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Total synthesis of the linear and angular 3-methylated regioisomers of the marine natural product Kealiiquinone and biological evaluation of related *Leucetta* sp. alkaloids on human breast cancer

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Abstract

A straightforward, concise, and building block-scalable, seven-step total synthesis of the unnatural 3-methylated regioisomer analog of the marine alkaloid kealiiquinone is described. The construction by a regioselective way of the 4-anisyl-3-methylbenzimidazolone core of this analog, as well as the homologation of the aromatic π-system through a 4π ring opening/6π ring closure sequence for the 1,4-quinoid ring construction by using the squaric acid chemistry, are the key features of this procedure. In light that only one total synthesis of this 3-methyl-*iso*-kealiiquinone has been described by using a Friedel−Crafts reaction as the key step, herein, we present our pericyclic-based strategy which was successfully concluded with the aforementioned alkaloid synthesis. The biological evaluation of these unnatural derivatives was carried out on MDA-MB231 human cancer cell lines of breast. The *cis*-platin (CDDP) was used as a positive control of activity.

Keywords Total synthesis · Kealiiquinone · Structural analogs · Marine alkaloids · Marine sponges *Leucetta sp*

Introduction

Marine natural products (Kijjoa and Sawangwong 2004; Mayer et al. 2010; Martins et al. 2014; Jimeno et al. 2004; Ramadoss et al. 2016) are an excellent and diversified source of potentially active chemical compounds whose preliminary essays have been oriented to the new drug discovery. Specifically, those secondary metabolites coming from marine sponges (Bergmann and Stempien 1957; Henkel et al. 1999; He et al. 1992; Faulkner 1992) have received in recent years special attention due to the displayed promising biological activity (Shady et al. 2017). From the *Leucetta* sp. sponge, several of these secondary metabolites have been already isolated, synthesized, and essayed (Koswatta et al. 2017). A representative common feature in these alkaloids is the presence of the imidazole moiety in different oxidation states (Fig. 1).

To date, only one synthesis of **2** has been reported by the Ohta group (Nakamura et al. 2001). In this strategy, a Friedel−Crafts-type reaction was the key step for the central ring construction of the unnatural analog of kealiiquinone. Additionally, several redox fluctuations such as protection–deprotection−protection of functional groups (at least three of them) can be pointed as limitants, and finally an overall 15 steps were necessary to conclude the route. Considering these drawbacks, and the great relevance of the structural related derivatives (unnatural or analogs) of a naturally occurring compound (Lee et al. 2013; Smith et al. 2002; Zurwerra et al. 2010; Evans et al. 1998; Bonazzi et al. 2013; Chen et al. 2013), we decided to establish a different synthetic protocol for accessing to **2** (Scheme 1).

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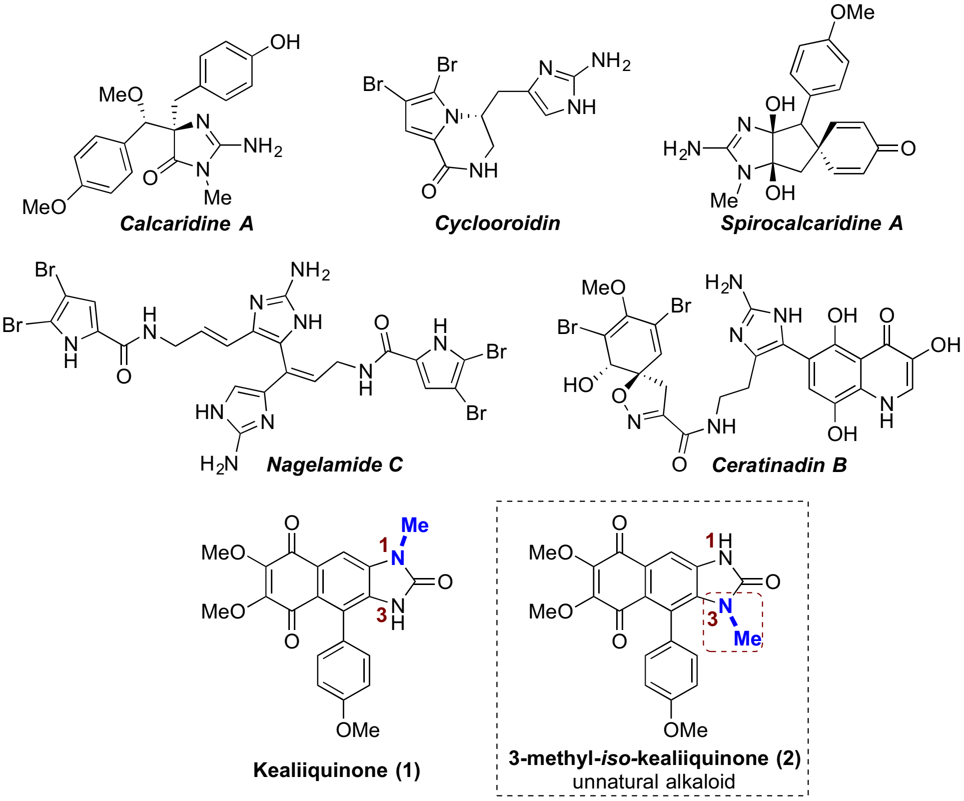


Fig. 1 Representative imidazole alkaloids isolated from *Leucetta* sp.

Materials and methods

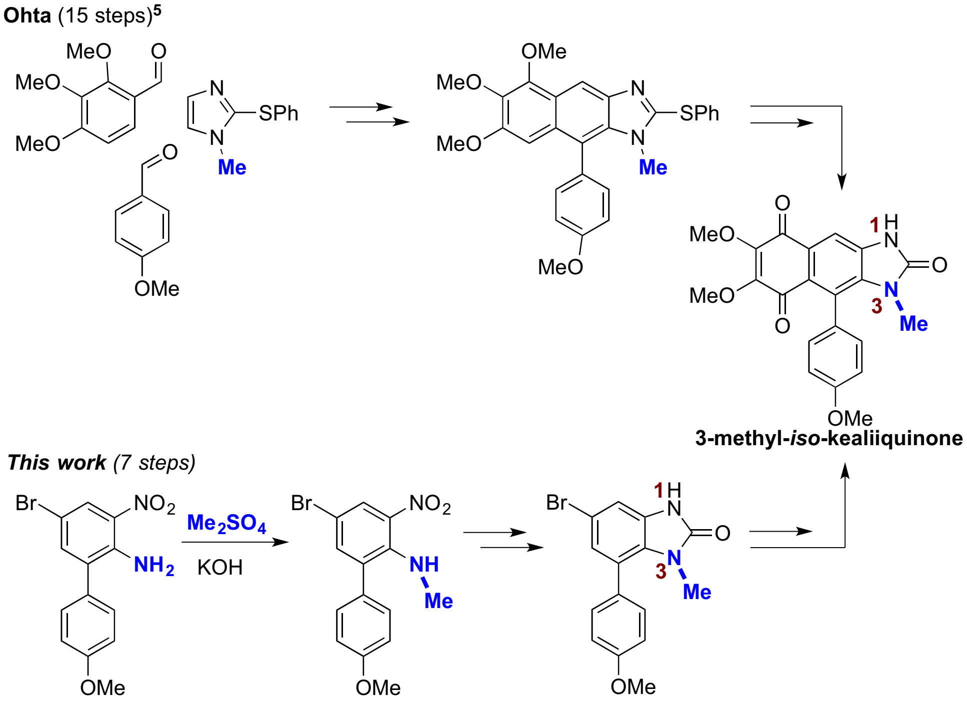
Chemical synthesis

General information

All moisture and oxygen-sensitive reactions were carried out in flame-dried round-bottom flasks under an inert atmosphere of nitrogen. Unless otherwise specified, all commercial materials were used as received without further purification. Anhydrous solvents were purchased from Sigma Aldrich in SureSeal® bottles. Column chromatography was performed using silica gel of size 100–200 and 230–400 mesh (Sigma Aldrich). Thin-layer chromatography was performed with TLC Silica gel 60 F256 plates, and visualization was affected with short-wavelength UV light (254 nm). Compounds were characterized using ^{1}H-NMR, ^{13}C-NMR. (Copies of ^{1}H-NMR and ^{13}C-NMR spectra are provided for all the compounds.) Data of known compounds were compared with existing literature characterization data and the references are given. ^{1}H and ^{13}C NMR spectra were recorded with 500-MHz and Bruker Advance 400-MHz instruments using deuterated solvents purchased from Sigma Aldrich like $CDCl_3$. ^{1}H spectra were referenced with tetramethyl silane (TMS, 0.0 ppm) or chloroform ($CDCl_3$, 7.26 ppm) and are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz), and integration. Chemical shifts of the ^{13}C NMR spectra were measured relative to $CDCl_3$ ($\delta = 77.16$ ppm). All the starting materials were synthesized according to reported procedures in the literature. High-resolution mass (HRMS) analysis was obtained using MAXIS IMPACT BRUKER. Chemical nomenclature was generated using Chemdraw. Infrared (IR) spectra were recorded using Perkin-Elmer system 2000 FT-IR spectrometer. Melting points of solids were measured using Fisher−Johns melting point apparatus.

Selective methylation on *N*-(5-bromo-4′-methoxy-3-nitro-[1,1′-biphenyl]-2-yl)amine

A dried 100-mL round-bottom flask was charged with *N*-(5-bromo-4′-methoxy-3-nitro-[1,1′-biphenyl]-2-yl)amine **3** (1.2 g, 3.738 mmol) dissolved in 25 mL of dry dimethylacetamide. To the reaction mixture, powdered KOH was added (0.523 g, 9.3457 mmol, 2.5 equiv) at 23 °C followed by the dropwise addition of dimethyl sulfate (0.55 mL, 5.6074 mmol, 1.5 equiv). The mixture was stirred at room temperature for 3 h and quenched by the addition of H_2O (20 mL). The crude was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and finally concentrated under reduced pressure. The resulting crude was purified by column chromatography (5% EtOAc/Hexane) to furnish **4a** (260 mg, 19%) as a pale yellowish solid, **4** (767 mg, 63%) as a yellowish solid.



Scheme 1 Strategies for accessing to 3-methyl-*iso*-kealiiquinone (**2**)

Spectral data for 5-bromo-4′-methoxy-*N,N*-dimethyl-3-nitro-[1,1′-biphenyl]-2-amine (4a)

The following compound was purified by 5% EtOAc/Hexane and the yield is 19% (260 mg) as a pale white solid. m.p. = 102–105 °C. $R_f = 0.4$ (20% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3078, 2963, 2939, 2839 (C−H, aromatic), 1649 (C−N), 1492, 1421 (C=C, aromatic). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.63 (s, 1H, C−H aromatic), 7.39 (s, 1H, C−H aromatic), 7.24 (d, $J = 8.0$ Hz, 2H, CH aromatic anisole), 6.90 (d, $J = 7.8$ Hz, 2H, CH aromatic anisole), 3.79 (s, 3H, OMe), 2.44 (s, 6H, NMe_2). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.5 (C−O), 146.7 (C−H aromatic), 143.6 (C−N aromatic), 142.0 (C−N aromatic), 137.9, 130.7, 129.6, 126.5, 114.3 (C−H, aromatics), 113.9 (C−Br), 55.5 (OMe), 42.1 (NMe). HRMS (ESI+): m/z calcd. for $C_{15}H_{16}BrN_2O_3$ $[M+H]^+ = 351.0344$, found 365.0502.

Regioselective synthesis of 5-bromo-4′-methoxy-*N*-methyl-3-nitro-[1,1′-biphenyl]-2-amine (4)

A dried 100-mL round-bottom flask was charged with *N*-(5-bromo-4′-methoxy-3-nitro-[1,1′-biphenyl]-2-yl)amine **3** (0.8 g, 2.4767 mmol) dissolved in 25 mL of dry dimethylacetamide. To the reaction mixture was added powdered KOH (0.277 g, 4.953 mmol, 2.0 equiv) at 23 °C followed by the dropwise addition of dimethyl sulfate (0.28 mL, 2.971 mmol, 1.2 equiv). The mixture was stirred at room temperature for 8 h and quenched by the addition of H_2O (20 mL). The crude was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and finally concentrated under reduced pressure. The resulting crude was purified by column chromatography (6% EtOAc/Hexane) to furnish **4** (705 mg, 85%) as a yellowish solid. m.p. 98–101 °C. $R_f = 0.45$ (20% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3257 (N−H), 2959, 2927, 2868 (C−H, aromatic), 1606 (C−N), 1495 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (s, 1H, C−H aromatic), 7.65 (s, 1H, N−H), 7.39 (s, 1H, C−H aromatic), 7.30 (d, $J = 7.9$ Hz, 2H, CH aromatic anisole), 6.95 (d, $J = 7.9$ Hz, 2H, CH aromatic anisole), 3.86 (s, 3H, OMe), 2.43 (s, 3H, NMe). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.5 (C−O), 145.4 (C–N aromatic), 141.3 (C−N), 136.4, 133.74, 131.5, 130.1, 127.8, 114.2 (C−H aromatics), 107.8 (C−Br), 55.5, 34.6. HRMS (ESI+): m/z calcd. for $C_{14}H_{13}BrN_2O_3Na$ $[M+Na]^+ = 361.0344$, found 361.0176.

5-Bromo-4′-methoxy-*N*^{2}-methyl-[1,1′-biphenyl]-2,3-diamine (5)

5-bromo-4′-methoxy-N-methyl-3-nitro-[1,1′-biphenyl]-2-amine **4** (1.9 g, 5.6547 mmol, 1 equiv) was charged in a 500-mL two-necked round-bottom flask and dissolved in MeOH (80 mL). To the resulting solution was added $NaBH_4$ (2.15 g, 56.547 mmol, 10 equiv) portionwise within 5 min at 0 °C and followed by $NiCl_2{\cdot}6H_2O$ (0.403 g, 1.6964 mmol, 0.3 equiv) that was added. The reaction mixture was stirred at 23 °C for 1 h. The reaction mixture was poured into ice-cold water. The resulting reaction mixture was extracted with EtOAc (200 mL) and water (200 mL). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography (15% EtOAc/Hexane) to furnish 5-bromo-4′-methoxy-N^2-methyl-[1,1′-biphenyl]-2,3-diamine **5** (1.56 g, 88%) of a yellowish solid. m.p. 59–61 °C. $R_f = 0.5$ (30% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3303, 3228 (N−H), 2954, 2915, 2850 (C−H, aromatic), 1608 (C−N), 1512, 1468 (C=C). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.24 (d, J = 7.8 Hz, 1H, C−H aromatic anisole), 6.97 (d, J = 7.8 Hz, 1H, C−H aromatic anisole), 6.84 (s, 1H, C−H aromatic), 6.75 (s, 1H, C−H aromatic), 3.95 (bs, 2H, NH amine), 3.86 (s, 3H, OMe), 2.58 (s, 3H, NMe). ^{13}C NMR (126 MHz, CDCl$_3$): δ 159.3 (C−O), 142.6 (C−N), 137.5 (C−N), 133.7, 131.1, 130.0, 122.9, 117.3 (C−H aromatics), 115.8 (C−Br), 114.3 (C−H aromatic), 55.5 (OMe), 34.7 (NMe). HRMS (ESI+): m/z calcd. for $C_{14}H_{16}BrN_2O$ $[M+H]^+$ = 307.0446, found 307.0455.

5-Bromo-7-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (6)

In a 250-mL two-necked round-bottom flask was added 5-bromo-4′-methoxy-N^2-methyl-[1,1′-biphenyl]-2,3-diamine **5** (1.50 g, 4.8859 mmol) and dissolved in 40 mL of DCE at room temperature. Then, a solution of phosgene 15 wt% in toluene (4.3 mL, 39.0879 mmol, 8.0 equiv) was added dropwise to the reaction mixture and stirred for 1 h. The reaction mixture was poured into ice-cold water (100 mL) and immediately a white solid was formed. The resulting solid was filtered-off and dried under vacuum to give **6** (1.6 g, 90%) as an off-white solid. The compound was used without purification for the next step. m.p. = 206–208 °C. $R_f = 0.3$ (40% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3368, 3102, 3006 (NH), 2979, 2961, 2941, 2833 (C−H aromatics), 1684 (C=O), 1610 (C−N), 1513, 1462 (C=C). ^{1}H NMR (500 MHz, CDCl$_3$): δ 10.77 (s, 1H, N−H), 7.25 (d, J = 8.3 Hz, 2H, C−H aromatic anisole), 7.23 (d, J = 1.1 Hz, 1H, C−H aromatic), 7.05 (d, J = 1.1 Hz, 1H, C−H aromatic), 6.95 (d, J = 8.4 Hz, 2H, C−H aromatic anisole), 3.86 (s, 3H, OMe), 2.98 (s, 3H, NMe). ^{13}C NMR (126 MHz, CDCl$_3$): δ 159.7 (C−O), 156.6 (C=O), 131.0, 129.8, 129.1, 127.4, 126.8, 126.4, 113.6, 113.6 (C−H aromatics), 111.7 (C−Br), 55.5 (OMe), 30.2 (NMe). HRMS (ESI+): m/z calcd. for $C_{15}H_{14.}BrN_2O_2$ $[M+H]^+$ = 333.0239, found 333.0225.

6-Bromo-1-(methoxymethyl)-4-(4-methoxyphenyl)-3-methyl-1,3-dihydro-2*H*-benzo-[*d*]imidazol-2-one (7)

A dried 100-mL two-necked round-bottom flask was charged with 6-bromo-4-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one **6** (0.630 g, 1.8973 mmol) and dissolved in 30 mL of dry dimethylacetamide. To the reaction mixture was added Cs_2CO_3 (3.08 g, 9.4879 mmol, 5.0 equiv), followed by addition of MOM-Cl (0.72 mL, 9.4865 mmol, 5.0 equiv) dropwise at room temperature and stirred at 50 °C for 12 h. The reaction mixture was quenched with H_2O and extracted with EtOAc (200 mL). The organic layers were collected and dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude was purified by column chromatography (27% EtOAc/Hexane) to afford the MOM-protected compound **7** (0.560 g, 77%) as a pale white solid. m.p. = 128–131 °C. R_f = 0.4 (50% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3390, 3000 (C−H aromatics), 2955, 2920, 2850 (C−H aliphatic), 1707 (C=O), 1615 (C−N), 1515, 1453 (C=C). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.29 (d, J = 1.9 Hz, 1H, C−H aromatic), 7.27 (d, J = 8.7 Hz, 2H, C−H aromatic anisole), 7.11 (d, J = 1.9 Hz, 1H, C−H aromatic), 6.96 (d, J = 8.6 Hz, 2H, C −H aromatic anisole), 5.29 (s, 2H, CH2), 3.87 (s, 3H, OMe anisole), 3.39 (s, 3H, OMe MOM), 2.97 (s, 3H, NMe). ^{13}C NMR (126 MHz, CDCl$_3$) δ 159.7 (C−O), 155.1 (C=O), 130.9 (C−H aromatic), 130.3 (C−N), 129.1 (C−N), 127.6, 126.7, 126.4, 113.7, 113.6 (C−H aromatics), 110.8 (C−Br), 72.9 (CH2), 56.6 (OMe anisol), 55.5 (OMe MOM), 30.5 (NMe). HRMS (ESI+): m/z calcd. for $C_{17}H_{18}BrN_2O_3[M+H]^+$ = 377.0501, found 377.0487.

Metal-halogen exchange and 1,2-addition of compound (8)

A flame-dried 250-mL two-necked round-bottom flask was charged with MOM-protected benzimidazolone **7** (0.37 g, 0.9840 mmol) dissolved in dry THF (40 mL) and stirred at −78 °C (acetone/dry ice bath). Afterwards, 1.6 M solution of *n*-BuLi in hexane (1.6 mL, 2.460 mmol, 2.5 equiv) was added dropwise. The clear solution was stirred at −78 °C temperature for an additional 15 min, then a solution of methyl squarate (0.1 g, 0.7021 mmol, 0.6 equiv) in dry THF (3 mL) was added dropwise to the reaction mixture, and the stirring was continued at −78 °C for 30 min. The reaction was quenched by addition of saturated NH_4Cl solution (10 mL) and stirred for 10 min and allowed to reach room temperature. The layers were separated, and the aqueous layer was extracted with EtOAc (100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give crude material. The resulting crude was purified by column chromatography (75% EtOAc/Hexane) to furnish the intermediate **8** (0.175 g, 40%) as a white sponge-like solid. m.p. = 71–74 °C. R_f = 0.3 (75% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3298 (O−H), 2995, 2948 (C−H aromatics), 2838 (C−H aliphatic), 1773 (C=O), 1686, 1632 (C−N), 1514, 1461 (C=C). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.37 (s, 1H, CH aromatic), 7.29 (d, J = 7.9 Hz, 2H, CH aromatic anisole), 7.08 (s, 1H, CH aromatic), 6.96 (d, J = 7.9 Hz, 2H, CH aromatic anisole), 5.32 (s, 2H, CH2), 4.09 (s, 3H, OMe cyclobutene), 4.01 (s, 3H, OMe anisole), 3.87 (s, 3H, OMe cyclobutene), 3.39 (s, 3H, OMe MOM), 2.98 (s, 3H, NMe). ^{13}C NMR (126 MHz,

$CDCl_3$): δ 183.9 (C=O cyclobutene), 165.9 (C−O), 159.6 (C=O), 155.5 (C−N), 135.7, 131.1, 130.9, 130.4, 130.1, 129.6, 127.7, 124.9, 122.6, 113.5 (C−H aromatics), 105.5 (C−O cyclobutene), 72.7 (CH2 MOM), 60.5 (OMe), 58.87 (OMe), 56.64 (OMe), 55.50 (OMe), 30.5 (NMe). HRMS (ESI+): m/z calculated for $C_{23}H_{25}N_2O_7[M+H]^+ =$ 441.1662, found 441.1638.

Thermolysis of 8

A flame-dried 20-mL glass vial was charged with the intermediate **8** (0.14 g). Without the addition of solvent, the vial was placed in a preheated oil bath for 1 h in which temperature was previously adjusted and fixed at 160 °C. After this period, the reaction mixture was removed from the hot bath, allowed to reach room temperature, and dissolved in DCM (20 mL). The DCM was evaporated under reduced pressure and the crude of reaction such obtained was purified by column chromatography (28% EtOAc/Hexane) to afford the angular compound **9** (12 mg, 8%) as a yellow solid and the linear isomer **10** (20 mg, 14%) as a yellow solid.

7,8-dimethoxy-3-(methoxymethyl)-4-(4-methoxyphenyl)-1-methyl-1*H*-naphtho[1,2-*d*]-imidazole-2,6,9(3*H*)-trione (9)

m.p. = 105–107 °C. $R_f = 0.5$ (50% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3000, 2952 (C−H aromatic), 2926, 2851 (C−H aliphatic), 1712 (C=O), 1664 (C=O), 1609 (C=O), 1514 (C−N), 1459 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (s, 1H, C−H aromatic), 7.27 (d, $J = 7.5$ Hz, 2H, C−H aromatic anisole), 6.99 (d, $J = 7.5$ Hz, 2H, C−H aromatic anisole), 5.86 (s, 2H, CH2 MOM), 4.09 (s, 3H, OMe quinone), 4.09 (s, 3H, OMe anisole), 3.88 (s, 3H, OMe quinone), 3.17 (s, 3H, OMe MOM), 3.02 (s, 3H, NMe). ^{13}C NMR (126 MHz, $CDCl_3$): δ 181.3 (C=O quinone), 181.3 (C=O quinone), 160.1 (C−O), 156.7 (C=O), 148.4 (C−OMe quinone), 146.2 (C−OMe quinone), 133.9 (C−N), 130.8, 128.8, 128.4, 127.4, 125.3, 124.8, 115.8, 113.9 (C−H aromatics), 75.6 (CH2 MOM), 61.4 (OMe quinone), 61.3 (OMe quinone), 56.3 (OMe), 55.5 (OMe), 31.3 (NMe). HRMS (ESI+): m/z calculated for $C_{23}H_{23}N_2O_7[M+H]^+ =$ 439.1505, found 439.1489.

6,7-dimethoxy-3-(methoxymethyl)-4-(4-methoxyphenyl)-1-methyl-1*H*-naphtho[2,3-*d*]-imidazole-2,5,8(3*H*)-trione (10)

m.p. = 77–79 °C. $R_f = 0.5$ (50% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3000, 2947 (C−H aromatic), 2837 (C–H aliphatic), 1717 (C=O), 1656 (C=O), 1622 (C=O), 1514 (C−N), 1455 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.93 (s, 1H, C−H aromatic), 7.15 (d, $J = 7.9$ Hz, 2H, C−H aromatic anisole), 6.98 (d, $J = 7.9$ Hz, 2H, C−H aromatic anisole), 5.37 (s, 2H, CH2 MOM), 4.05 (s, 3H, OMe quinone), 3.96 (s, 3H, OMe anisole), 3.88 (s, 3H, OMe quinone), 3.41 (s, 3H, OMe MOM), 2.77 (s, 3H, NMe). ^{13}C NMR (126 MHz, $CDCl_3$): δ 181.8 (C=O quinone), 181.7 (C=O quinone), 159.6 (C−O), 155.2 (C=O), 148.0 (C−OMe quinone), 145.6 (C−OMe quinone), 132.8 (C−N), 132.2, 130.4, 127.8, 127.5, 125.3, 124.5, 113.7, 106.5 (C−H aromatics), 72.9 (CH2 MOM), 61.4 (OMe quinone), 61.3 (OMe quinone), 56.9 (OMe), 55.4 (OMe), 30.1 (NMe). HRMS (ESI+): m/z calculated for $C_{23}H_{23}N_2O_7[M+H]^+ =$ 439.1505, found 439.1509.

3-Methyl-*iso*-kealiiquinone (Angular). 7,8-dimethoxy-4-(4-methoxyphenyl)-3-methyl-1*H*-naphtho[1,2-*d*]imidazole-2,6,9(3*H*)-trione (11)

A 25-mL two-necked round-bottom flask was charged with 7,8-dimethoxy-3-(methoxymethyl)-4-(4-methoxyphenyl)-3-methyl-1*H*-naphtho[1,2-*d*]imidazole-2,6,9(3 *H*)-trione **10** (8 mg, 0.01822 mmol) and was dissolved in trifluoroacetic acid (3 mL). The reaction mixture was stirred at 55 °C for 12 h and it was neutralized with 20 mL of sat.$NaHCO_3$ solution followed by extraction with EtOAc (30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by a crystallization method to afford an angular isomer of iso-kealiiquinone **11** (5 mg, 72%) as a pale-yellow solid. m.p. 190–193 °C. $R_f = 0.4$ (50% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3224 (N−H), 2998 (C−H aromatic), 2950, 2839 (C−H aliphatic), 1716 (C=O), 1622 (C=O), 1602 (C=O), 1515 (C−N), 1455 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.61 (s, 1H, N−H), 7.68 (s, 1H, C−H aromatic), 7.29 (d, $J = 7.4$ Hz, 1H, C−H aromatic anisole), 7.00 (d, $J = 7.5$ Hz, 1H, C−H aromatic anisole), 4.11 (s, 6H, 2OMe quinone, anisole), 3.88 (s, 3H, OMe quinone), 3.02 (s, 2H, NMe). ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.3 (C=O quinone), 181.2 (C=O quinone), 160.2 (C−O), 155.62 (C=O), 148.6 (C−OMe quinone), 147.3 (C−OMe quinone), 133.9 (C−N), 130.8, 128.8, 128.7, 128.3, 124.3, 123.3, 113.9, 110.7 (C−H aromatics), 61.8 (OMe), 61.7 (OMe), 55.5 (OMe), 30.3 (NMe). HRMS (ESI): m/z calcd. for $C_{21}H_{19}N_2O_6[M+H]^+ = 395.1243$, found 395.1240.

3-Methyl-*iso*-kealiiquinone (Linear). 6,7-dimethoxy-4-(4-methoxyphenyl)-3-methyl-1*H*-naphtho[2,3-*d*]imidazole-2,5,8(3*H*)-trione (2)

A 25-mL two-necked round-bottom flask was charged with 7,8-dimethoxy-3-(methoxymethyl)-4-(4-methoxyphenyl)-3-methyl-1*H*-naphtho[1,2-*d*]imidazole-2,6,9(3 *H*)-trione 9 (15 mg, 0.0341 mmol) and was dissolved in trifluoroacetic acid (6 mL). The reaction mixture was stirred at 55 °C for

48 h and it was neutralized with 20 mL of sat.$NaHCO_3$ solution followed by extraction with EtOAc (30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by a crystallization method to afford isokealiiquinone **2** (9.5 mg, 73%) as a yellow solid. m.p. 264–266 °C. $R_f = 0.4$ (50% EtOAc/Hexane). IR (neat, ν/cm^{-1}): 3511 (N−H), 2998, 2949 (C−H aromatic), 2840 (C−H aliphatic), 1716 (C=O), 1656 (C=O), 1622 (C=O), 1606 (C−N), 1515 (C=C). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 11.73 (s, 1H, N−H), 7.56 (s, 1H, C−H aromatic), 7.18 (d, $J = 7.9$ Hz, 2H, C−H aromatic anisole), 6.97 (d, $J = 7.8$ Hz, 2H, C−H aromatic anisole), 3.92 (s, 3H, OMe quinone), 3.83 (s, 3H, OMe anisole), 3.83 (s, 3H, OMe quinone), 2.57 (s, 3H, NMe). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 181.2 (C=O quinone), 181.0 (C=O quinone), 158.6 (C−O), 154.8 (C=O), 147.9 (C−OMe quinone), 145.2 (C−OMe quinone), 133.1 (C−N), 132.1, 130.6, 127.8, 126.3, 123.9, 122.9, 113.0, 105.6 (C−H aromatics), 60.9 (OMe), 60.8 (OMe), 55.0 (OMe), 28.9 (NMe). HRMS (ESI+): m/z calcd. for $C_{21}H_{19}N_2O_6[M+H]^+ = 395.1243$, found 395.1245.

Biological evaluation of activity

Experimental procedure for biological evaluation

Cell lines and culture conditions The human cancer cell lines of breast (MDA-MB231), obtained from ATCC (Manassas, VA, USA), were used in this study. Cells were grown at 37 °C and 5% CO_2 in RPMI with 7% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 pg/mL streptomycin).

MTT assay Cell lines were seeded at 5000 cells/well in 96-well microplates. After 24 h of incubation, cells were incubated with the positive control cisplatin (CDDP) or compounds at concentrations ranging from 0.1 to 100 μM. After 48 h of treatment, the MTT assay was performed following the protocol described by Jacobo-Salcedo. Optical density (O.D.) was recorded at 590 nm. IC_{50} values were calculated by linear regression and indicate the concentration that inhibits 50% of viability on the cell line.

Results and discussion

Retrosynthetic analysis

In our proposal, we designed a shorter, imidazole non-dependent and concise sequence that uses only one protecting group and was based upon our previous experience synthesizing the kealiiquinone (**1**) (Ramadoss et al. 2018a) and some of its structural analogs (Ramadoss et al. 2018b). Therefore, the synthetic strategy is outlined as follows (Scheme 2).

Synthesis of 3-methylated linear and angular analogs of Kealiiquinone

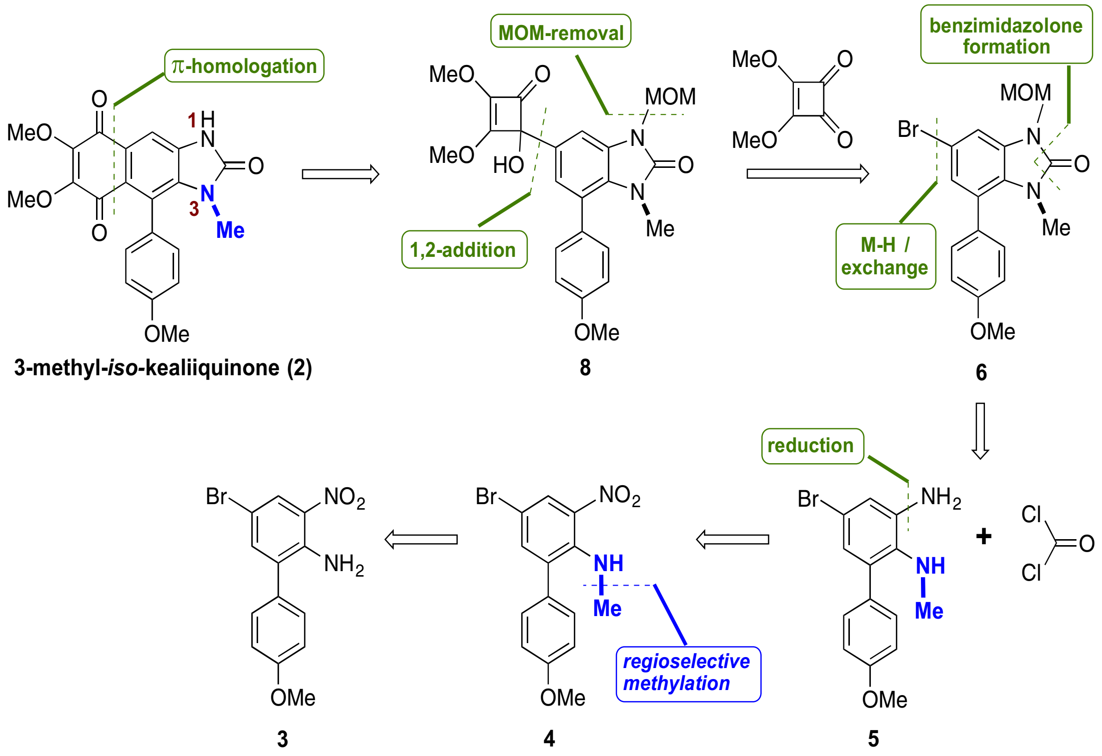
The 3-methyl-*iso*-kealiiquinone **2** was obtained from the tertiary alcohol **8** through the π-homologation of the aryl moiety after the acidic removal of the MOM group. The compound **8** was prepared from the 1,2-addition of the lithium anion generated in **6** over the dimethyl squarate. This regiodifferentiated 4-anisyl-3-methylbenzimidazolone was synthesized from **5** by the MOM-protecting group introduction after the phosgene treatment to construct the benzimidazolone ring. The functionalized *o*-phenylendiamine **5** is obtained from the *o*-nitroaniline **4** by nitro group reduction. Finally, **4** is synthesized by substrate-controlled alkylation of **3**. This step allowed us the introduction of the methyl group in the desired position to get the kealiiquinone derivative **2**. Even though in **3**, the only possible position to be alkylated is the nitrogen of the amino group, this resulted at the end of the route in its regioselective introduction.

According to the proposed synthetic protocol, we started with the methylation of compound **3** which has been previously synthesized (Ramadoss et al. 2018a; Ramadoss et al. 2018b). This resulted in a necessary short optimization (Table 1).

This methylation was carried out by adapting the previously described conditions for similar systems (Reddy et al. 2007). The use of 1.5 equivalents of dimethyl sulfate and 2.5 equivalents of potassium hydroxide yielded a mono- and bis-methylation mixture of **4** and **4a** in 63% and 19%, respectively (entry 1). With 2.3 base equivalents without change in the alkylating reagent, a dramatical ratio change to 70% and 8%, respectively, of **4** and **4a** was found (entry 2). Finally, by adjusting to 1.2 equivalents of the methylating reagent and 2.0 equivalents of base, we only observed that the desired mono-methylation product **4** was obtained in 85% (entry 3).

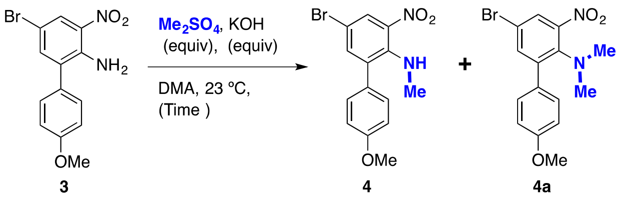
Once the optimization of this first step was successfully achieved, we continued with the rest of the route (Scheme 3).

This seven-step total synthesis started with the methylation of **3** under our optimized conditions. Thus, this first step consisted of the *N*-methylation of **3** producing **4** in 85%. The nitro group reduction under nickel catalysis (Hassanloie et al. 2014; Back et al. 1992) gave rise to the 1,2-diamine **5** in 88% for completing the second step. Next, the phosgene addition produced the benzimidazole ring formation yielding **6** in 90%. The following regioselective MOM protection at the first nitrogen position afforded **7** in 77%, leading to the completion of the fourth step. The next short sequence is a two-step one-pot reaction. Therefore, the metal-halogen exchange on **7** produced the intermediate **7a**



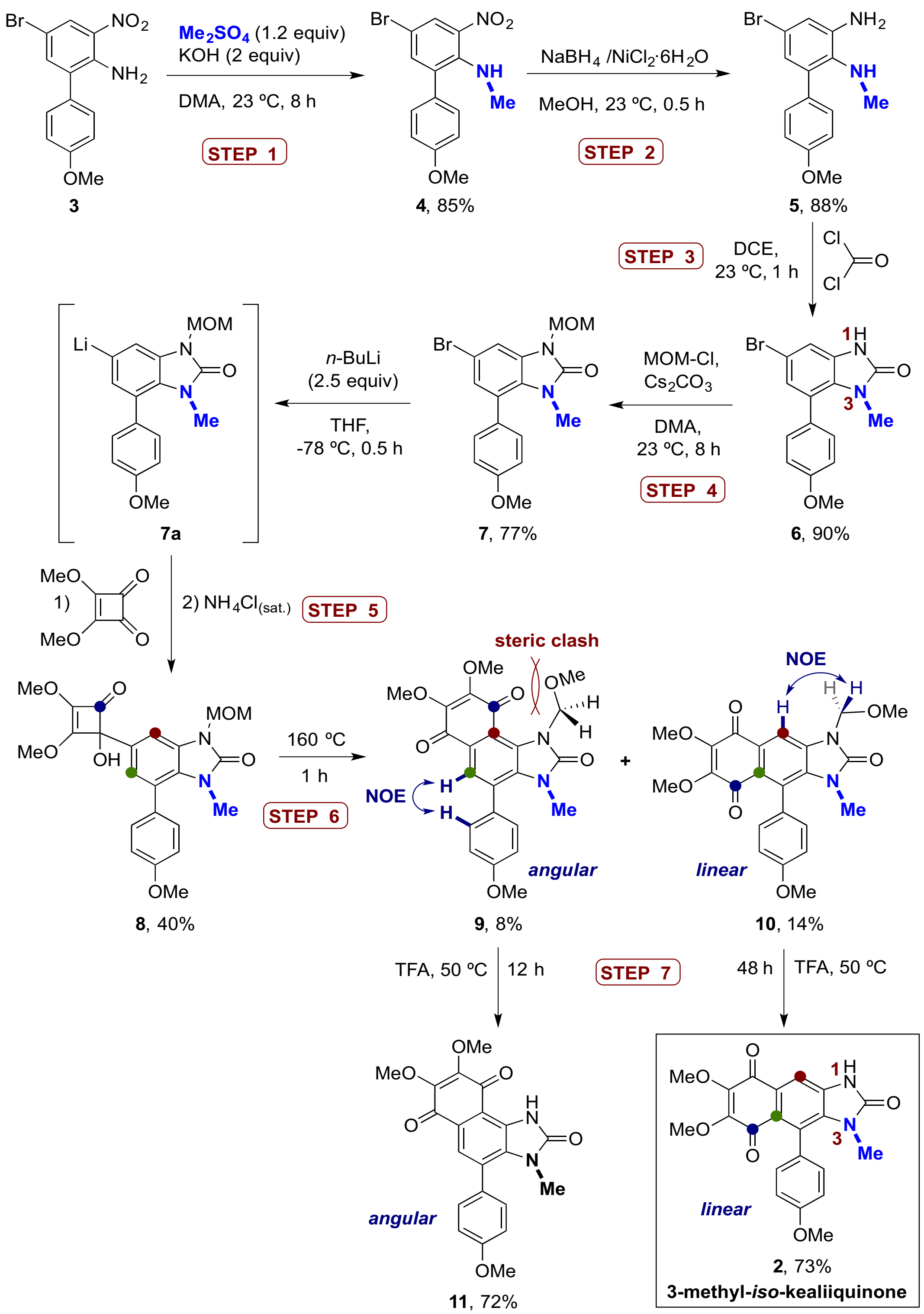
Scheme 2 Retrosynthetic analysis of 3-methyl-*iso*-kealiiquinone

Table 1 Optimization of the mono-methylation of **3**



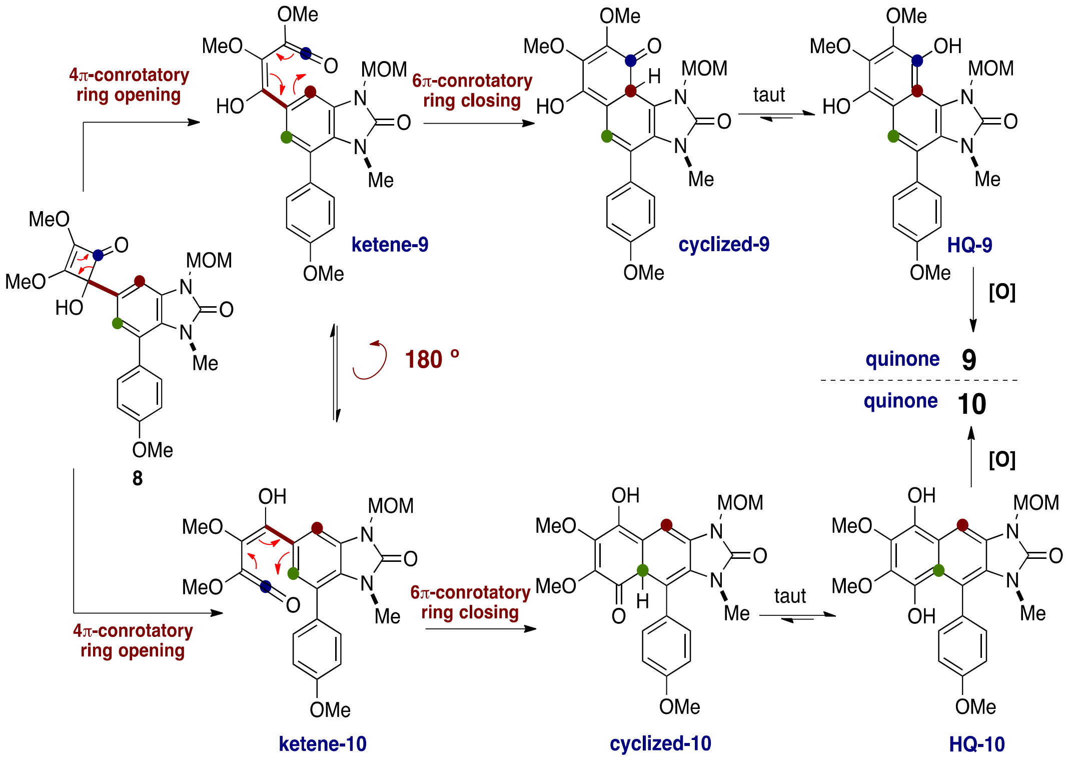
Entry	Me_2SO_4 (equiv)	KOH (equiv)	Time (h)	Yield (%) 4 + 4a
1	1.5	2.5	3	63 + 19
2	1.5	2.3	5	70 + 8
3	1.2	2.0	8	88

which reacted in situ with dimethyl squarate to give the tertiary alcohol **8** in modest 40% of yield after acid quench. From this key intermediate, the homologation of the π-system at the aromatic moiety took place. In such a way, **8** was thermolyzed at 160 °C along 1 h. In consequence the 1,4-quinoid ring was formed by the sequence 4π ring opening/6π ring closing. This pericyclic transformation produced two different regiosomers, **9** and **10** in 8% and 14%, respectively. These isomers were perfectly separated and characterized. For convenience, they are mentioned as angular (dark blue-red connection) and linear (dark blue-green connection) isomers. Their regiochemistry was unequivocally assigned by NOESY experimentation (see SI for full details). Finally, the last synthetic seventh step was completed by the MOM-group removal using TFA as the solvent at 50 °C. This deprotection condition afforded the angular quinoid derivative **11** in 72% of yield and the desired 3-methyl-*iso*-kealiiquinone analog **2** in 73%. It is worth to mention that compound **11** is another angular 3-methyl-*iso*-kealiiquinone analog that presents a chemically interesting naphtho[1,2-*d*]imidazole core. To the best of our knowledge, this is the first time describing this class of structure.



Scheme 3 Synthesis of the unnatural alkaloid 3-methyl-*iso*-kealiiquinone (**2**)

At this point, it is important to discuss the following highlighting aspects: (1) Even though the obtained yields could appear low, they are the overall result of the sequence hydroquinone formation–oxidation to quinone (see Scheme 4). Then these yields are an average corresponding to a 28% and 37% for **9** and **10**, respectively, calculated in a linear manner, (2) we got almost a 1:2 yield ratio for **9** and **10**. This ratio that disfavors **9** can be explained mainly in terms of steric effects. Herein, it is necessary to consider that one of the carbonyl groups from the quinoid ring in **9** is very near to



Scheme 4 Mechanism of the reaction to get quinones **9** and **10**

the MOM group generating steric clash (see Scheme 3) which is reflected in the lowest yield of both regioisomers.

To finish the explanation of the completed synthesis, it is important to describe the operating mechanism in the π-homologation of the aryl system to get **9** and **10** (Scheme 4).

According to the previous reports for this chemistry (Liebeskind et al. 1988; Trost et al. 2003; Moore and Perri 1998; Perri and Moore 1987), the mechanism starts with the thermally promoted 4π-conrotatory ring opening of the cyclobutanone **8** to get the **ketene-9** and **ketene-10** which are rotamers (see the 180° spin in red bond) (Allen et al. 1995; Cerioni et al. 1996). These evolved by a 6π-conrotatory ring closure giving rise to the nonaromatic **cyclized-9** and **cyclized-10** intermediates. The following tautomerization led to the formation of aromatic hydroquinone **HQ-9** and hydroquinone **HQ-10**. Finally, the spontaneous air oxidation of these electron-rich naphthoimidazols gives rise to **quinones 9** and **10** which are the direct MOM-protected precursors of the desired unnatural derivatives.

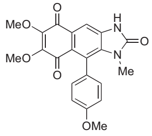
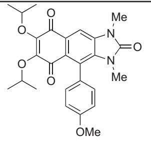
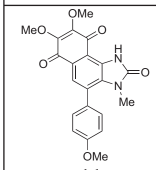
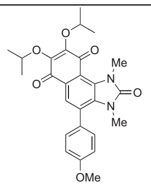
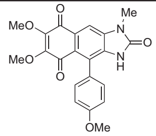
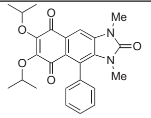
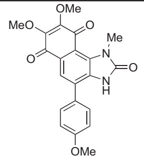
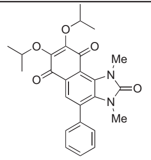
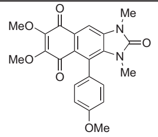
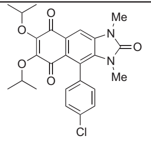
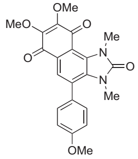
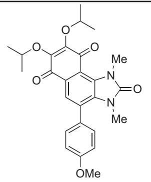
In this way, we were able to complete this total synthesis of the unnatural 3-methylated analog of marine imidazole alkaloid kealiiquinone (**1**). Regarding the regioselectivity on the methyl group position introduction, it is very worthy to mention that a significant synthetic challenge was addressed. To support this comment, it is enough to look at the references reported by Lovely group (Lima et al. 2012; 2014). In all of them, after several failures due to the

Table 2 Comparison of ^{1}H and ^{13}C NMR between the 3-methyl-*iso*-kealiiquinone reported by Ohta and this work

3-Methyl-*iso*-kealiiquinone (2)			
^{1}H NMR		^{13}C NMR	
Ohta	This work	Ohta	This work
3.33 (s, 3H)	2.57 (s,3H)	29.1	28.9
3.83 (s, 3H)	3.83 (s, 3H)	55.3	55.0
3.83 (s, 3H)	3.83 (s, 3H)	60.9	60.8
3.92 (s, 3H)	3.92 (s, 3H)	60.9	60.8
6.97 (d, 2H)	6.97 (d, 2H)	105.8	105.8
7.19 (d, 2H)	7.18 (d, 2H)	113.2	113.0
7.55 (s, 1H)	7.56 (s, 1H)	123.1	122.9
11.73 (s, 1H)	11.73 (s, 1H)	124.1	123.9
		126.5	126.3
		127.9	127.8
		130.8	130.6
		132.2	132.1
		133.3	133.1
		145.4	145.2
		148.1	147.9
		155.0	154.8
		158.8	158.6
		181.1	181.0
		181.3	181.2

The spectra were obtained in DMSO-d_6

Table 3 Biological evaluation of compounds **2** and **11** as well as some structurally related derivatives

Compound	MDA-MB231 IC_{50} (μM)	HaCaT IC_{50} (μM)	Compound	MDA-MB231 IC_{50} (μM)	HaCaT IC_{50} (μM)
 **2**	143.2 ± 17.8	> 100	 **15**	153.4 ± 19.2	> 100
 **11**	> 200	> 100	 **16**	> 200	> 100
 **1**	177.12 ± 23.8	> 100	 **17**	142.5 ± 17.7	> 100
 **12**	> 200	> 100	 18	> 200	> 100
 **13**	171.8 ± 20.9	> 100	 19	> 200	> 100
 **14**	> 200	> 100	 **20**	> 200	> 100
CDDP	6.7 ± 1.2	2.6 ± 0.3			

difficulty for the regioselective introduction of the methyl group at the first nitrogen position, some analogous derivatives of **1** were obtained. After some experimentation, it was progressively found the right way for the regioselective introduction of such methyl group in the synthetic route of the kealiiquinone. Also, Ohta (Nakamura et al. 2001) found this challenge in the introduction of such methyl group in the synthesis of kealiiquinone and the regioisomer of kealiiquinone synthesis which was summarized in a long synthetic route, including several protecting groups. Compared with these admirable efforts, it is relevant to mention that our procedure to the 3-methyl-*iso*-kealiiquinone analog contains a similar synthetic challenge that was a developed shorter route.

After the successful completion of this total synthesis, we compared the characterization of our synthetic alkaloid with those reported by Ohta (Nakamura et al. 2001) (Table 2).

We found an excellent correlation in the ^{1}H and ^{13}C spectra obtained by the Ohta (Nakamura et al. 2001) group and our results. There is only one signal not matching in the

^{1}H spectrum. While it seems that such signal in the Ohta's work was missing, certainly, the NOESY and the rest of the characterization we carried out undoubtedly confirmed the completion of the 3-methyl-*iso*-kealiiquinone (**2**) by our laboratory.

Biological evaluation of activity

Finally, to complete this work, the biological evaluation of the synthesized linear and angular 3-methylated analogs of kealiiquinone was carried out on MDA-MB231 human cancer cell lines of breast. Some structural analogs (**12**–**20**) containing the naphtho[1,2- or 2,3-*d*]imidazole alkaloid core of the *Leucetta* sp. sponge, which were previously obtained (Ramadoss et al. 2018a; Ramadoss et al. 2018b) in our group by chemical synthesis were also tested on this cell line, including the kealiiquinone (**1**). It is important to mention that all of these alkaloids have not been previously assayed to determine the activity in breast cancer (Table 3).

The 3-metyl-*iso*-kealiiquinone **2**, as well as the linear analogs **13**, **15**, and **17** showed modest biological activity ranging from 142 to 171 μM. It is worth mentioning that the unnatural derivative **2** showed slightly higher activity than the natural kealiiquinone (**1**). The rest of alkaloid derivatives did not exhibit any promising results. Interestingly, the linear derivatives showed in general higher activity in comparison with angular compounds.

Conclusion

In summary, we developed an imidazole nondependent, straightforward, and concise total synthesis of the unnatural linear and angular 3-methylated regioisomer analog of kealiiquinone. The route was completed in seven steps by using a regioselective substrate-controlled methyl group introduction as one of the key and significative transformations. The use of phosgene allowed us the benzimidazolone ring formation, while the squaric acid chemistry led to the formation of the 1,4-quinoid ring by the π-homologation of the aryl system in the molecule. The ^{1}H, ^{13}C, and mainly NOESY spectra, unequivocally confirmed, we obtained the 3-methyl-*iso*-kealiiquinone (**2**). The biological evaluation of these derivatives showed modest biological activity for a linear compound, while the angular derivative did not show a promising result.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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