Bioorganic & Medicinal Chemistry Letters 23 (2013) 6183–6187

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Jayanta Das, Arunoday Bhan, Subhrangsu S. Mandal, Carl J. Lovely *

Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019, United States

ARTICLE INFO

Article history:
Received 15 May 2013
Revised 20 August 2013
Accepted 26 August 2013
Available online 6 September 2013

Keywords:
Naphthimidazole
Biomimetic
Oxidation
Cytotoxicity
Leucetta alkaloids

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.

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 (Fig. 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 SAR[7] 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 alkaloids[11] and herein we report the synthesis and evaluation of synthetic versions of several naphthimidazole-natural products, precursors and analogs as potential anti-cancer molecules.

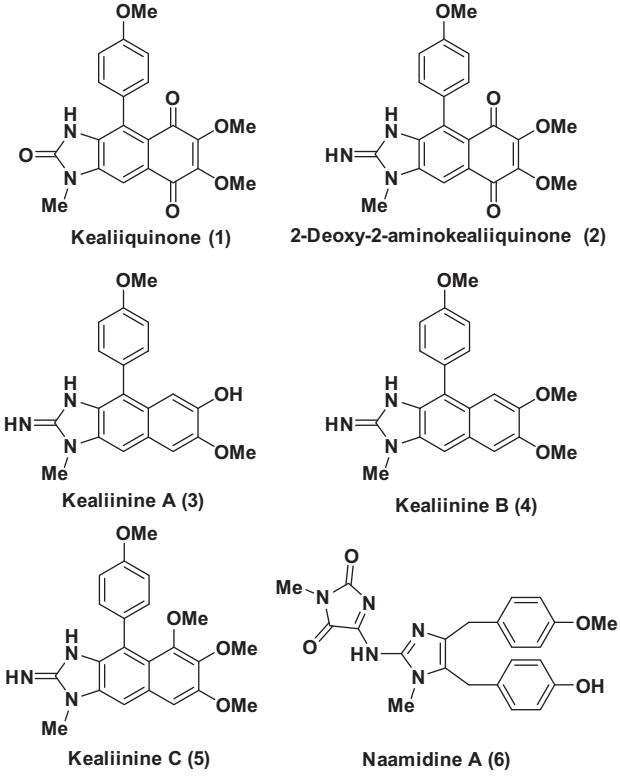
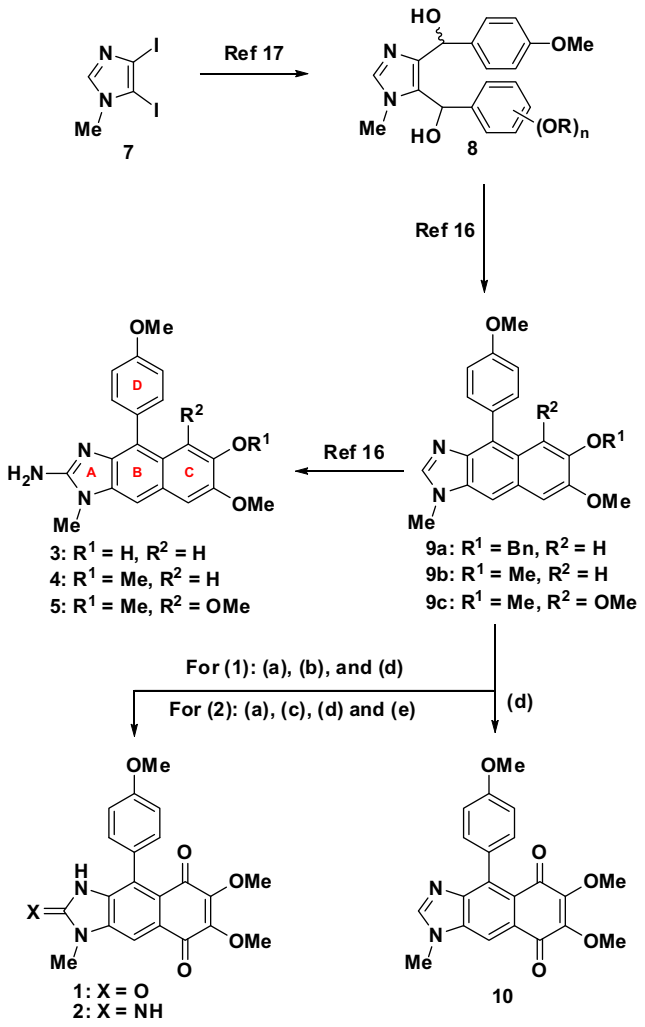


Figure 1. Various *Leucetta*-derived alkaloids.

* Corresponding author. Tel.: +1 817 272 5446; fax: +1 817 272 3801.
E-mail address: lovely@uta.edu (C.J. Lovely).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.08.093

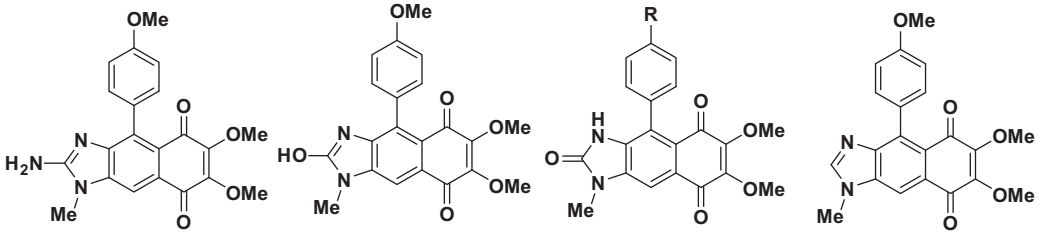


Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; (b) $(TMSO)_2$, 59%; (c) $TrisN_3$, −78 °C to rt, 69%; (d) H_2O_2, MeOH (for **1** = 60%; for **10** = 67%); (e) Pd–C, H_2, MeOH, 75% (two steps from **9c**).

Several years ago Clardy and Scheuer reported the isolation of kealiiquinone (**1**) from a *Leucetta*-derived sponge possessing a unique imidazobenzoquinone framework (Fig. 1).[12] 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-aminokealiiquinone (**2**), but no bioactivity was reported for this molecule.[14] More recently, Proksch and co-workers 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 (**14**) from diiodoimidazole **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 kealiiquinone (**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 kealiiquinone (**1**) and the 2-amino congener **2**, the latter for the first time in synthetic form. Specifically, for synthesis of kealiiquinone (**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 kealiiquinone (**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 $TrisN_3$ provided the corresponding 2-azido derivative, which was converted to the kealiiquinone derivative by oxidation with hydrogen

Table 1
Comparative ^1H NMR data for natural and synthetic 2-deoxy-2-aminokealiiquinone (**2**) and kealiiquinone (**1**) and related compounds acquired at 500 MHz in DMSO-d_6



2-Deoxy-2-amino-kealiiquinone (2) | **Kealiiquinone (1') (as isolated)** | **R = OMe; Kealiiquinone (1) (synthetic)** R = OH; 7'-Desmethyl kealiiquinone (11) R = H; 4'-Desmethoxy kealiiquinone (12) | **2-Deoxykealiiquinone (10)**

2-Deoxy-2-aminokealiiquinone		Kealiiquinone			7′-Desmethyl kealiiquinone	4′-Desmethoxy-kealiiquinone	2-Deoxykealiiquinone[e]
Synthetic	Natural	Synthetic (this work)	Natural	Synthetic (Ohta)			
		11.02[b]		11.03[b]	10.95[b]	8.11[b]	
			8.14[c]		9.41[d]		8.24
7.75	7.75	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

[a] Two proton signal due to NH_2.
[b] One proton signal due to NH.
[c] One proton due to OH.
[d] Phenolic OH.
[e] Recorded in $CDCl_3$.

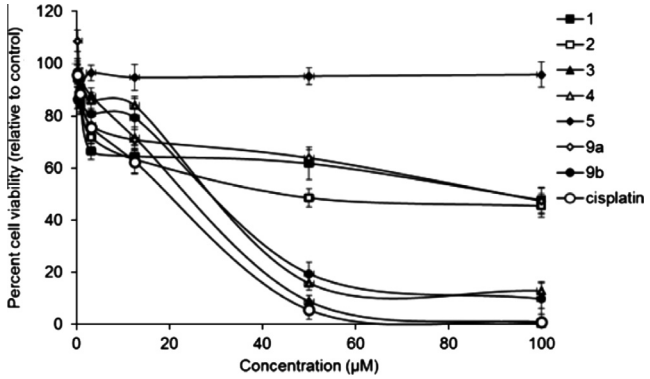
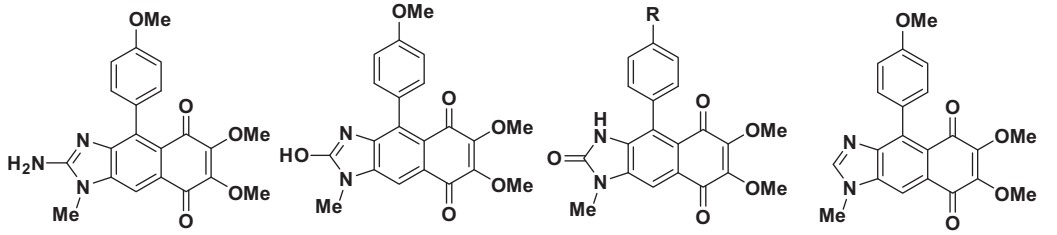


Figure 2. Cytotoxicity plots of selected compounds **1**–**5** and **9a**–**b** and cisplatin.

peroxide.[21] Reduction of the azide by catalytic hydrogenation then delivered 2-deoxy-2-aminokealiiquinone (**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-aminokealiiquinone (**2**) matched exactly with that reported by Schmitz and co-workers (Tables 1 and 2),[14] however this proved not to be the case for the NMR data reported for the sponge-derived material for kealiiquinone (**1**). Our data were generally a good match with that obtained by Ohta with the exception of two signals in the ^{13}C 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

Table 2
Comparative ^{13}C NMR data for natural and synthetic 2-deoxy-2-aminokealiiquinone and kealiiquinone and related compounds acquired at 125 MHz in DMSO-d_6



2-Deoxy-2-amino-kealiiquinone (2) | **Kealiiquinone (1') (as isolated)** | **R = OMe; Kealiiquinone (1) (synthetic)** **R = OH; 7'-Desmethyl kealiiquinone (11)** **R = H; 4'-Desmethoxy kealiiquinone (12)** | **2-Deoxykealiiquinone (10)**

2-Deoxy-2-aminokealiiquinone		Kealiiquinone			7′-Desmethyl kealiiquinone	4′-Desmethoxy kealiiquinone	2-Deoxykealiiquinone[a]
Synthetic	Natural	Synthetic (this work)	Natural	Synthetic (Ohta)			
182.0	182.0	181.3	182.4	181.3	181.9	181.9	182.3
181.5	181.4	181.1	181.8	181.1	181.7	181.5	182.0
158.6	158.5	158.5	159.0	158.5	157.2		159.1
157.9	157.8	154.8	158.3	154.8	155.3	154.2	148.9
147.9	147.8	147.8	148.2	147.8	148.4	147.6	147.4
147.5	147.4	145.2	147.8		145.6	146.0	146.5
145.6	145.5	134.0	146.0	134.0	134.4	135.3	136.8
137.5	137.5	132.7	137.9	132.6	133.1	134.1	130.6
130.7	130.6	129.9	131.1	129.9	130.3	131.6	130.2
130.2	130.2	127.7	130.6	127.7	127.0	129.1	128.8
129.0	129.0	126.4	129.4	126.50	126.4	128.3	128.3
				126.46		128.1	
123.7	123.6	123.5	124.1	123.5	124.5	127.8	122.8
122.5	122.5	122.6	122.9	122.6	123.2	124.3	114.5
112.9	112.8	113.9	113.3	113.9	115.8	123.0	113.8
105.1	105.1	104.5	105.5	104.6	104.9	105.5	108.8
60.7	60.6	60.8	61.0	60.8	61.2	61.5	61.4
60.6	60.6	60.8	61.0	60.8	61.2	61.4	61.3
55.0	55.0	55.0	55.4	55.0			55.3
28.8	28.7	26.8	29.2	26.8	27.3	27.4	31.7

[a] These data were acquired in $CDCl_3$.

Table 3
IC_{50} values of test compounds derived from MMT growth assays in MCF7 cells

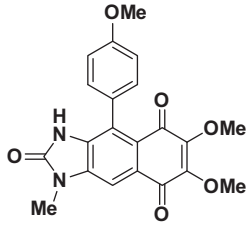
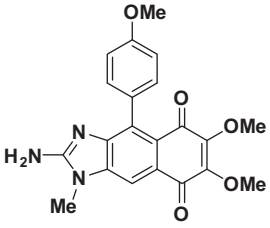
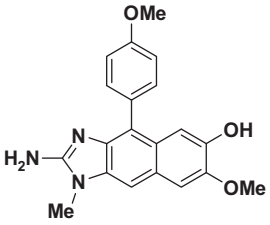
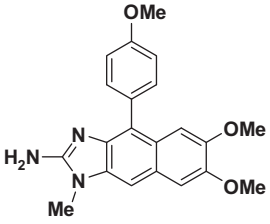
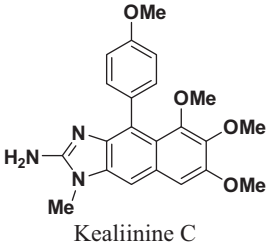
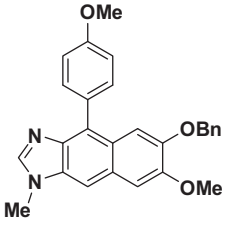
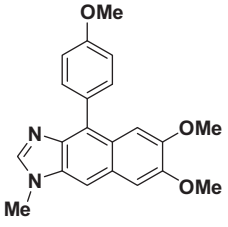
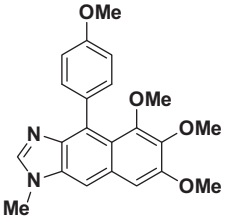
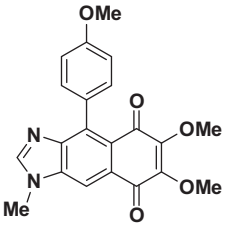
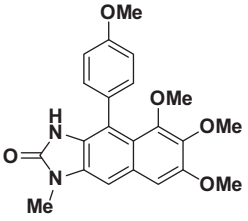
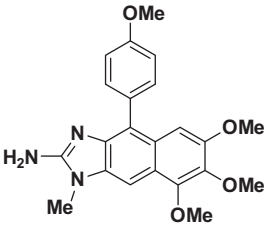
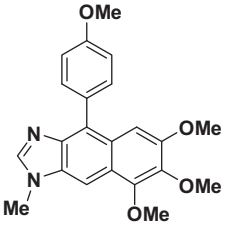
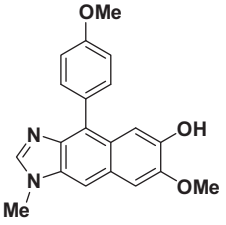
Compound	Structure	IC_{50} (μM)	SEM (μM)
1	 Kealiiquinone	91.9	±0.5
2	 2-Deoxy-2-aminokealiiquinone	43.8	±0.4
3	 Kealiinine A	23.4	±1.9
4	 Kealiinine B	28.8	±1.2
5	 Kealiinine C	>100	N/A
9a		92.0	±2.2
9b		28.0	±1.3

Table 3 (*continued*)

Compound	Structure	IC_{50} (μM)	SEM (μM)
9c		99.8	±0.9
10	 2-Deoxykealiiquinone	41.1	±0.1
13		29.9	±0.3
14	 Isokealiinine C	22.7	±1.0
15		68.2	±1.6
16		>100	N/A

appearance of a peak in the ^{1}H 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 ^{13}C NMR spectrum is characteristic for a 2-imidazolone.[22] Careful comparison of the ^{13}C 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 kealiiquinone group of molecules we have prepared two other kealiiquinone-like structures **11** and **12** via a completely different strategy involving an intramolecular Diels–Alder reaction and we find that the ^{13}C 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 (IC_{50}: 19.4 μM).[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 μM (Fig. 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.

Acknowledgments

This work was supported in part by the NIH (ES019129 (S.S.M.) and in part by GM065503 (C.J.L.)) and the Robert A. Welch Foundation (Y-1362 (C.J.L.)). Spectroscopic data were obtained on NMR instruments purchased through grants provided by the NSF (CHE-0234811 and CHE-0840509).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.093.

References and notes

1. Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2012**, *29*, 144.
2. Morris, J. C.; Phillips, A. J. *Nat. Prod. Rep.* **2011**, *28*, 269.
3. Koswatta, P. B.; Lovely, C. J. *Nat . Prod. Rep.* **2011**, *28*, 511.
4. Sullivan, J. D.; Giles, R. L.; Looper, R. E. *Curr. Bioact. Compd.* **2009**, *5*, 39.
5. Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193.
6. Aberle, N. S.; Lessene, G.; Watson, K. G. *Org. Lett.* **2006**, *8*, 419.
7. Aberle, N. S.; Catimel, J.; Nice, E. C.; Watson, K. G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3741.
8. Copp, B. R.; Fairchild, C. R.; Cornell, L.; Casazza, A. M.; Robinson, S.; Ireland, C. M. *J. Med. Chem.* **1998**, *41*, 3909.
9. LaBarbera, D. V.; Modzelewska, K.; Glazar, A. I.; Gray, P. D.; Kaur, M.; Liu, T.; Grossman, D.; Harper, M. K.; Kuwada, S. K.; Moghal, N.; Ireland, C. M. *Anticancer Drugs* **2009**, *20*, 425.
10. James, R. D.; Jones, D. A.; Aalbersberg, W.; Ireland, C. M. *Mol. Cancer Ther.* **2003**, *2*, 747.
11. Lovely, C. J., *Strategies and Tactics in Organic Synthesis*. Academic Press, 2012; Vol. 8, p 199.
12. Akee, R. K.; Carroll, T. R.; Yoshida, W. Y.; Scheuer, P. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 1944.
13. Nakamura, S.; Tsuno, N.; Yamashita, M.; Kawasaki, I.; Ohta, S.; Ohishi, Y. *J. Chem. Soc. Perkin Trans. 1* **2001**, 429.
14. Fu, X.; Barnes, J. R.; Do, T.; Schmitz, F. J. *J. Nat. Prod.* **1997**, *60*, 497.
15. Hassan, W.; Edrada, R.; Ebel, R.; Wray, V.; Berg, A.; Van Soest, R.; Wiryowidagdo, S.; Proksch, P. *J. Nat. Prod.* **2004**, *67*, 817.
16. Das, J.; Koswatta, P. B.; Yousufuddin, M.; Jones, J. D.; Lovely, C. J. *Org. Lett.* **2012**, *14*, 6210.
17. For an alternative approach to kealiinines B and C see: Gibbons, J. B.; Gligorich, K. M.; Welm, B. E.; Looper, R. E. *Org. Lett.* **2012**, *14*, 4734.
18. Kawasaki, I.; Taguchi, N.; Yamashita, M.; Ohta, S. *Chem. Pharm. Bull.* **1997**, *45*, 1393.
19. Lima, H. M.; Sivappa, R.; Yousufuddin, M.; Lovely, C. J. *Org. Lett.* **2012**, *14*, 2274.
20. Lipshutz, B. H.; Hagen, W. *Tetrahedron Lett.* **1992**, *33*, 5865.
21. Orita, H.; Shimizu, M.; Hayakawa, T.; Takehira, K. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1652.
22. Lima, H. M.; Lovely, C. J. *Org. Lett.* **2011**, *13*, 5736.
23. Lima, H. M. *Total Synthesis of Marine Derived Natural Products: Kealiiquinone and Ageliferin*; Ph.D. Dissertation, The University of Texas at Arlington: Arlington, 2011.
24. Dvorák, Z.; Štarha, P.; Šindelár, Z. k.; Trávnícek, Z. *Toxicol. in Vitro* **2012**, *26*, 480–484.