. The resulting cellular growth over the 48 h treatment period was determined using the SRB assay and graphed as a percent of growth compared to the time of drug addition (y = 0) and vehicle-treated controls (y = 100). The resulting concentration response to growth inhibition was graphed as arbitrary units (AU) comparing the effects of compound in the pellet fraction with or without tubulin to provide an indication of whether a potent compound was able to specifically coprecipitate with purified tubulin. We found that a potent, antiproliferative compound specifically coprecipitated with the microtubule pellet from our stocks of 3, but not 4 or 5, demonstrating that the antiproliferative effects of the latter two compounds do not appear to be due to contamination with a small amount of **3**. No antiproliferative activity was observed in the undiluted pellet fraction of **5** with tubulin.