

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/265694172>

# Bioactivity Data for Eunicellin-Based Diterpenes Isolated from *Acalycigorgia* Sp

Article in *International Journal of Applied Chemistry* · January 2013

CITATIONS

0

READS

120

6 authors, including:



**Kwaku Kyeremeh**

University of Ghana

26 PUBLICATIONS 170 CITATIONS

[SEE PROFILE](#)



**Isaac Tuffour**

Noguchi Memorial Institute for Medical Research

29 PUBLICATIONS 51 CITATIONS

[SEE PROFILE](#)



**Regina Appiah-Opong**

Noguchi Memorial Institute for Medical Research

73 PUBLICATIONS 583 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



In vitro investigation of the relationship between schistosomiasis and prostate cancer [View project](#)



Mitochondrial function and Medicinal Plant Extracts [View project](#)

## Bioactivity Data for Eunicellin-Based Diterpenes Isolated from *Acalycigorgia* Sp

Kwaku Kyeremeh<sup>1</sup>, Asamoah Obed<sup>1</sup>, Botchie S. Kojo<sup>1</sup>,  
Isaac Tuffour<sup>2</sup>, Takuhiro Uto<sup>3</sup> and Regina Appiah-Opong<sup>2</sup>

<sup>1</sup>*Marine and Plant Research Laboratory of Ghana,  
Department of Chemistry, University of Ghana, Box LG 56, Ghana*

<sup>2</sup>*Department of Clinical Pathology,  
Noguchi Memorial Institute for Medical Research,  
University of Ghana, Box LG 581, Ghana*

<sup>3</sup>*Department of Pharmacognosy, Faculty of Pharmaceutical Science,  
Nagasaki International University, 2825-7 Huis Ten Bosch,  
Sasebo, Nagasaki, 859-3298, Japan  
kkyeremeh@ug.edu.gh Tel: +233207891320*

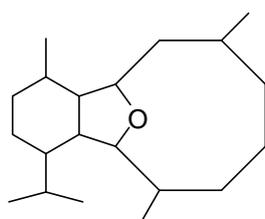
### Abstract

The cytotoxicity of five compounds previously isolated from the gorgonian *Acalycigorgia* sp was evaluated against human myeloid leukemia cells HL-60 and T-lymphocyte Jurkat cells. The tetrazolium-based colorimetric (MTT) assay was used to assess cytotoxicity of the compounds. They were found to be cytotoxic against the cell lines tested, and their IC<sub>50</sub> values were comparable to that of curcumin which had IC<sub>50</sub> values of 9.0 $\mu$ M and 6.8 $\mu$ M in HL-60 and Jurkat cells, respectively. Compound 1 was the most cytotoxic with IC<sub>50</sub> values of 15.0 $\mu$ M and 21.9 $\mu$ M in HL-60 and Jurkat cells, respectively. The least cytotoxic was compound 5 which had IC<sub>50</sub> values of 92.0 $\mu$ M and 123.8 $\mu$ M in HL-60 and Jurkat cells, respectively. The lower cytotoxicity of compound 5 could be attributed to the bulky fatty acid moiety attached at C6 of the main eunicellin skeleton. The GST activity of the five compounds was also studied and the results showed that the compounds had only moderate GST inhibitory activities. Compound 3 showed the strongest GST inhibitory activity with IC<sub>50</sub> value of 0.67mM compared to the IC<sub>50</sub> value of 0.01mM given by ETA which was used as a positive control. In addition, the five compounds tested showed very mild radical scavenging activity in the DPPH test.

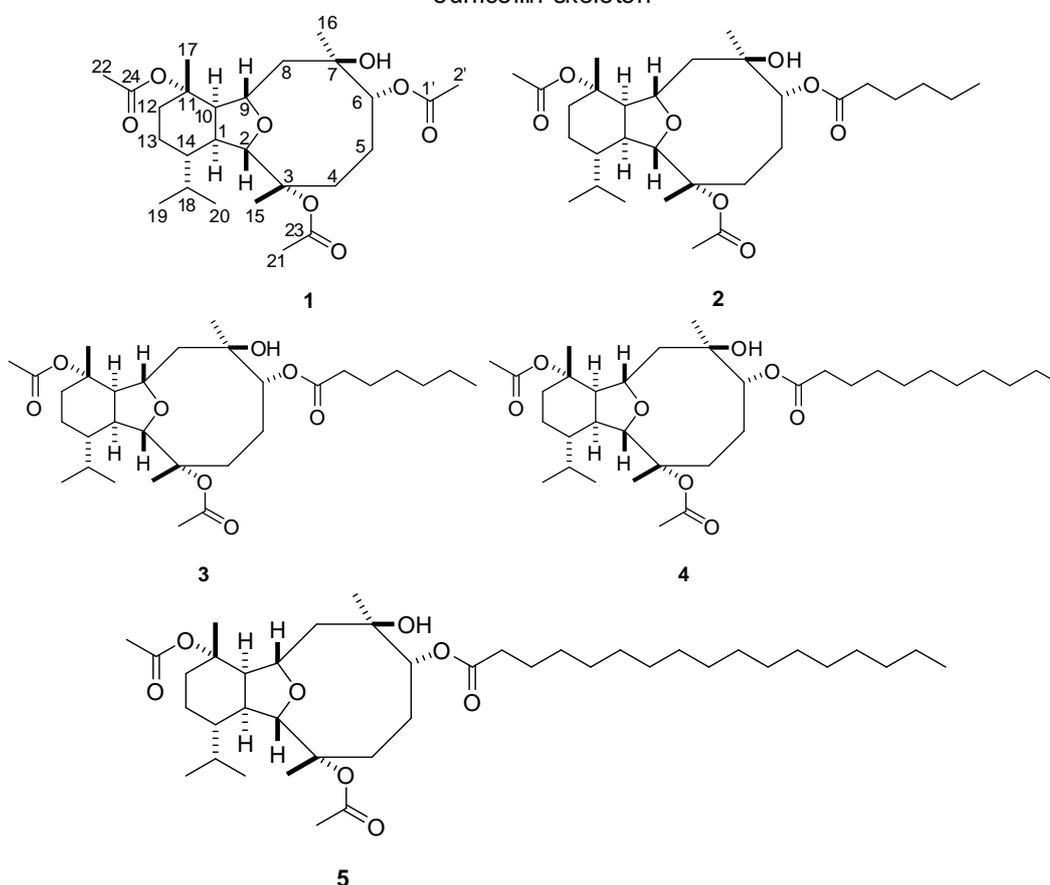
**Keywords:** HL-60 cell, Jurkat cell, Cytotoxicity

## Introduction

Many species from the genera *Eunicella* [1], *Sarcodictyon* [2], *Eleutherobia* [3], *Solenopodium* [4], *Sclerophytum* [5], *Alcyonium* [6] especially *Briareum* [7-9] and *Cladiella* [10-16] produce a wide variety of marine diterpenes derived from the eunicellin skeleton.



eunicellin skeleton



These eunicellin-based diterpenes have generated much interest as a result of the fact that sclerophytin A [5] has shown potent antileukemic activity while eleutherobin [3] and sarcodictyin [2] including their various synthetic analogs [17,18] have shown similar mechanisms of action as paclitaxel (Taxol). Eunicellin-based diterpenes possess ecological and agrochemical-related biological activities [10] but, the activity profiles of sclerophytins, eleutherobins, sarcodictyins and asbestinins have inspired

more research into their antitumor and anti-inflammatory potential. Current research suggests that, these compounds also have the potential to act as anti-migratory and anti-invasive agents which has further exacerbated interests in the prospects of these molecules as future anticancer drug prototypes [19,13]. Previously, we reported an entire homologous series of eunicellin-based diterpenes from *Acalycigorgia* sp [20] and tested compounds 1, 2 and palmonine B *in vitro* for their differential cytotoxicity in the soft agar assay. These compounds were found to be inactive against the murine colon adenocarcinoma 38 (MC-38) cells. The three compounds exhibited no murine solid tumor selectivity relative to murine normal cells. However, the continued isolation of eunicellin-based diterpenes and the current data on the anti-migratory and anti-invasive properties of these compounds in prostate cancers [11,15] have inspired further examination of the biological activity of the compounds previously isolated from *Acalycigorgia* sp. In this study, the cytotoxicity, free radical scavenging activity and the glutathione S-transferase (GST) inhibitory activity of compounds 1-5 were evaluated. Cytotoxicity of the compounds was evaluated using a slight modification of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay) method described by Mosmann (1983). The cancer cells used for this assay were HL-60 cells which are acute myeloid leukaemia cells and Jurkat cells which are T-lymphocyte cells. Radical scavenging activity was studied using a slight modification of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by [21].

The GSTs are phase II biotransformation enzymes that play a major role in the detoxification of endogenous substances and xenobiotics in the body [22]. However, investigations have revealed that high concentrations of these enzymes are found in cancer cells [23]. They have been implicated in drug resistance in cancer chemotherapy [24]. Therefore inhibition of GST activity is considered important in cancer therapy.

Glutathione S-transferase assay measures GST activity by measuring the conjugation of the substrate 1-chloro-2,4-dinitrobenzene (CDNB) to reduced glutathione (GSH) in the presence of GST enzymes. The CDNB-GSH conjugation results in an increase in the UV absorbance of CDNB at  $\lambda_{\max}$  340 nm. The rate of increase is directly proportional to the GST activity in the sample while a decrease in absorbance indicates inhibition of GST activity.

## Materials and Methods

### Materials

Methanol, ethanol, ethacrynic acid (ETA), butylated hydroxyl toluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and isopropanol were obtained from Sigma (MO, USA). Rose Park Memorial Institute (RPMI)-1640 culture medium, Fetal Bovine Serum (FBS), Triton X-100 and curcumin were purchased from Sigma-Aldrich (Germany). Dimethylsulfoxide (DMSO) and penicillin streptomycin glutamate (PSG) were obtained from WAKO Pure Chemical industry (Osaka, Japan). The HL-60 and Jurkat cell lines were donated by Dr. Uto, Nagasaki International University, Japan, and rat

liver cytosolic fractions were provided by the Clinical Pathology Department, Noguchi Memorial Institute for Medical Research. Eunicellin-based diterpenes compound used in the work were provided by Dr. K. Kyeremah (Accra, Ghana).

All other chemicals and reagents used were of analytical grade and obtained from standard suppliers.

### **Marine invertebrate**

The sample of *Acalycigorgia* sp. was collected by the Australian Institute of Marine Science in Thailand at 07 41.2 N; 98 46.4 E on 1 June 1990 at 15 m deep. Taxonomic identification was carried out by Phil Aderslade at the Museum of the Northern Territories and voucher specimens (COO6609) were kept at both the National History Museum in Washington DC, USA and at the Queensland Museum in Brisbane, Australia. The specimens collected were stored at 20 °C until used. The five compounds 1-5 are among the many secondary metabolites isolated from this species.

### **Cancer cells**

The HL-60 and Jurkat cancer cell lines were used in the cytotoxicity assay. The cells were maintained in continuous culture and checked at regular intervals for their confluence levels and condition. The culture medium consisted of RPMI medium with 1% PSG and 10% FBS. Cells were only used when they were about 80% confluent and in good condition.

### **MTT Assay**

The compounds 1-5 and curcumin as positive control were solubilized with absolute DMSO to make 20mM stock solutions. The 20mM sample stock solutions, was diluted 10 times with culture media to obtain a 2000µM solution. From this solution, 2-fold serial dilutions of the compounds were prepared to obtain 4 different concentrations, 1000µM, 500 µM, 250 µM and 125 µM of sample each containing 10% DMSO. Twenty milliliters cell suspension containing  $1 \times 10^5$  cells/milliliter was prepared and 100µL aliquots of the cell suspension were transferred into wells in a 96-well plate. The cells were treated with the various dilutions of the compounds in solution; the plates were incubated for 72hours at 37°C with 5% CO<sub>2</sub>. Positive and negative control experiments were also set up. Triplicate experiments were performed. After incubation, 20µL of 2.5 mg/ml MTT was added to each well and the plates were incubated for another 4hours. The reaction was stopped with acidified isopropanol and the plates were kept in the dark at room temperature overnight. The absorbance was read at a wavelength of 570nm and a graph of percentage cell viability was plotted against concentration of samples. The formula used for calculating the percentage cell viability is as shown below:

$$\% \text{Cell Viability} = \frac{\text{Mean abs of sample} - \text{abs of compound control}}{\text{Mean abs of negative control} - \text{abs of blank}} \times 100 \%$$

### **Free radical scavenging activity-DPPH assay**

The antioxidant activities of the compounds 1-5 were determined using the DPPH

method with slight modification [21]. A 0.5mM DPPH solution was prepared in methanol. For a positive control, 20.0mM BHT solution was also prepared in methanol. Two-fold serial dilutions of each of the compounds (1-5) and BHT were prepared to obtain the concentrations 0.625mM, 1.25mM, 2.5mM, 5.0mM, and 10.0mM for each compound, and 0.0195mM, 0.0391mM, 0.078mM, 0.1563mM, 0.3125mM, 0.625mM, 1.25mM, 2.5mM, 5.0mM, and 10.0mM for BHT. Aliquots of 100 $\mu$ L of the each dilution was transferred into wells of a 96-well microtitre plate in duplicate and treated with 100 $\mu$ L DPPH solution. Negative control experiments were also prepared with methanol and DMSO. The plates were incubated for 20min at room temperature in the dark and then absorbance was read at a wavelength of 517nm. Percentage antioxidant activity was calculated using the formula shown below:

$$\% \text{Cell Viability} = \frac{\text{Mean abs of blank} - \text{abs of compound}}{\text{Mean abs of blank}} \times 100 \%$$

A graph of percentage antioxidant activity against concentration of compounds was plotted from the results obtained.

### GST inhibition assay

The GST inhibitory activity of the compounds was assessed as earlier described with slight modification [25]. The incubation mixture for the assay consisted of 160  $\mu$ L of buffer, 10  $\mu$ L of 10mM GSH, 5  $\mu$ L of 16mM CDNB, 10.3  $\mu$ L of each sample dilution, and 20  $\mu$ L of 1.28 mg/ml protein rat liver cytosolic fraction containing GST enzymes were transferred into the wells. Ethacrynic acid (ETA) was used as positive control. Triplicate experiments were performed for each concentration. Absorbance was read at a wavelength of 340nm. A graph of percentage inhibition of GST activity against concentration was plotted from the results obtained. The formula used for calculating percentage inhibition of GST activity is as shown below:

$$\% \text{Cell Viability} = \frac{\text{Mean abs of negative control} - \text{abs of compound}}{\text{Mean abs of negative control}} \times 100 \%$$

## Results

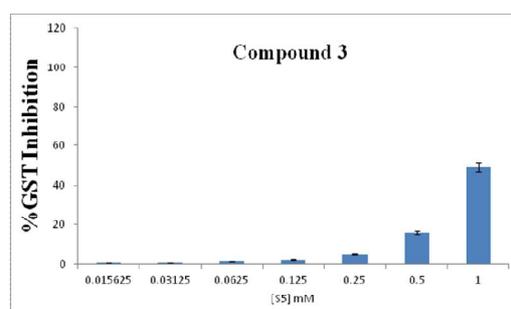


Figure 1: GST inhibitory activity of compound 3

## Discussion

All the compounds tested showed very weak antioxidant activity (data not included). Compound 1 showed a percentage antioxidant activity of about 20%, whilst the value for the remaining compounds 2-5 showed lower activities. These results suggest that the compounds tested lack the potential to act as free radicals scavengers in cancer cells. On the other hand, compounds 1-4 showed very interesting cytotoxicity profiles, compared to the standard which in this case was curcumin. The IC<sub>50</sub> values obtained for the compounds are shown below in Table 1 but, the graph of percentage cell viability plotted against various concentrations of the test samples are for simplicity not shown.

**Table 1:** Measured IC<sub>50</sub> values for compounds with respect to HL-60 and Jurkat cells (IC<sub>50</sub> values obtained from measurements in triplicates)

COMPOUND	Observed Mass in HRESIMS	Molecular formula [M+Na] <sup>+</sup>	Side Chain Molecular formula [RCOO] <sup>-</sup>	IC <sub>50</sub> μM	
				HL-60 Cells	Jurkat Cells
Curcumin				9.0	6.8
1	505.2741	C <sub>26</sub> H <sub>42</sub> O <sub>8</sub> Na	C <sub>2</sub> H <sub>3</sub> O <sub>2</sub>	15.0	21.9
2	561.3398	C <sub>30</sub> H <sub>50</sub> O <sub>8</sub> Na	C <sub>6</sub> H <sub>11</sub> O <sub>2</sub>	22.3	21.6
3	575.3572	C <sub>31</sub> H <sub>52</sub> O <sub>8</sub> Na	C <sub>7</sub> H <sub>13</sub> O <sub>2</sub>	22.7	23.3
4	631.4180	C <sub>35</sub> H <sub>60</sub> O <sub>8</sub> Na	C <sub>11</sub> H <sub>21</sub> O <sub>2</sub>	20.2	27.8
5	715.5102	C <sub>41</sub> H <sub>72</sub> O <sub>8</sub> Na	C <sub>17</sub> H <sub>33</sub> O <sub>2</sub>	92.0	123.8

Compounds 1-5 are structural homologues differing only by virtue of the length of the side chains at C6 on the main eunicellin skeleton. Hence, the IC<sub>50</sub> values obtained for compound 5 shows clearly a relationship of the cytotoxicity profile for these compounds to the length of the side chain at C6. It appears that a shorter less bulky side chain at C6 as in compound 1 enhances the cytotoxicity of these compounds to HL-60 and Jurkat cell lines. This observation is interesting since Hassan *et al.* (2011) have shown that varying the nature of substituents at C6, C11, C16 and C17 produces changes in the anti-invasive and anti-migratory activity of these eunicellin-based diterpenes. The present results in combination with that from Hassan *et al.* (2011) continues to reinforce the view that eunicellin-based diterpenes possess biological activities that can vary over a wide range depending on the kind of substituent present at different points on the eunicellin skeleton.

Compared to ETA which was used as a positive control in the GST inhibitory assay, the activity of the compounds 1-5 in this assay was moderate. Compound 3 exhibited the highest GST activity with percent inhibition of about 50% at the highest concentration tested (1mM) compared to 100% inhibition by ETA at the same concentration, which produced an IC<sub>50</sub> value of 0.01mM. The interesting feature of the GST activity measured for these compounds is the gradual increase in activity with increasing concentration of the compounds (Figure 1). It appears that the

compounds 1-5 become more active at concentrations of 1mM and above.

Cancer cells are normally characterized by increased rates of proliferation and metabolism which normally results in the generation of many reactive oxygen species (ROS) or reactive nitric oxides (RNO) alongside other harmful substances [26]. GST enzymes are involved in the detoxification of cells by their ability to catalyze reactions that lead to the removal and neutralization of harmful substances from the cells. The activity of GSTs has been linked to the development of resistance of cancer cells to chemotherapeutic drugs [27]. Due to the over expression of GSTs in tumor cells, drug substrates and other xenobiotics are rapidly detoxified which gives these tumor cells an innate resistance [28]. It is plausible that GSTs serve two distinct roles in the development of drug resistance via direct detoxification as well as acting as inhibitors of the MAP kinase pathway [29]. Hence, it is not surprising that high levels of GSTs have been reported in a large number of tumor types.

Hence, compounds that are able to inhibit the action of GSTs in cancer cells will be of great benefit to the treatment of cancers. The activity profiles shown by compounds 1-5 confirm their suitability for further investigation as future drug prototypes. It is not surprising therefore that, eunicellin-based diterpenes are currently listed among the most promising marine anticancer, anti-inflammatory and anti-infectious (malaria) compounds that constitute potential drug molecules [20,30].

This research gives a first time account of highly oxygenated and highly acetylated eunicellin-based diterpenes that have shown strong cytotoxicity against HL-60 and Jurkat cells. Currently, this is the first report where eunicellin based diterpenes have shown good activity towards HL-60 and Jurkat cells which constitute very important cells for the study of many anticancer agents and cancer related phenomena. It is also of great importance to observe that compounds 1-5 are inactive against the murine colon adenocarcinoma 38 (MC-38) cells but, have proved to be highly cytotoxic to HL-60 and Jurkat cells in the current work. Hence, the apparent lack of activity of eunicellin diterpenes in one cytotoxicity assay should not discourage further testing in different assays while further testing of these molecules should eventually provide an in-depth understanding of their bioactivity.

## **Conclusion**

Eunicellin-based diterpenes are capable of a wide range of cytotoxicity activity directly related to their structures. Even though the huge structural versatility of these compounds makes it difficult to directly correlate which structures are likely to be active against a particular cancer cell line, it appears that lymphoma and leukemia cell lines respond more positively to these compounds than carcinoma and sarcoma cell lines. The results in this report reinforces this idea where compounds 1-5 are inactive against the murine colon adenocarcinoma 38 (MC-38) cells but, have proved to be highly cytotoxic to HL-60 and Jurkat cells in the current work. The trend where these compounds are completely inactive towards prostate cancers but are able to act as anti-migratory and anti-invasive agents for this cancer therefore suggests that, continued biomedical investigation of eunicellin-based diterpenes is likely to yield new anticancer agents.

## Acknowledgement

The group is delighted to acknowledge the University of Ghana Research Fund which provided seed-funding for most of the marine invertebrates and microbial research in our laboratories. We thank the staff of Clinical Pathology department for their technical support.

## References

- [1] Mancini, I., Guella, G., Zibrowius, G., Pietra, F., 2000, "Configuration, Conformation and Reactivity of Highly Functionalized Eunicellin Diterpenes Isolated from the Gorgonian Eunicella cavolini and Eunicella singu," *Helv. Chim. Acta.*, 83, pp. 1561-1575. 10.1002/1522-2675(20000705)83:7<1561::AID-HLCA1561>3.0.CO;2-H
- [2] D'Ambrosio, M., Guerriero, A., Pietra, F., 1987, "Sarcodictyin A and Sarcodictyin B, Diterpenordic Alcohols Esterified by Methyluronic acid, from *Stolonifer Sarcodictyon roseum*," *Helv. Chim. Acta.*, 70, pp. 2019-2027. 10.1002/hlca.19870700807
- [3] Lindel, T., Jensen, P. R., Fenical, W., Long, B. H., Casazza, A. M., Carboni, J., Fairchild, C. R., 1997, "Eleutherobin, a New Cytotoxin that Mimics Paclitaxel (Taxol) by Stabilizing Microtubules," *J. Am. Chem. Soc.*, 119, pp. 8744-8745. 10.1021/ja9717828
- [4] Groweiss, A., Look, S. A., Fenical, W., 1988, "Solenolides, an Anti-inflammatory and Antiviral Diterpenoid from a Marine Octocoral of genus *Solenopodium*," *J. Org. Chem.*, 53(11), pp. 2401-2406. 10.1021/jo00246a001
- [5] Sharma, P., Alam, M., 1988, Sclerophytins A and B, Isolation and Structure of Novel Cytotoxic Diterpenes from the Marine Coral *Sclerophyllum capitalis*," *J. Chem. Soc. Perkin Trans.*, 1(0), pp. 2537-2540. 10.1039/P19880002537
- [6] Lin, Y. C., Bewley, C. A., Faulkner, D. J., 1993, The Valdivones, Anti-inflammatory Diterpene Esters from the South African Soft Coral *Alcyonium valdivae*," *Tetrahedron*, 49, pp. 7977-7984. [http://dx.doi.org/10.1016/S0040-4020\(01\)88021-2](http://dx.doi.org/10.1016/S0040-4020(01)88021-2)
- [7] Roderiguez, A. D., Cobar, O. M., 1993, "Structures and Bioactivities of New Asbestinin Diterpenoids from the Caribbean Gorgonian Octocoral *Briareum asbestinum*," *Tetrahedron*, 49, pp. 319-328. [http://dx.doi.org/10.1016/S0040-4020\(01\)80301-X](http://dx.doi.org/10.1016/S0040-4020(01)80301-X)
- [8] Roderiguez, A. D., Cobar, O. M., 1995, Studies on Minor Constituents of Caribbean Gorgonian Octocoral *Briareum asbestinum pallas*. Isolation and Structure Determination of Eunicellin-based Diterpenoids Briarellins E-I," *Chem. Pharm. Bull.*, 43, pp. 1853-1858. <http://dx.doi.org/10.1248/cpb.43.1853>
- [9] Joyner, P. M., Waters, A. L., Williams, R. B., Powell, D. R., Janakiram, N. B., Rao, C. V., Cichewicz, R. H., 2011, "Briarane Diterpenes Diminish COX-2 Expression in Human Colon Adenocarcinoma cells," *J. Nat. Prod.*, 74(4), pp. 857-861. <http://dx.doi.org/10.1021/np100775a>

- [10] Yamada, K., Ogata, N., Ryu, K., Miyamoto, T., Komori, T., Higuchi, R., 1997, "Bioactive Terpenoids from Octocorallia. 3. A New Eunicellin-based Diterpenoid from the Soft Coral *Cladiella sphaeroides*," *J. Nat. Prod.*, 60(4), pp. 393-396. 10.1021/np9605605
- [11] Hassan, H. M., Khanfar, M. A., Elnagar, A. U., Mohammed, R., Shaala, L. A., Youssef, D. T. A., Hifnawy, M. S., El Sayed, K. A., 2010, "Pachycladins A-E, Prostate Cancer Invasion and Migration Inhibitory Eunicellin-based Diterpenoids from the Red Sea Soft Coral *Cladiella pachyclados*," *J. Nat. Prod.*, 73(5), pp. 848-853. 10.1021/np900787p
- [12] Williams, D. E., Amiani, A., Dewi, A. S., Patrick, B. O., van Ofwegen, L., Mui, A. L. F., Anderson, R. J., 2010, "Australin E Isolated from the Soft Coral *Cladiella* sp collected in Pohnpei Activates the Inositol-5-phosphate SHIP1," *Aust. J. Chem.*, 63(6), pp. 895-900. 10.1071/CH10053
- [13] Chen, B. W., Chang, S. M., Huang, C. Y., Chao, C. H., Su, J. H., Wen, Z. H., Hsu, C. H., Dai, C. F., Wu, Y. C., Shu, J. H., 2010, Hirsutalins A-H, Eunicellin-based Diterpenoids from the Soft Coral *Cladiella hirsute*, *J. Nat. Prod.*, 73(11), pp. 1785-1791. 10.1021/np100401f
- [14] Tai, C. J., Su, J. H., Huang, M. S., Wen, Z. H., Dai, C. F., Sheu, J. H., 2011, "Bioactive Eunicellin-based Diterpenoids from the Soft Coral *Cladiella krempfi*," *Mar. Drugs*, 9, pp. 2036-2045. 10.3390/md9102036
- [15] Hassan, H. M., Elnagar, A. U., Khanfar, M. A., Sallam, A. A., Mohammed, R., Shaala, L. A., Youssef, D. T. A., Hifnawy, M. S., El Sayed, K. A., 2011, "Design of Semi-synthetic Analogues and 3D-QSAR Study of Eunicellin-based Diterpenoids as Prostate Cancer Migration and Invasion Inhibitors, " *Eur. J. Med. Chem.*, 46(4), pp. 1122-1130. <http://dx.doi.org/10.1016/j.ejmech.2011.01.028>
- [16] Cai, Y-S., Yao, L-G., Pascal, A. D., Irace, C., Mollo, E., Tagliatela-Scfati, O., Guo, Y-W., 2013, "Polyoxygenated Diterpenoids of the Eunicellin-type from the Chinese Soft Coral *Cladiella krempfi*," *Tetrahedron*, 69(9), pp. 2214-2219. <http://dx.doi.org/10.1016/j.tet.2012.12.051>
- [17] Chandrasekhar, S., Jagadeshwar, V., Narsihmulu, Ch., Sarangapani, M., Krishna, D. R., Vidyasagar, J., Vijay, D., Sastry, G. N., 2004, "Design, Synthesis and Cytotoxicity Studies on the Simplified Oxy analog of Eleutherobin, *Biorg. Med. Chem. Letters.*, 14(14), pp. 3687-3689. <http://dx.doi.org/10.1016/j.bmcl.2004.05.017>
- [18] Beumer, R., Bayón, P., Bugada, P., Ducki, S., Mongelli, N., Sirtori, F. R., Telsler, J., Gennari, C., 2003, "Synthesis of Novel Simplified Sarcodictyin/Eleutherobin Analogs with Potent Microtubule-Stabilizing Activity, using Closing Metathesis as the Key-Step," *Tetrahedron*, 59(44), pp. 8803-8820. <http://dx.doi.org/10.1016/j.tet.2003.08.057>
- [19] Vinothkumar, S., Parameswaran, P. S., 2013, "Recent Advances in Marine Drug Research," *Biotech Adv*, In Press, Corrected Proof is Available online. <http://dx.doi.org/10.1016/j.biotechadv.2013.02.006>
- [20] Kwaku Kyeremeh, Thomas C. Baddeley, Bridget K. Stein, Marcel Jaspars, 2006, "A homologous series of Eunicellin-based diterpenes from *Acalycigorgia*

- sp. characterised by tandem mass spectrometry", *Tetrahedron*, 62(37), pp. 8770-8778
- [21] Blois, M. S., 1958, "Antioxidant Determinations by the Use of a Stable Free Radical," *Nature*, 181, pp. 1199-1200. 10.1038/1811199a0
- [22] Klaassen, C. D., 2001, *The Basic Science of Poisons. Toxicology* (6<sup>th</sup> edn). McGraw-Hill Medical Pub. Division: New York.
- [23] Atkinson, J. H., Babbitt, P. C., 2009, "Glutathione Transferases are Structural and Functional Outliers in the Thioredoxin Fold," *Biochemistry*, 48(46), pp. 11108-11116. 10.1021/bi901180v
- [24] Lo, H. W., Ali-Osman, F., 2007, "Genetic Polymorphism and Function of Glutathione S-Transferase in Tumor Drug Resistance," *Curr. Opin. Pharmacol.*, 7(4), pp. 367-374. <http://dx.doi.org/10.1016/j.coph.2007.06.009>
- [25] Appiah-Opong, R., Commandeur, J. N. M., Istyastono, E., Bogaards, J. J., Vermeulen, N. P. E., 2009, "Inhibition of Human Glutathione S-transferases by Curcumin and Analogues," *Xenobiotica*, 39(4), pp. 302-11. 10.1080/00498250802702316
- [26] Hussain, S. P., Hofseth, L. J., Harris, C. C., 2003, "Radical Causes of Cancer," *Nat. Rev. Cancer*, 3(4), pp. 276-285. 10.1038/nrc1046
- [27] Hayes, J. D., Flanagan, J. U., Jowsey, I. R., 2005, "Glutathione-S-Transferases," *Ann. Rev. Pharmacol. Toxicol.*, 45(1), pp. 51-58. 10.1146/annurev.pharmtox.45.120403.095857
- [28] McIlwain, C. C., Townsend, D. M., Tew, K. D., 2006, "Glutathione S-Transferase Polymorphisms: Cancer Incidence and Therapy," *Oncogene*, 25, pp. 1639-1648. 10.1038/sj.onc.1209373
- [29] Laborde, E., 2010, "GST as Mediators of Signaling Pathways Involved in Cell Proliferation of Cell Death," *Cell Death Differ.*, 17(9), pp. 1373-1380. 10.1038/cdd.2010.80
- [30] Chen, Y-H., Tai, C-Y., Hwang, T-L., Weng, C-F., Li, J-J., Fang, L-S., Wang, W-H., Wu, Y-C., Sung, P-J., 2010, "Cladielloides A and B: New Eunicellin-Type Diterpenoids from an Indonesian Octocoral *Cladiella* sp.," *Mar. Drugs*, 8, pp. 2936-2945. 10.3390/md8122936