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New Candidaspongiolides, Tedanolide Analogues That Selectively Inhibit Melanoma Cell Growth

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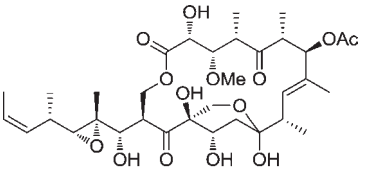
ABSTRACT

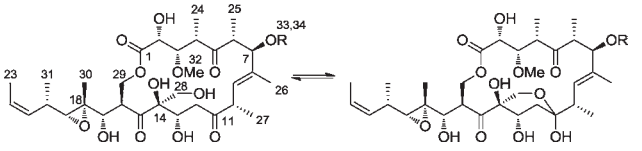


candidaspongiolide B

Extracts of the sponge genus *Candidaspongia* showed selective cytotoxicity toward melanoma cells in the NCI 60-cell-line screen. Continued investigation of the *Candidaspongia* sp. extracts led to the isolation of three new tedanolide analogues, precandidaspongiolides A (1) and B (2) and candidaspongiolide B (4), as well as candidaspongiolide A (3) and tedanolide (5). Semisynthetic derivatives were also generated to develop SAR. Candidaspongiolides A/B were the most potent and showed low nanomolar activity against several melanoma cell lines.

Melanoma is the most common life-threatening form of skin cancer, and lifetime incidence rates have been steadily rising over the past 30 years. Metastatic melanoma is notoriously resistant to a wide range of chemotherapeutic agents, and patient prognosis is generally poor.[1] Consequently, the search for novel melanoma-specific agents continues.

Several extracts of the sponge genus *Candidaspongia* were identified as potently cytotoxic and showed selectivity toward melanoma cells in the NCI 60-cell-line screen. Further examination of these *Candidaspongia* extracts yielded a complex mixture of acyl esters of a tedanolide-related macrolide, the candidaspongiolides.[2] Lipase-catalyzed hydrolysis of the complex mixture resulted in the isolation and identification of the macrolide core, named herein candidaspongiolide A (**3**). In the NCI 60-cell-line screen, the candidaspongiolide acyl ester mixture and candidaspongiolide A (**3**) exhibited GI_{50}'s of ~14 nM and < 4 nM, respectively, against seven melanoma cell lines (Tables S7 and S8, Supporting Information). The candidaspongiolides belong to a small class of tedanolide macrolides, which includes tedanolide (**5**),[3] 13-deoxytedanolide,[4] and tedanolide C,[5] all of which are cytotoxic in the subnanomolar to nanomolar range against various cancer cell lines.



1 R = H
3 R = Ac

2 R = H
4 R = Ac

Continued investigation of the Papua New Guinea *Candidaspongia* sp. extracts for more melanoma-selective agents led to the isolation of three new compounds, precandidaspongiolides A (**1**) and B (**2**) and candidaspongiolide B (**4**), as well as candidaspongiolide A (**3**),

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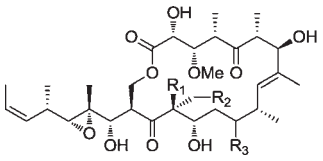
(not previously isolated from natural sources) and (+)-tedanolide (**5**). Semisynthetic derivatives (**6**–**8**) of precandidaspongiolide A (**1**) were also prepared to assess the importance of the hemiketal/hydroxy ketone for melanoma inhibitory activity (Figure 1).



	R_1	R_2	R_3
5	H	H	=O
6	OH	OAc	=O
7	OH	OH	OH
8	OH	OH	OH

Figure 1. Tedanolide (**5**) and semisynthetic precandidaspongiolides (**6**–**8**).

Mass-guided fractionation of the aqueous *Candidaspongia* extracts utilizing size-exclusion chromatography, reversed-phase column chromatography, and reversed-phase HPLC resulted in the isolation of **1**–**5**.

Precandidaspongiolides A (**1**) and B (**2**) were isolated as an inseparable mixture of two isomers in equilibrium, clearly related to the tedanolides, with the major isomer containing a primary alcohol (**1**) and the minor isomer containing a hemiketal (**2**) (ratio **1**:**2** = 4.5:1). Interestingly, the myriaporones, isolated from a bryozoan and structurally related to the southern hemisphere of the tedanolides, have also been isolated as an equilibrium mixture (Figure 2).[6] In subsequent synthetic studies of the myriaporones, equilibrium mixtures were also reported.[7,8]

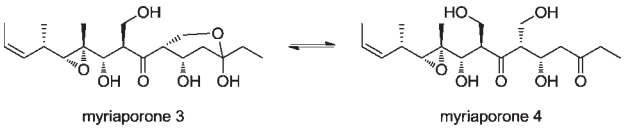


Figure 2. Myriaporones **3** and **4**.

Therefore, structure determination of the two isomers was carried out on the mixture of **1** and **2**. HRESIMS data (m/z 665.3146 $[M + Na]^+$, Δ = 0.3 ppm) indicated that the molecular formula for both **1** and **2**, was $C_{32}H_{50}O_{13}$; two oxygens more than the molecular formula for tedanolide (**5**), and 42 Da less than the molecular weight of candidaspongiolide A (**3**). The aforementioned data suggested that **1** and **2** lacked the large fatty acid esters, as well as the C-7 acetoxy moiety present in the original candidaspongiolide mixtures. Analysis of the combined NMR data confirmed this supposition (Table 1).

The major isomer, precandidaspongiolide A (**1**), clearly lacked the acetyl group at C-7, as indicated by the upfield shift of H-7 (δ_H 4.04) compared to **3** (δ_H 5.39). Additionally, only one ester was present in **1**; the macrolide lactone (δ_c 173.4, C-1). The presence of a primary alcohol was also apparent in **1**, as evidenced by the degenerate chemical shifts of the C-28 methylene (δ_H 3.75, s). The NMR data (Table 1) supported the remaining structure of precandidaspongiolide A (**1**), (or 7-deacetylcandidaspongiolide A), as drawn.

The minor isomer, precandidaspongiolide B (**2**), also lacked the acetoxy group at C-7 (Table 1) and had a large number of chemical shift deviations from **1**, particularly in the C-10 to C-14 portion of the molecule. Compound **2** contained only two ketones (δ_c 217.2, 212.1), which were identified as C-5 and C-15, respectively, and suggested a modification to the C-11 ketone. Additionally, the diastereotopicity of the C-28 methylene (δ_H 3.63, 3.53) and a downfield quaternary carbon (δ_C 100.5) supported the presence of a hemiketal ring in **2**. The hemiketal identity was confirmed based on HMBC correlations from H-28a/b, H-10, and H-12a/b to C-11. The NMR data (Table 1) corroborated the remaining portion of **2**, the tedanolide core, as drawn.

Candidaspongiolides A (**3**) and B (**4**) were also isolated as an equilibrium mixture (ratio **3**:**4** = 1.7:1). HRESIMS data (m/z 707.3235 $[M + Na]^+$, Δ = 2.1 ppm) indicated that the molecular formula for **3** and **4**, was $C_{34}H_{52}O_{14}$, matching the molecular formula for candidaspongiolide A, originally isolated from lipase catalyzed hydrolysis of the candidaspongiolide acyl esters.[2] The molecular formula and NMR data (Table 1) suggested that **3** was the acetylated version of **1**. Comparison of the spectroscopic data for the macrolide core (candidaspongiolide A)[2] and the major isomer **3** (in acetone-d_6, Table S1, Supporting Information,[9]) confirmed the identity and structure, as drawn.[10]

The minor isomer, candidaspongiolide B (**4**), closely resembled precandidaspongiolide B (**2**), with the major differences appearing around C-7. Analysis of the NMR data (Table 1) confirmed the identity of candidaspongiolide B (**4**) as the C-7 acetyl analog of **2**.

HRESIMS data (m/z 633.3232 $[M + Na]^+$, Δ = 2.2 ppm) supported a molecular formula of $C_{32}H_{50}O_{11}$ for **5**, identical to that of tedanolide. Compound **5** was confirmed as (+)-tedanolide upon comparison of spectroscopic data with those reported in the literature.[3,11]

Analysis of the coupling constants and ROESY data for **1**–**4** suggested that their relative configurations were identical to tedanolide,[3] 13-deoxytedanolide,[4] and the original candidaspongiolides.[2] Biosynthetic principles support **1**–**4** having the same absolute configuration as (+)-tedanolide (**5**), given that **1**–**4** were concurrently isolated with **5**.

Within the small tedanolide class, there are a number of structural features that are unique to the candidaspongiolides,

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(9) Minor revisions have been made to the NMR assignments of candidaspongiolide A (**3**) in acetone-d_6.

(10) There was no evidence of equilibrium isomers in the NMR data for the lipase-derived macrolide core reported previously.[2]

(11) See the Supporting Information for a complete assignment of (+)-tedanolide (**5**) in $CDCl_3$ and CD_3OD.

Table 1. NMR Data for Precandidaspongiolides A (**1**) and B (**2**) and Candidaspongiolides A (**3**) and B (**4**) (600 MHz, CD_3OD)

	precandidaspongiolide A (**1**)		precandidaspongiolide B (**2**)		candidaspongiolide A (**3**)		candidaspongiolide B (**4**)	
no.	δ_C	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)
1	173.4		173.3		173.2		173.1	
2	73.1	3.76[a] (2.0)	72.7	3.60, d (1.9)	73.0	3.77, d (1.9)	72.7	3.57, d (1.9)
3	84.8	3.81, dd (9.5, 2.0)	84.9	3.66, dd (9.5, 1.9)	85.4	3.80, dd (9.4, 1.9)	85.1	3.68, dd (9.8, 1.9)
4	49.6	3.15,[a] (9.5, 7.1)	48.5	3.34, dq (9.5, 7.2)	48.9	3.21, dq (9.4, 7.0)	48.2	3.31,[d] (9.8, 6.9)
5	217.8		217.2		216.1		215.4	
6	51.3	3.15,[a] (10.0, 7.0)	51.6	3.15, dq (10.2, 6.9)	50.0	3.41, dq (10.2, 7.1)	49.7	3.35,[c] (6.7)
7	80.0	4.04, d (10.0)	79.2	4.15, d (10.2)	81.4	5.39, d (10.2)	81.3	5.48,[c]
8	139.1		135.8		134.7		131.4	
9	129.9	5.33, br d (9.5)[b]	132.5	5.40, br d (10.0)	133.3	5.45, br d (9.8)	135.9	5.55 br d (10.0)
10	46.7	3.38, dq (9.5, 6.8)	44.3	2.35, dq (10.0, 6.9)	46.6	3.35, dq (9.8, 6.8)	44.6	2.32, dq (10.0, 6.8)
11	212.9		100.5		212.2		100.4	
12a	44.4	2.75, dd (17.6, 9.6)	41.1	2.01, dd (13.6, 4.3)	44.5	2.70, dd (17.6, 9.6)	41.2	1.99, dd (13.8, 4.2)
12b		2.24, dd (17.6, 1.7)		1.03,[c] (13.6, 12.7)		2.24, dd (17.6, 1.9)		1.00,[c] (13.8, 12.6)
13	69.5	4.44, dd (9.6, 1.7)	71.9	4.22, dd (12.7, 4.3)	69.4	4.46, dd (9.6, 1.9)	71.9	4.21, dd (12.6, 4.2)
14	85.4		79.1		85.5		79.1	
15	216.6		212.1		216.5		212.1	
16	48.9	4.08, ddd (11.2, 10.8, 4.1)	51.9	4.36,[c] (11.7, 10.4, 3.8)	49.0	4.04, ddd (11.0, 10.7, 3.9)	52.0	4.35,[c] (11.9, 10.4, 3.9)
17	78.5	3.21, d (10.8)	78.9	3.02, d (10.4)	78.4	3.23, d (10.7)	78.9	3.00, d (10.4)
18	64.1		63.9		63.7		63.8	
19	67.5	2.64, d (9.4)	67.3	2.66, d (9.2)[b]	67.5	2.64, d (9.2)	67.3	2.62, d (9.2)
20	32.6	2.48, ddq (10.7, 9.4, 6.6)	32.6	2.64,[c] (9.2, 7.2)	32.5	2.48, ddq (10.7, 9.2, 6.5)	32.5	2.50,[c]
21	131.8	5.33, ddq (10.9, 10.7, 1.5)[b]	132.0	5.32,[c] (1.6)	131.8	5.35, ddq (10.9, 10.7, 1.5)	132.0	5.32, ddq (10.9, 10.6, 1.4)
22	126.3	5.51, dq (10.9, 6.8)	126.1	5.50,[c] (6.9)	126.3	5.51, dq (10.9, 6.7)	126.1	5.50,[c] (6.8)
23	13.7	1.63, dd (6.8, 1.5)	13.7	1.60, dd (6.9, 1.6)	13.7	1.63, dd (6.7, 1.5)	13.7	1.60, dd (6.8, 1.5)
24	15.2	1.23, d (7.1)	15.5	1.25, d (7.2)[b]	15.0	1.23, d (7.0)	15.6	1.15, d (6.9)
25	15.7	1.26, d (7.0)	15.2	1.14, d (6.9)	14.8	1.18, d (7.1)	14.5	1.13, d (6.7)
26	10.5	1.66, br s	10.4	1.51, br s	11.0	1.66, br s	10.6	1.50, br s
27	15.8	1.04, d (6.8)	13.0	0.95, d (6.9)	15.6	1.00, d (6.8)	12.8	0.91, d (6.8)
28a	65.8	3.75,[a] s	66.8	3.63, d (11.2)	65.8	3.75, s	66.8	3.62, d (11.2)
28b				3.53, d (11.2)				3.53, d (11.2)
29a	65.3	4.34, dd (10.8, 4.1)	65.4	4.31, dd (11.7, 10.0)	65.0	4.33, dd (10.8, 3.9)	65.4	4.31, dd (11.9, 10.0)
29b		3.93, dd (11.2, 10.8)		3.98, dd (10.0, 3.8)		3.95, dd (11.0, 10.8)		3.98, dd (10.0, 3.9)
30	11.6	1.35, s	11.5	1.38, s	11.6	1.35, s	11.5	1.35, s
31	18.8	1.11, d (6.6)	18.9	1.12, d (7.2)[b]	18.9	1.10, d (6.5)	18.9	1.10, d (6.5)
32	61.5	3.39, s	61.2	3.31, s	61.5	3.41, s	61.3	3.32, s
33					171.9		172.0	
34					21.0	2.03, s	21.0	2.03, s

[a] Signals overlapped. [b] Measured in acetone-d_6. [c] Signal obscured by major/minor isomer. [d] Buried under CD_3OD signal.

particularly the hemiketal. Three semisynthetic analogs were generated from the equilibrium mixture of **1**/**2** to develop SARs among the candidaspongiolides, and assess the importance of the hemiketal for melanoma cytotoxicity. The primary alcohol of **3** was selectively acetylated,[12] using AcCl and 2,4,6-trimethylpyridine, to give 28-acetyl-precandidaspongiolide (**6**), and the 11-keto group was preferentially reduced[13] using $NaBH_4$ in MeOH to yield a pair of diastereomers, 11*R*- and 11*S*- dihydro-precandidaspongiolide A (**7**, **8**), respectively. The configurations of C-11 in **7** and **8** were assigned on the basis of interpretation of ROESY data, coupling constants, and comparison of ^{1}H NMR chemical shifts with (11*R*)- and (11*S*)-dihydro-13-dexoytedanolide.[12]

ROESY cross peaks between H-10/H-11 and H-11/H-13 suggested an 11*R* stereochemistry for **7**, while cross peaks between H-9/H-11 and a number of upfield ^{1}H NMR shifts in **8** compared to **7** implied a 11*S* stereochemistry for **8**. In addition, the 11*R* diastereomer was reported as the major product from the reduction of 13-deoxytedanolide (confirmed by Mosher's analysis),[12] which is in agreement with the major product (**7**-11*R*) from the reduction of **1**.

Precandidaspongiolides A (**1**) and B (**2**) showed excellent selectivity against melanoma cell lines in the NCI 60-cell-line screen (Figures S3 and S4, Supporting Information). The LC_{50} values for **1**/**2** against melanoma cell lines were significantly lower than other tumor cell lines; seven of the nine melanoma cell lines in the panel had nanomolar LC_{50} values (19−174 nM), while a majority of the other tumor cell lines had LC_{50}'s greater than 100 μM (Figure S4, Supporting Information).

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Table 2. Biological Activity of **1-8**

	IC_{50} (nM)[a]				
	melanoma			breast	lung
compd	UACC257	LOX-IMVI	M14	MCF7	NCI-H460
precandidaspongiolide A/B (**1/2**)	14.2 ± 0.2	6.9 ± 1.0	17.9 ± 4.3	8.3 ± 0.5	12.3 ± 1.0
candidaspongiolide A/B (**3/4**)	1.6 ± 0.5	<2.0	7.5 ± 1.5	<2.0	3.4 ± 0.2
tedanolide (**5**)	5.9 ± 0.1	2.5 ± 0.4	8.6 ± 2.4	3.6 ± 0.3	7.0 ± 3.9
28-acetylprecandidaspongiolide A (**6**)	103.8 ± 3.4	34.2 ± 9.4	261.0 ± 63.9	98.6 ± 12.5	96.8 ± 15.7
(11*R*)-dihydroprecandidaspongiolide A (**7**)	16.7 ± 2.2	10.3 ± 0.3	25.1 ± 0.5	12.4 ± 1.2	43.7 ± 16.6
(11*S*)-dihydroprecandidaspongiolide A (**8**)	423.1 ± 57.4	275.3 ± 20.0	505.6 ± 94.0	368.8 ± 19.1	433.4 ± 17.5

[a] IC_{50} cytotoxicity values were determined as the drug concentration that reduced cell growth to 50% of the untreated control.

To develop SARs, compounds **1**–**8** were tested against three NCI-60 melanoma cell lines (UACC-257, LOX-IMVI, and M14), as well as a breast (MCF7) and lung cancer (NCI-H460) cell line (Table 2). Compounds **1**–**8** showed nanomolar activity among the various cell lines. Identical patterns were observed between melanoma, breast and lung tumor cell lines, which are still consistent with the original NCI 60-cell line data for **1**/**2** (Figures S3 and S4, Supporting Information). In the interest of preserving the limited amount of material available for **3**–**8**, the compounds were tested in the IC_{50} range (= NCI 60-cell line GI_{50}, see page S7, Supporting Information) and not the LC_{50} range. It is likely that the LC_{50} values and melanoma selectivity of **3**–**8** would mimic those of **1**/**2**. Candidaspongiolides A/B (**3**/**4**) were the most active against all cell lines, while **8** was the least active. Comparing the activities for **1**/**2** and the semisynthetic derivatives (**6**–**8**), it appears that the hemiketal is not essential for activity, as **7** retains the same level of potency as **1**/**2**. However, potency is affected when the primary alcohol is substituted, as in **6**, which is ∼5–15 times less potent than **1**/**2**. This is consistent with the NCI 60-cell line data for the candidaspongiolide acyl esters and candidaspongiolide A (**3**) (Tables S7 and S8, Supporting Information); compound **3** is over 3× more potent than the candidaspongiolide fatty acid esters. Interestingly, compound **7** is significantly more potent than **8**. Fusetani et al. reported that the 11*S* diastereomer of 13-deoxytedanolide was more potent than the 11*R* in p388 murine leukemia cells.[12] This apparent discrepancy may be due to different tumor cell type specificity, and would be an interesting area for further investigation. Additionally, when comparing **1**/**2** and **3**/**4**, it appears that C-7 acetylation increases potency. However, differences in the equilibrium mixture ratios may also contribute to the potency differences. The somewhat simpler tedanolide (**5**) retains low nanomolar activity without the oxygenation at C-14 and C-28, the hemiketal moiety, and acetylation at C-7.

The 60-cell-line data for **1**/**2** revealed a number of candidaspongiolide insensitive cell lines (NCI/ADR-RES; HCT-15) that are known to express P-glycoprotein (P-gp), a multidrug resistance transporter. To test whether **1**/**2** were P-gp substrates, KB-3-1 (adenocarcinoma, P-gp-deficient) and KB-V1 (P-gp-overexpressing) cells were treated with **1**/**2** (Figure S10, Supporting Information). KB-3−1 cells were sensitive to **1**/**2**, while KB-V1 cells were resistant. Resistance is defined as the ratio of cytotoxicity against P-gp-expressing cell pairs versus parental cells (resistance ratio (RR) = 26.7). Both cell lines were coincubated with **1**/**2** and tariquidar (TQR), a specific P-gp inhibitor. TQR did not affect the toxicity of **1**/**2** against the P-gp deficient KB-3-1 cells but completely reversed resistance in the KB-V1 cells, confirming that **1**/**2** are in fact P-gp substrates. P-gp is, however, not thought to play a role in drug-resistant melanoma.[14]

The similarities between the myriaporones and the new candidaspongiolides offers further evidence in support of microorganisms as producers of these compounds. 13-Deoxytedanolide[15] and candidaspongiolide A (**3**)[16] both inhibit protein synthesis. However, the underlying reasons for the candidaspongiolides' melanoma selectivity have yet to be determined and are currently being investigated.

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Supporting Information Available. General experimental details, organism collection, isolation details, reaction conditions, bioassay methods, spectral data, and NCI-60 cell line data. This material is available free of charge via the Internet at http://pubs.acs.org.

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