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Isolation and Structure Determination of an Imidazo-pyrimidine, 5-Chlorocavernicolin, Maleimide oximes and Nucleosides from a Marine Sponge Extract

Roshan R. Kulkarni[1], Jang Hoon Kim[1], Young Ho Kim[1], Sangtaek Oh[2], and MinKyun Na[1]*

[1]College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
[2]Department of Bio and Fermentation Convergence Technology, Kookmin University, Seoul 136-702, Korea

Abstract – In a continuation of our studies to discover bioactive secondary metabolites from marine sources, we further investigated samples from a tryptamine and phenyl-alkane producing sponge, which resulted in the isolation of four uncommon small molecules and five nucleosides. Their structures were determined to be 7,8-dihydroimidazo[1,5-*c*]pyrimidin-5(6*H*)-one (**1**), 5-chlorocavernicolin (**2**), maleimide-5-oxime (**3**), 3-methylmaleimide-5-oxime (**4**), uridine (**5**), 2'-deoxyuridine (**6**), thymidine (**7**), adenine (**8**), and adenosine (**9**) by spectroscopic analyses. The isolated compounds were evaluated for inhibitory activity against soluble epoxide hydrolase (sEH) as well as the Wnt/β-catenine signaling pathway.
Keywords – Imidazo-pyrimidine, 5-Chlorocavernicolin, Maleimide-5-oxime, Nucleoside, Soluble epoxide hydrolase, Wnt/β-catenine signaling

Introduction

The oceans represent a vast untapped source of natural products with astonishing structural diversity and diverse biological properties.[1-4] A look at recent drug trials reveals contributions marine organisms have provided as leads for drug development in several disease areas.[5] It has been appreciated that marine invertebrates produce chemical structures with the potential to be developed into drug candidates.[2] Of particular note are the relatively simple natural products spongothymidine and spongouridine originally isolated from the sponge *Tethya crypta*, and later developed into cytarabine and vidarabine as anticancer and antiviral agents, respectively.[4] Our previous work on marine invertebrates of phylum Porifera viz. *Verongula rigida*, *Smenospongia aurea*, and *Smenospongia cerebriformis* bears testimony to the above observations. New tryptamine derivatives with antidepressant activity,[6] a new biological property for this scaffold were isolated, so as a novel phenyl-alkene representing a new class of source material with cytotoxicity against the HL-60 human leukemia cancer cell lines.[7]

Recently, there has been an increase in the discovery of new and clinically relevant therapeutic drug targets.[8] This fact coupled with a yet to be fully explored chemical diversity of ocean inhabitants presents a unique and exciting area of drug discovery. Encouraged by our previous findings and motivated by the prospects, we sought to further investigate sponge extracts remaining from the previous study. Indeed, this work led to the isolation of nine metabolites of which the structures of four compounds were not common. The structures were identified as 7,8-dihydroimidazo[1,5-*c*]pyrimidin-5(6*H*)-one (**1**), 5-chlorocavernicolin (**2**), maleimide derivatives maleimide-5-oxime (**3**) and 3-methyl-maleimide-5-oxime (**4**), nucleosides uridine (**5**), 2'-deoxyuridine (**6**), thymidine (**7**), purine adenine (**8**) and adenosine (**9**). Compound 2 belongs to the class of compounds called cavernicolins, which are bicyclic compounds derived from halogenated tyrosine derivatives and are mainly reported from the genus *Aplysina*.[16] The maleimide-5-oxime is interesting as it has also recently been isolated from terrestrial sources.[18]

In recent years, stabilization of the endogenous anti-inflammatory mediators, epoxyeicosatrienoic acids, through the inhibition of enzyme soluble epoxide hydrolase (sEH) has gained importance as a strategy in the management of pain,[9] inflammation,[10] as well as co-morbidities associated with diabetes.[11] The structural features necessary for an inhibitor to show activity have been well characterized,

*Author for correspondence
Prof. MinKyun Na, College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Tel: +82-42-821-5925; E-mail: mkna@cnu.ac.kr

and it is recognized that a hydrogen bond acceptor such as a carbonyl group and a hydrogen bond donor such as an amine group are simultaneously necessary for activity.[11] Except for compounds 8 and 9, all of the isolated compounds from the sponge extracts displayed these structural features; hence, the isolated compounds were evaluated for their inhibition of sEH.

The Wnt/β-catenine pathway is increasingly recognized as being upregulated in various cancers, and mediators that downregulate this pathway have the potential to be used as anticancer agents.[12,13] As none of the isolated compounds have thus far been described as downregulators of the Wnt/β-catenine pathway, they were also subjected to the Wnt/β-catenine assay system for evaluation.

Herein, we report details on the isolation and structure determination of nine compounds and the evaluation of the isolated compounds on sEH inhibition and Wnt/β-catenine expression.

Experimental

General experimental procedures – Vacuum liquid chromatography (VLC) was performed using silica gel (Merck, 70 - 230 mesh). Medium pressure liquid chromatography (MPLC) was carried out with BiotageIsolera™ and reversed phase C_{18} SNAP Cartridges KP-C18-HS (120 g and 340 g, Biotage). Preparative reversed phase HPLC (prep HPLC) separation was carried out on a YMC C_{18} column (250 × 20.0 mm, 5 μm) at a flow rate of 5 mL/min. ^{1}H (600 MHz, 400 MHz, 300 MHz), ^{13}C (150 MHz, 100 MHz, 75 MHz) NMR spectra were recorded on a Bruker Avance Ultrashield spectrometers and 2D NMR data (COSY, HSQC, HMBC, NOESY) was recorded on a Bruker Avance Ultrashield (600 MHz) spectrometer equipped with a 5 mm direct detection PFG probe. Prep HPLC was carried out using HPLC grade solvents (Merck, LTD), while the solvents used for VLC and MPLC separation were distilled prior to use. For the sEH bioassay, sEH and PHOME were purchased from Cayman (A61510, A60932) respectively. Bruker SMART APEX CCD diffractometer using Mo Kα radiation was used for single crystal X-ray crystallography.

Isolation of compounds – The collection and extraction of sponges and their extraction has been described in our earlier work.[6,7] The dried ethanol extract (3.6 kg) was subjected to silica gel vacuum liquid chromatography (VLC) eluting with a stepwise gradient of hexane/acetone/methanol/water to give 13 fractions (Fr. 1 - 13). Fraction 8 (2.1 g) was further separated into 13 fractions (Fr. 8 - 1 to 8 - 13) using MPLC with a gradient of MeOH from 80 to 100% at a flow rate of 40 mL/min. Fraction 8 - 1 (480.0 mg) was separated by repetitive prep HPLC cycles using a gradient of methanol in water from 0 to 50% over 60 min to yield pure compounds **3** (14.5 mg, t_R = 23.3 min,), **4** (1.9 mg, t_R = 29.7 min,), **2** (0.8 mg, t_R = 37.8 min,), and mixtures of compounds **5** with **6** (t_R = 33.4 min), and compounds **1** with **7** (t_R = 41.1 min). The mixture of **5** and **6** was subjected to further repetitive prep HPLC using a gradient of methanol in water from 0 to 30% over 60 min to isolate pure compounds **5** (16.3 mg, t_R = 39.0 min) and **6** (2.5 mg, t_R = 39.8 min). The mixture of compounds **1** and **7** was separated by prep HPLC using 20% MeOH: H_2O as mobile phase to isolate **7** (9.5 mg, t_R = 23.8 min) and **1** (5.2 mg, t_R = 25.0 min). The combined fractions 9, 10, 12, and 13 from the VLC (19.1 g) were further subdivided into 16 fractions using MPLC with a MeOH gradient from 10 to 100% at a flow rate of 65 mL/min. Fraction 5 from the above chromatography (220.5 mg) was separated by repetitive cycles of prep HPLC using a gradient of methanol in water from 0 to 50% over 60 min to yield pure compounds **8** (5.6 mg, t_R = 32.3 min) and **9** (4.0 mg, t_R = 42.3 min).

7,8-Dihydroimidazo[1,5-*c*]pyrimidin-5(6*H*)-one (1) – Colorless crystals. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 8.60 (1H, s, H-8), 7.02 (1H, s, H-6), 3.77 (2H, t, J = 6.4 Hz, H_2-3), 2.77 (2H, t, J = 6.4 Hz, H_2-4); ^{13}C NMR (pyridine-d_5, 75 MHz): δ 149.9 (C-1), 135.3 (C-8), 128.0 (C-6), 126.0 (C-5), 39.3 (C-3), 20.4 (C-4).

Crystals of compound **1** were grown by slow evaporation of a methanol solution. A white crystal of approximate size 0.16 × 0.14 × 0.04 mm^3 was used for data collection on a Bruker SMART APEX CCD diffractometer using Mo Kα radiation. Exposure/frame = 16.0 s/frame, crystals belong to the monoclinic space group P 21/n, a = 7.3963 (9) Å, b = 11.4988 (14) Å, c = 7.7103 (10) Å, V = 617.02 (13) Å^3, Z = 3, Dc = 1.220 g/cm^3, μ(Mo Kα) = 0.71073 Å, T = 296 K, 5365 reflections measured, R value 0.0392, wR^2 = 0.0902. The unit cell parameters were in agreement with those reported in the literature.[14]

5-Chlorocavernicolin (2) – Colorless gummy mass. ^{1}H NMR (pyridine-d_5, 600 MHz): δ 7.39 (1H, bs, H-4), 4.49 (1H, m, H-7a), 3.28 (1H, dd, J = 16.4, 2.8 Hz, H-7'), 3.16 (1H, d, J = 16.4 Hz, H-3'), 3.07 (1H, d, J = 16.4 Hz, H-3"), 3.03 (1H, dd, J = 16.4, 6.3 Hz, H-7"); ^{13}C NMR (pyridine-d_5, 150 MHz): δ 189.4 (C-6), 174.1 (C-2), 146.6 (C-4), 132.3 (C-5), 74.8 (C-3a), 60.7 (C-7a), 45.6 (C-3), 41.8 (C-7).

Maleimide-5-oxime (3) – White amorphous powder. ^{1}H NMR (D_2O, 300 MHz): δ 7.54 (1H, d, J = 7.7 Hz, H-4), 5.81 (1H, d, J = 7.7 Hz, H-3); ^{13}C NMR (D_2O, 75

MHz): δ 167.4 (C-2), 153.0 (C-5), 143.3 (C-4), 101.0 (C-3).

3-Methyl-maleimide-5-oxime (4) – White amorphous powder. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 7.28 (1H, s, H-4), 1.97 (3H, s, Me); ^{13}C NMR (pyridine-d_5, 75 MHz): δ 166.5 (C-2), 153.6 (C-5), 138.1 (C-4), 109.2 (C-3), 12.8 (Me).

Uridine (5) – White amorphous powder. ^{1}H NMR (pyridine-d_5, 600 MHz): δ 8.55 (1H, d, J = 8.1 Hz, H-3), 6.83 (1H, d, J = 4.0 Hz, H-1'), 5.81 (1H, d, J = 8.1 Hz, H-2), 4.91 (2H, dt, J = 9.2, 5.0 Hz, H-2', H-3'), 4.67 (1H, dt, J = 5.0, 2.5 Hz, H-4'), 4.32 (1H, dd, J = 12.1, 2.4 Hz, H-5'a), 4.21 (1H, dd, J = 12.1, 2.3 Hz, H-5'b); ^{13}C NMR (pyridine-d_5, 150 MHz): δ 164.8 (C-1), 152.5 (C-5), 141.4 (C-3), 90.6 (C-1'), 86.5 (C-4'), 76.4 (C-2'), 71.5 (C-3'), 62.0 (C-5').

2'-Deoxyuridine (6) – Colorless gum. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 4.49 (1H, m, H-4'), 4.21 (1H, d, J = 11.7 Hz, H-5'a), 4.14 (1H, d, J = 11.7 Hz, H-5'b), 2.70 (1H, m, H-2'a), 2.59 (1H, m, H-2'b); ^{13}C NMR (pyridine-d_5, 75 MHz): δ 142.0 (C-3), 102.2 (C-2), 86.7 (C-1'), 85.5 (C-4'), 70.5 (C-3'), 62.6 (C-5'), 38.6 (C-2').

Thymidine (7) – White amorphous powder. ^{1}H NMR (pyridine-d_5, 400 MHz): δ 8.17 (1H, s, H-3), 7.05 (1H, t, J = 6.7 Hz, H-1'), 4.49 (1H, q, J = 3.0 Hz, H-4'), 4.24 (1H, dd, J = 11.9, 2.9 Hz, H-5'a), 4.15 (1H, dd, J = 11.9, 2.8 Hz, H-5'b), 2.78-2.60 (2H, m, H-2'), 1.88 (3H, s, Me); ^{13}C NMR (pyridine-d_5, 100 MHz): δ 165.4 (C-1), 152.4 (C-5), 137.0 (C-3), 110.8 (C-2), 89.2 (C-1'), 85.6 (C-4'), 71.7 (C-3'), 62.6 (C-5'), 41.6 (C-2'), 13.0 (Me).

Adenine (8) – White amorphous powder. ^{1}H NMR (methanol-d_4, 600 MHz): δ 8.18 (1H, s, H-3), 8.11 (1H, s, H-7); ^{13}C NMR (methanol-d_4, 150 MHz): δ 156.8 (C-1), 153.7 (C-3), 151.2 (C-5), 141.3(C-7), 119.3 (C-9).

Adenosine (9) – White amorphous powder. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 8.63 (1H, s, H-3), 8.37 (1H, s, H-7), 6.73 (1H, d, J = 5.9 Hz, H-1'), 5.52 (1H, t, J = 5.4 Hz, H-2'), 5.08 (1H, dd, J = 4.8, 3.1 Hz, H-4'), 4.81- 4.74 (1H, m, H-3'), 4.32 (1H, dd, J = 12.1, 2.4 Hz, H-5'a), 4.21 (1H, dd, J = 12.1, 2.3 Hz, H-5'b); ^{13}C NMR (pyridine-d_5, 75 MHz): δ 156.8 (C-1), 153.1 (C-3), 151.2 (C-5), 140.4 (C-7), 119.3 (C-9), 90.7 (C-1'), 87.6 (C-4'), 75.3 (C-2'), 72.2 (C-3'), 62.8 (C-5').

sEH assay – Briefly, 50 μL of sEH (140 ng/mL) and 20 μL of different concentrations of the inhibitors dissolved in MeOH were mixed in a 96 white well plate containing 80 μL of 25 mM bis-Tris HCl buffer (pH 7.0) containing 0.1% BSA, and then 50 μL of 20 μM PHOME were added to each well as a substrate. The mixture was incubated at 37 ºC, and the products of hydrolysis were monitored at excitation and emission wavelengths of 330 and 465 nm after one hour.

$$\text{Enzyme activity (\%)} = [(S_{60} - S_2 / C_{60} - C_2] \times 100,$$

where C_{60} and S_{60} are the fluorescence of the control and inhibitor after 60 min, and S_2 and C_2 are the fluorescence of inhibitor and control after 2 min.

sEH kinetic assay – 50 μL of sEH and 20 μL of various concentrations of the inhibitors dissolved in MeOH were added to a 96 white well plate containing 80 μL of 25 mM bis-Tris HCl buffer (pH 7.0) containing 0.1% BSA, and then 50 μL of PHOME in concentrations ranging from 10 to 80 μM as a substrate were added to each well. After starting the enzyme reaction at 37 ºC, products of hydrolysis were monitored at excitation and emission wavelengths of 330 and 465 nm at ten min intervals.

Wnt/β-catenine inhibition – Compounds were evaluated for their downregulation of the Wnt/β-catenine pathway by the previously published protocol.[12,13] Briefly, to screen for small-molecule inhibitors of Wnt/β-catenin signaling, HEK293 cells were stably transfected with a TOPFlash reporter and the hFz-1 expression plasmid to make reporter cells. The HEK293 reporter cell line was then inoculated into 96-well plates at 15,000 cells/well in duplicate and grown for 24 h. Medium previously enriched with Wnt3a was then added, and the test compounds were added to the wells at a final concentration of 20 mM. After 15 h, the plates were assayed for firefly luciferase activity and cell viability.

Results and Discussion

Compound **1** was obtained as colorless crystals. The ^{1}H NMR spectrum showed signals for two singlet protons at δ_H 8.60 (1H, s, H-8) and 7.02 (1H, s, H-6) as well as two triplet protons at δ_H 2.77 (2H, t, J = 6.4 Hz, H-4) and 3.35 (2H, t, J = 6.4 Hz, H-3). The singlet protons at δ_H 8.60 and 7.02 indicated an imidazole ring. The ^{13}C NMR and DEPT spectra showed six resonances corresponding to two methylene, two methine, and two quaternary carbons (Fig. S1, see supplementary data). The two methylene groups at δ_C 20.4 and 39.3 indicated an aliphatic chain terminated by a heteroatom. To assign the structure, 2D NMR data (Fig. S2, see supplementary data) were recorded that showed an HMBC correlation of δ_H 3.35 and δ_H 8.60 with δ_C 149.9. The upfield shifted quaternary carbon at δ_C 149.9 indicated a carbonyl group attached to two nitrogen atoms. However in the absence of any other HMBC correlations with the later posed an ambiguity in the structure determination. The observed 1D and 2D

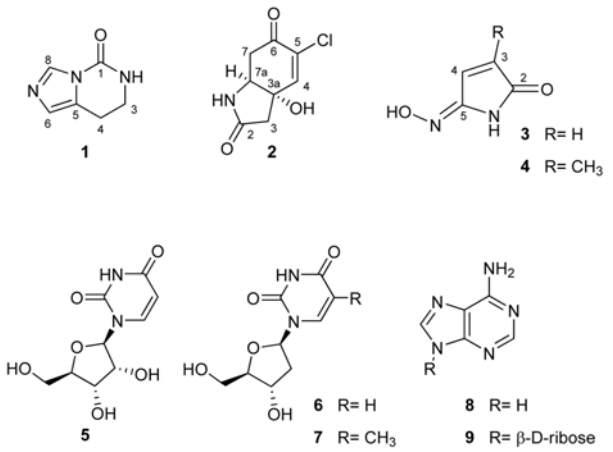

Fig. 1. The structures of isolated compounds.

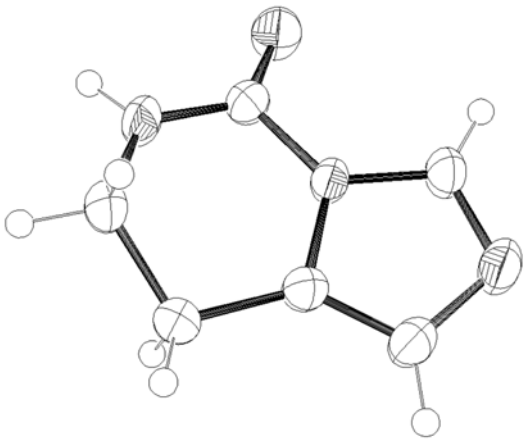

Fig. 2. ORTEP diagram of compound **1**. Ellipsoids are drawn at 50% probability.

NMR data suggested compound **1** to be an imidazole derivative fused with a pyrimidine. Hence, to confirm the structure, the compound was crystalized in methanol and was subjected to single crystal x-ray analysis (Fig. 2) that revealed compound **1** to be 7,8-dihydroimidazo[1,5-*c*] pyrimidin-5(6*H*)-one. The crystal unit cell parameters were in agreement with literature values.[14] The compound is known synthetically[14] and has been isolated from Verongida sponges.[15] Compound **1** is used as an intermediate in the synthesis of histamine analogues, but has not been reported to possess any biological activity.

Compound **2** was obtained as a colorless, gummy mass. The ^{1}H NMR spectrum showed signals for a highly downfield shifted proton at δ_H 9.4 (1H, bs, N*H*), a singlet proton at δ_H 7.39 (1H, s, H-4), a proton at δ_H 4.49 (1H, m, H-7a) as well as signals corresponding to two methylene groups at δ_H 3.03 (1H, dd, J = 16.4, 6.3, H_2-7a), 3.28 (1H, dd, J = 16.4, 4.8, H_2-7b), 3.07 (1H, d, J = 16.4, H_2-3a), and 3.16 (1H, d, J = 16.4, H_2-3b). The ^{13}C NMR and DEPT spectra showed eight resonances corresponding to two methylene, two methine, two heteroatom substituted quaternary carbons at δ_C 60.7 and 74.8 as well as two carbonyl carbons at δ_C 174.1 and 189.4 (Fig. S3, see supplementary data). The carbonyl carbon at δ_C 189.4 and an olefinic group at δ_C 132.3 and 146.6 (δ_H 7.39) hinted towards an α,β-unsaturated carbonyl moiety. The downfield shifted quaternary olefinic carbon at δ_C 132.3 indicated the presence of a halogen atom. The above 1D NMR observations suggested compound **2** being 5-chlorocavernicolin. The structure was further elucidated using COSY, HSQC and HMBC analyses (Fig. 3, Fig. S4, see supplementary data) as follows: the methylene protons at δ_H 3.03 and 3.28 (H_2-7, δ_C 41.8) showed COSY correlations with a methine proton at δ_H 4.49 (H-7a, δ_C 60.7) identifying the later as position 7a. The olefinic methine proton at δ_H 7.39 (H-4, δ_C 146.6) showed three bond correlations with the methine carbon at δ_C 60.7 and a carbonyl carbon at δ_C 189.4 identifying the later as position 6. Additionally, an NH proton at δ_H 9.4 showed a correlation with a quaternary carbon at δ_C 74.8 (C-3a). The methylene protons at δ_H 3.07 and 3.16 (H_2-3) showed two bond correlations with a carbonyl carbon at δ_C 174.1 (C-2) and the quaternary carbon at 74.8 (C-3a) as well as a three bond correlation with the olefinic methine δ_C 146.6 (C-4). Thus, from detailed analysis of the 2D NMR spectra, compound **2** was identified as 5-chlorocavernicolin.[16] Cavernicolins are rare in nature and thus far have not shown any biological activity.

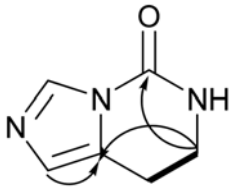

Fig. 3. Key HMBC (↷) and COSY (▬) correlations for compound **1**.

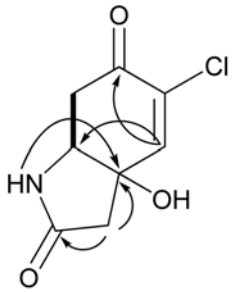

Fig. 4. Key HMBC (↷) and COSY (▬) correlations for compound **2**.

Compound **3** was isolated as a white amorphous powder. The quaternary carbons at δ_C 167.4 and 153.0 indicated a maleimide structure. Together with *ortho* substituted protons at δ_H 5.81 (1H, d, J = 7.7, H-3) and 7.54 (1H, d, J = 7.7, H-4) compound **3** was identified as maleimide-5-oxime.[17] Compound **3** has also been described in the *Zingiber officinale*.[18] Compound **4** was isolated as a white amorphous powder. The quaternary carbons at δ_C 166.5 and 153.6 indicated a maleimide structure similar to compound **3**. In compound **4**, the *ortho* substituted ^{1}H NMR features collapsed into a singlet at δ_H 7.28. With a singlet methyl proton at δ_H 1.97 (δ_C 12.8), compound **4** was identified as 3-methylmaleimide-5-oxime.[19] The maleimide oximes, although simple compounds are relatively rare, and compounds **3** and **4** have been evaluated for antifungal and anticancer activities.[17,19] These compounds, however, did not show any significant inhibition in the current assays. Isolated nucleosides were identified as uridine (**5**), 2'-deoxyuridine (**6**), thymidine (**7**), adenine (**8**), and adenosine (**9**).

As mentioned above, all of the compounds except **8** and **9** had structural features of hydrogen bond acceptor, a carbonyl group and a hydrogen bond donor, an amine group that suggested their potential as sEH inhibitors; thus, they were evaluated in the sEH assay. However, contrary to our hypothesis, none of the isolated compounds inhibited the sEH enzyme even at a higher concentration of 60 μM. The absence of the activity can be explained by the absence of a long aliphatic chain assisting and stabilizing active site-ligand interactions. These compounds likewise failed to provide any downregulation of the Wnt/β-catenine pathway at a higher concentration of 60 μM.

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References

(1) Perdicaris, S.; Vlachogianni, T.; Valavanidis, A. *Nat. Prod. Chem. Res*. **2013**, *1*, 114 doi:10.4172/2329-6836.1000115.
(2) Leal, M. C.; Puga, J.; Serodio, J.; Gomes, N. C. M.; Calado, R. *PLoS ONE* **2012**, *7*, e30580. doi:10.1371/journal.pone.0030580.
(3) Kingston, D. G. I. *J. Nat. Prod.* **2011**, *74*, 496-511.
(4) Montaser, R.; Luesch, H. *Future Med. Chem*. **2011**, *3*, 1475-1489.
(5) Mayer, A. M. S.; Glaser, K. B.; Cuevas, C.; Jacobs, R. S.; Kem, W.; Little, R. D.; McIntosh, J. M.; Newman, D. J.; Potts, B. C.; Shuster, D. E. *Trends Pharmacol. Sci.* **2010**, *31*, 255-265.
(6) Kochanowska, A. J.; Rao, K. V.; Childress, S.; El-Alfy, A.; Matsumoto, R. R.; Kelly, M.; Stewart, G. S.; Sufka, K. J.; Hamann, M. T. *J. Nat. Prod*. **2008**, *71*, 186-189.
(7) Hwang, I. H.; Oh, J.; Kochanowska-Karamyan, A.; Doerksen, R. J.; Na, M.; Hamann, M. T. *Tetrahedron Lett*. **2013**, *54*, 3872-3876.
(8) Zhu, F.; Han, B.; Kumar, P.; Liu, X.; Ma, X.; Wei, X.; Huang, L.; Guo, Y.; Han, L.; Zheng, C.; Chen, Y. *Nucleic Acids Res*. **2010**, *38(database issue)*, D787-D791.
(9) Hammock, B. D.; Wagner, K.; Inceoglu, B. *Pain Manage.* **2011**, *1*, 383-386.
(10) Pillarisetti, S.; Khanna, I. *Inflamm. Allergy Drug Targets* **2012**, *11,* 143-158.
(11) Shen, H. C.; Hammock, B. D. *J. Med. Chem*. **2012**, *55*, 1789-1808.
(12) Park, S.; Yun, E.; Hwang, I. H.; Yoon, S.; Kim, D.-E.; Kim, J. S.; Na, M.; Song, G. -Y., Oh, S. *Mar. Drugs* **2014**, *12*, 3231-3244.
(13) Park, S.; Gwak, J.; Cho, M.; Song, T.; Won, J.; Kim, D. -E.; Shin, J. -G.; Oh, S. *Mol. Pharmacol.* **2006**, *70*, 960-966.
(14) Durmus, S.; Garrison, J. C.; Panzner, M. J.; Tessier, C. A.; Youngs, W. J. *Tetrahedron* **2005**, *61*, 97-101.
(15) Ciminiello, P.; Costantino, V.; Fattorusso, E.; Magno, S.; Mangoni, A. *J. Nat. Prod*. **1994**, *57*, 705-712.
(16) Lira, N. S.; Montes, R. C.; Tavares, J. F.; da Silva, M. S.; da Cunha, E. V. L.; de Athayde-Filho, P. F.; Rodrigues, L. C.; da Silva Dias, C.; Barbosa-Filho, J. M. *Mar. Drugs* **2011**, *9*, 2316-2368.
(17) Wattanadilok, R.; Sawangwong, P.; Rodrigues, C.; Cidade, H.; Pinto, M.; Pinto, E.; Silva, A.; Kijjoa, A. *Mar. Drugs* **2007**, *5*, 40-51.
(18) Bao, L.; Deng, A.; Li, Z.; Du, G.; Qin, H. *Zhongguo Zhong Yao Za Zhi.* **2010**, *35*, 598-601.
(19) Kijjoa, A.; Bessa, J.; Wattanadilok, R.; Sawangwong, P.; Nascimento, M. S. J.; Pedro, M.; Silva, A. M. S.; Eaton, G.; Van Soest, R.; Herz, W. *Z. Naturforsch*. **2005**, *60b*, 904-908.

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