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Total syntheses and cytotoxicity of kealiiquinone, 2-deoxy-2-aminokealiiquinone and analogs

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Abstract

Concise syntheses of two *Leucetta*-derived naphthimidazole alkaloids, kealiiquinone and 2-deoxy-2-aminokealiiquinone, are described based on a biosynthetic-guided hypothesis. Advanced intermediates containing the full naphthimidazole framework are constructed through Friedel-Crafts chemistry followed by oxidation of the electron rich C-ring with hydrogen peroxide. The cytotoxicity of these alkaloids in a breast cancer cell line along with several closely related marine-derived natural products kealiinines A-C and analogs are reported.

Keywords

Naphthimidazole; Biomimetic; Oxidation; MCF7; Keyword_5

Marine sponges produce an array of structurally unique secondary metabolites that exhibit interesting biological activities and as such serve as lead compounds in drug discovery programs.^{1, 2} Sponges of the *Leucetta* and *Clathrina* families are known to produce various types of natural products, including several examples of imidazole-containing alkaloids.^{3, 4} In the course of their isolation bioactivity-guided fractionation schemes are used and as a result some biological information emerges. However, it is oftentimes limited in scope based on the screens available in a particular lab and because of the nature of the isolation process the purity of the isolated material can be questionable. While some limited structure-activity relationship information can be obtained in these efforts through the isolation of related molecules, it is restricted to (usually) modest structural changes. Such is the case in the *Leucetta* and *Clathrina* alkaloids, wherein a number of different groups of natural products have been isolated and their biological activities assessed (Figure 1), but few direct comparative studies have been performed and limited in depth investigations have been undertaken.⁴ A notable exception to this is naamidine A (**6**),^{5, 6} for which both SAR⁷ and mechanism of action data have been obtained.⁸⁻¹⁰ Our lab has developed a number of total syntheses of various family members of the *Calcarous* family of alkaloids¹¹ and herein we report the synthesis and evaluation of synthetic versions of several naphthimidazole-natural products, precursors and analogs as potential anti-cancer molecules.

Several years ago Clardy and Scheuer reported the isolation of kealiiquinone (**1**) from a *Leucetta*-derived sponge possessing a unique imidazobenzoquinone framework (Figure 1).¹²

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At the time of this report, no bioactivity data were reported, but in a subsequent synthetic effort it was determined that it was cytotoxic, with potentially a unique mechanism of action based on the inhibition profile in a multi cell line screen.¹³ Schmitz reported the isolation of 2-deoxy-2-aminokealiquinone (**2**), but no bioactivity was reported for this molecule.¹⁴ More recently, Proksch and coworkers reported the isolation of three related naphthimidazole derivatives for which only limited investigations were performed using the brine shrimp toxicity assay.¹⁵ Our group has developed concise synthetic methods for the total syntheses of all of these natural products.^{16, 17} The general strategy that we have adopted involves the elaboration of simple imidazoles and as such provides the opportunity to evaluate C2-deletion derivatives as well as to prepare other analogs.

We have described recently the synthesis of kealiinines A-C (**3-5**) and isokealiinine C (**11**) from diiodimidazole **7** utilizing a Friedel-Crafts/dehydration sequence to construct the B-ring, these efforts are summarized in Scheme 1.¹⁶ The Ohta lab has published a total synthesis of kealiquinone (**1**) via the oxidation of intermediate related to **9c**.^{18, 19} Taking our lead from this latter report, we have streamlined this sequence substantially to provide access to both kealiquinone (**1**) and the 2-amino congener **2**, the latter for the first time in synthetic form. Specifically, for synthesis of kealiquinone (**1**) the imidazole C2-position was oxidized by lithiation with *n*-BuLi followed by treatment with TMS-peroxide, according to the protocol of the Lipshutz group.²⁰ Subsequent oxidation of the C-ring with hydrogen peroxide resulted in the formation of the corresponding benzoquinone and thus kealiquinone (**1**) (Scheme 1).²¹ While in many respects our synthesis is broadly similar to the one reported by Ohta in terms of general strategy, it is substantially shorter and protecting group free.^{18, 19} In addition, we have employed extremely simple conditions for the introduction of the benzoquinone moiety via hydrogen peroxide mediated oxidation. For the preparation of the 2-amino congener, lithiation of the imidazole C2 position with *n*-BuLi and trapping with TrisN₃ provided the corresponding 2-azido derivative, which was converted to the kealiquinone derivative by oxidation with hydrogen peroxide.²¹ Reduction of the azide by catalytic hydrogenation then delivered 2-deoxy-2-aminokealiquinone (**2**) (Scheme 1). The C2-deletion analog **10** was prepared from **9c** by oxidation with hydrogen peroxide (Scheme 1).²¹

The spectroscopic data for 2-deoxy-2-aminokealiquinone (**2**) matched exactly with that reported by Schmitz and coworkers (Table 1 and Table 2),¹⁴ however this proved not to be the case for the NMR data reported for the sponge-derived material for kealiquinone (**1**). Our data were generally a good match with that obtained by Ohta with the exception of two signals in the ¹³C NMR spectrum (vide infra).¹⁸ The Ohta lab showed through X-ray crystallography that they had obtained the 2-oxo derivative rather than the 2-hydroxy derivative described for the natural product.¹⁸ Interestingly, in the isolation report an X-ray structure was obtained for the natural material that indicated that it was the 2-hydroxy form.¹² Further support for this assignment was derived from the appearance of a peak in the ¹H NMR spectrum at 8.14 ppm that was attributed to the enolic OH (2-hydroxy moiety, see **1'**, Table 1).¹² Our data are also consistent with the synthesis of the 2-oxo form, in particular an absorption at 154.8 ppm in the ¹³C NMR spectrum is characteristic for a 2-imidazolone.²² Careful comparison of the ¹³C NMR data obtained by us and the Ohta lab indicated that one signal at 145.2 ppm was missing and an extra absorbance appears at 126.5 ppm in the Ohta report. In the course of our studies towards the kealiquinone group of molecules we have prepared two other kealiquinone-like structures **11** and **12** via a completely different strategy involving an intramolecular Diels-Alder reaction and we find that the ¹³C NMR data are an excellent match for **1** (Table 2).^{19, 23} Based on these observations we conclude that the Ohta lab may have inadvertently misreported one signal, unfortunately we have been unable to obtain copies of the original spectroscopic data to confirm or refute this hypothesis. The preparation of the 2-oxo isomer through both

approaches is presumably a reflection of its thermodynamic stability. Interestingly, examination of the spectroscopic data for amino and hydroxyl tautomers shows a correlation with the chemical shift of the N-methyl group being further downfield than for the corresponding 2-oxo derivative. Unfortunately we have been unable to secure a sample of the natural material to confirm the original structural assignment and to establish whether it can indeed be converted into the 2-oxo form.

Each of the synthetic natural products **1-5** along with the pre-C2 functionalized intermediates **9a-c** were evaluated against breast cancer cell line MCF7 using an MTT growth assay, using cisplatin as a positive control (IC₅₀: 19.4 μ M).²⁴ In addition, isokealiinine C (**14**), isokealiinine C C2-deletion compound **15**, and two precursors en route to kealiiquinone **10** and **13** were assayed. All of the natural products exhibit low micromolar cytotoxicity with the exception of kealiinine C (**5**), which is inactive up to 100 μ M (Figure 2 and Table 3). The Looper lab has recently evaluated the bioactivity of synthetic versions of kealiinine B and C and obtained broadly similar results to ours; specifically kealiinine C was devoid of cytotoxicity.¹⁷ All of the substrates which were unsubstituted at C2 were less active, a trend that we have observed with other series in the *Leucetta* family of alkaloids, suggesting that there may be a key binding interaction with the C2-amino moiety or oxo group in the case of compound **13**, but there is greater tolerance to substitutions elsewhere in the molecular framework. Notably, kealiinine B precursor **9b** was an exception to this trend. Of particular note was the observation that the non-natural congener, isokealiinine C (**14**) was substantially more active than the corresponding natural isomer, in fact it is comparable to the two other kealiinine derivatives. Such activity patterns an observation reported by Ohta's group where isokealiiquinone is more active than kealiiquinone.¹³ As far as our studies with the benzoquinone group are concerned, kealiiquinone is the least active with the 2-amino derivative **2** and the C2-deletion analog 2-deoxykealiiquinone (**10**) being comparably active. At the present time no information is available regarding the mechanism of action of these compounds. In their report of the synthesis of kealiiquinone and isokealiiquinone, the Ohta lab suggest that these molecules act via a unique mechanism, based on the profile these compounds exhibit in a Japanese version of the NCI 60 cancer cell line panel.¹³ Likewise, the Looper group has ruled out some of the common pathways for cytotoxicity for kealiinine **B(4)**.¹⁷

The syntheses of kealiiquinone (**1**) and 2-deoxy-2-aminokealiiquinone (**2**) are described, the latter for the first time through a short sequence of reactions inspired by biosynthetic considerations. These two natural products, along with a small series of precursors and related naphthimidazole natural products were evaluated in cell growth assays against a hormone dependent breast cancer cell line. In general terms these derivatives showed modest levels of growth inhibition, with a 2-amino substituent necessary for enhanced levels of activity, but the substituents on the C-ring appear to be less critical.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Various *Leucetta*-derived alkaloids

Figure 2. Cytotoxicity plots of selected compounds 1–5 and 9a–b and cisplatin

Scheme 1. Reagents and conditions

(a) *n*-BuLi, THF, -78°C . (b) $(\text{TMSO})_2$, 59%. (c) TrisN₃, -78°C to rt, 69%. (d) H₂O₂, MeOH (for **1** = 60%; for **10** = 67%). (e) Pd-C, H₂, MeOH, 75% (two steps from **9c**)

Comparative ¹H NMR data for natural and synthetic 2-deoxy-2-aminokealiquinone (2) and kealiquinone (1) and related compounds acquired at 500 MHz in DMSO-*d*₆.

Table 1

2-Deoxy-2-aminokealiquinone		Kealiquinone		7'-Desmethyl kealiquinone		4'-Desmethoxy kealiquinone		2-Deoxykealiquinone ^e	
Synthetic	Natural	Synthetic (This work)	Natural	Synthetic (Ohta)					
		11.02 ^b		11.03 ^b		10.95 ^b	8.11 ^b		
7.75	7.75		8.14 ^c			9.41 ^d		8.24	
		7.68	7.69	7.68		7.63	7.74	8.00	
7.14	7.14	7.13	7.12	7.13		6.96	7.49-7.43	7.28	
7.13	7.10	6.98	6.88	6.98		6.75	7.22-7.21	7.02	
6.90 ^a	6.90 ^a								
3.94	3.94	3.94	3.92	3.94		3.90		4.10	
3.85	3.85	3.86	3.83	3.85			4.07	4.01	
3.80	3.80	3.83	3.78	3.82		3.82	3.97	3.95	
3.61	3.60	3.40	3.58	3.39		3.35	3.47	3.86	

^aTwo proton signal due to NH₂.

^bOne proton signal due to NH.

^cOne proton due to OH.

^dPhenolic OH.

^eRecorded in CDCl₃.

Table 2

Comparative ^{13}C NMR data for natural and synthetic 2-deoxy-2-aminoketone and related compounds acquired at 125 MHz in $\text{DMSO}-d_6$.

2-Deoxy-2-aminokealiquinone				Kealiquinone		7'-Desmethyl kealiquinone		4'-Desmethoxy kealiquinone		2-Deoxykealiquinone ^a	
Synthetic	Natural	Synthetic (This work)	Natural	Synthetic (Ohta)	Natural	Synthetic (Ohta)					
182.0	182.0	181.3	182.4	181.3	181.3	181.9	181.9	181.9	182.3		
181.5	181.4	181.1	181.8	181.1	181.1	181.7	181.7	181.5	182.0		
158.6	158.5	158.5	159.0	158.5	158.5	157.2	157.2		159.1		
157.9	157.8	154.8	158.3	154.8	154.8	155.3	155.3	154.2	148.9		
147.9	147.8	147.8	148.2	147.8	147.8	148.4	148.4	147.6	147.4		
147.5	147.4	145.2	147.8			145.6	145.6	146.0	146.5		
145.6	145.5	134.0	146.0	134.0	134.0	134.4	134.4	135.3	136.8		
137.5	137.5	132.7	137.9	132.6	132.6	133.1	133.1	134.1	130.6		
130.7	130.6	129.9	131.1	129.9	129.9	130.3	130.3	131.6	130.2		
130.2	130.2	127.7	130.6	127.7	127.7	127.0	127.0	129.1	128.8		
129.0	129.0	126.4	129.4	126.50	126.50	126.4	126.4	128.3	128.3		
				126.46	126.46			128.1			
123.7	123.6	123.5	124.1	123.5	123.5	124.5	124.5	127.8	122.8		
122.5	122.5	122.6	122.9	122.6	122.6	123.2	123.2	124.3	114.5		
112.9	112.8	113.9	113.3	113.9	113.9	115.8	115.8	123.0	113.8		
105.1	105.1	104.5	105.5	104.6	104.6	104.9	104.9	105.5	108.8		
60.7	60.6	60.8	61.0	60.8	60.8	61.2	61.2	61.5	61.4		
60.6	60.6	60.8	61.0	60.8	60.8	61.2	61.2	61.4	61.3		
55.0	55.0	55.0	55.4	55.0	55.0				55.3		

2-Deoxy-2-amino-kealiquinone (2)

Kealiquinone (1*)

Kealiquinone (11)

Kealiquinone (12)

2-Deoxykealiquinone (10)

R = OMe; Kealiquinone (1)

R = OH; 7'-Desmethyl kealiquinone (11)

R = H; 4'-Desmethoxy kealiquinone (12)

^aThese data were acquired in CDCl₃.

Table 3
IC₅₀ values of test compounds derived from MMT growth assays in MCF7 cells

Compound	Structure	IC ₅₀ μ M	SEM μ M
1	Kealiquinone	91.9	± 0.5
2	2-Deoxy-2-aminokealiquinone	43.8	± 0.4
3	Kealiinine A	23.4	± 1.9
4	Kealiinine B	28.8	± 1.2
5	Kealiinine C	>100	N/A
9a		92.0	± 2.2

Compound	Structure	IC ₅₀ μ M	SEM μ M
9b		28.0	± 1.3
9c		99.8	± 0.9
10		41.1	± 0.1
13	2-Deoxykealiquinone	29.9	± 0.3
14		22.7	± 1.0
15	Isokealiinine C	68.2	± 1.6
16		>100	N/A