Review

Recent Discovery of Bioactive Natural Products from Taiwanese Marine Invertebrates

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Abstract The secondary metabolites from Taiwanese marine soft corals and sponges have attracted much attention because they possess considerable potential biological activities. To explore the origin of bioactivity, many cytotoxic natural products were isolated and characterized in the past few years. For examples, The lipophilic extracts from marine sponges *Petrosia elastica* and *Ircinia formosana* were found active against several human tumor cells. The investigation of the gorgonian *Junceela* has also resulted in the discovery of a series of new juncenolides. Bioassay-directed fractionation of *Clavularia viridis* yielded seven new prostanoids. These compounds have been tested and evaluated as potential antitumor agents. The soft corals of the genus *Cespitularia* produced novel secondary metabolites with diverse chemical structures and interesting biological activities. Four new norditerpenoids, designated cespitulactones and cespihypotins were isolated from *Cespitularia hypotentaculata*. Cespitulactones are novel structures having a bond cleavage between C-10 and C-11. In addition, three novel diterpenes were isolated from *C. taeniata* and designated cespitulactams A, B and C having a phenylethyl amino side chain.

Key words : Taiwanese marine invertebrates, natural products, secondary metabolites, biological activity

Introduction

Cancer is the first cause of death in Taiwan. The most effective anticancer drugs (Taxol and camptothecins, etc.) have been developed from natural products. There are several important marine natural products under clinical investigation as potential anti-cancer drugs[5, 10]. These compounds include ecteinacidin 743, dolastatin 10, and bryostatin 1, and so on. Ecteinacidin 743, an alkaloid from the tunicate *Ecteinacidia turbinate* has a broad-spectrum anti-tumor activity and is especially effective against solid tumors such as sarcoma and breast cancers [11]. Ecteinacidin 743 is by far the most advanced anti-cancer drug in the phase III of clinical trials [3]. Dolastatin 10 was isolated as a short and uncommon peptide from the sea hare *Dolabella auricularia*. It is now in the phase II of clinical trials. Bryostatin 1, obtained from the bryozoa *Bugula neritina* was subjected to in combination therapy phase II clinical trials [6,9]. Other important marine natural products such as eleutherobine and sarcodictyn, both isolated from soft corals (*Eleutherobia* sp. and *Sarcodictyon* sp., respectively) are currently under preclinical trials for the treatment of cancers [4,7]. Significantly, eleutherobine is a diterpene glycoside, which showed to be a potent cancer cell inhibitor with an IC_{50} at 10 nM. Eleutherobin was also found to stabilize microtubules by competing for Taxol binding site due to their structural similarity [7].

In the past few years we have collected more than 200 species of marine sponges and soft corals in the southern reef of Taiwan. More than 25% of collected species showed significant cytotoxicity (IC50<10 μg/ml) against human tumor cells. To explore the origin of bioactivity, many cytotoxic natural products were isolated. For examples, parahigginols, parahigginine and parahigginone were isolated from *Parahigginsia sheni* [1]. Meroditerpenoid, strongylophorines and polyacetylenic compounds were isolated from the *Strongylophora durissima* [21]. The sesquiterpenoids,

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hippochromins A and B have been afforded from *Hippospongia metachromia* [12]. Chemical investigation of *Ircinia formosana* afforded four novel linear C_{22}-sesterterpenes, designated irciformonins A-D (**1-4**) [20]. Two of the isolated metabolites **1** and **2** have diene linear structure with furan ring at one end and a five-membered lactone ring at the other, while **3** and **4** are devoid of furan rings, having a lactone at either side of the linear skeleton. Pharmacological study revealed that compounds **3** and **4** had a mild activity against human colon adenocarcinoma (WiDr) tumor cells. The lipophilic extracts from marine sponges *Petrosia elastica* [22], *Ircinia* and other unknown spp. were found active against several human tumor cells.

In continuation of our interest in the chemistry and potential medicinal importance of briaranes, we investigated the gorgonian octocoral *Junceella juncea* collected in Taiwan. Several juncins, gemmacolides and junceelolides have been reported from this species. The current study resulted in the isolation of the seven novel briarane-type diterpenoidal compounds, juncenolides A-G (**5-11**) from the acetone extract of *Junceella juncea* [8,17,18]. Chromatographic investigation of an acetone extract of the octocoral *Xenia florida* afforded three new xenicane diterpenes, namely florxenilide A (**12**), florxenilide B (**13**) and florxenilide C (**14**) [2]. However, from another batch of collection three new diterpenoids, designated xeniolactones A (**15**), B (**16**) and C (**17**), were isolated from *Xenia* sp [16]. Compound **15** possesses a novel structure having a heterotricyclic skeleton in cyclononane system. Its structure was elucidated through spectroscopic analysis, especially 2D NMR. The absolute configuration of **12** was determined by NOESY, CD and Mosher's methods. Florxenilides A (**12**) and B (**13**) exhibited cytotoxicity against human colon cancer (WiDr) cells at 4.5 and 3.7 μM, respectively.

Bioassay-directed fractionation of *Clavularia viridis* yielded seven new prostanoids (**18-24**) [13]. Moreover, bioassay-guided chromatography of *Clavularia inflata* has led to the isolation of new dolabellane-type compounds. These compounds have been tested and evaluated as potential antitumor agents.

Of particular interest is the recent discovery of a series of norditerpenes, which appear to be biogenically derived from geranylgeranyl pyrophosphate and 1S-verticillene via loss of a methyl unit. The southern coast of Taiwan has long been a habitat of soft corals. Among them, specimens of *Cespitularia* are occationally encountered and have different color variants similar to species of *Xenia*. The polyps of *Cespitularia* are like those of *Xenia,* but are not restricted to the branch ends. A novel diterpenoid designated cespitulactone A (**25**) with an unusual bond cleavage between C-10 and C-11, and having a 14- membered lactone ring connection between C-10 and C-12 has been isolated from *Cespitularia taeniata* May [14]. In our continuing investigation of norditerpenoids from marine resources, we isolated three additional novel diterpenes designated cespitulactams A (**26**), B (**27**) and C (**28**) having a phenylethyl amino side chain and two known related compounds, cespitularins D and F from *Cespitularia taeniata* [19].

A collection of same genus but different species from same area has resulted in the isolation of four novel compounds, cespihypotins A (**29**), B (**30**), C (**31**) and D (**32**) from *Cespitularia hypotentaculata*[15]. Compound **29** contains a 13 membered lactone ring connection between C-10 and C-11. Compound **30** is an acetate analogue of cespitulactone A.

A plausible biogenetic pathway of **26-28** was proposed based on recently published cespitularines C, D and F and biosynthesis of taxane diterpenes (Scheme 1). From a biogenetic point of view, geranylgeranyl pyrophosphate and 1S-verticillene are precurors of cespitulactams A-C (**26-28**), which might be tranformed from cespitularine C through several intermediates via oxidation, hydration, amino transfer, and amide formation.

Scheme 2 illustrated a plausible biogenetic pathway for compounds **29-32**. The precursor geranylgeranyl diphosphate is transformed to an intermediate, 1S-verticillene by the enzyme cyclase. Subsequent steps involving rearrangement and oxidation yield intermediate **a**, then decarboxylation and hydroxylation produce intermediates **b** and **c**, which might precusors of cespihypotins A (**31**) and B (**32**). Cespitulactones C (**29**) and D (**30**) might be derived from the norditerpenes **d** and **e** via epoxidation, hydration, oxidation and lactonization, which involves attack of the C-12 hydroxy or C-11 hydroxy on the carbonyl at C-10 and subsequent bond cleavage between C-10 and C-11.

The structures of cespitulactams A (**26**), B (**27**) and C (**28**) are closely related to 3,8-seco-taxoids, but with an unusual N-phenylethyl-butyrolactamyl moiety. Four human cancer cell lines (KB, Hepa, Daoy and WiDr)

were chosen to test *in vitro* cytotoxic potentialities. Cespitulactam A exhibited significant cytotoxicity against human Widr and Daoy cancer cells at IC_{50} 2.72 and 6.34 μg/ml, respectively, while its acetate was inactive toward four tumor cells (> 20 μg/ml). The preliminary biological test revealed that the free hydroxyl function at C-6 in **1** is critical because acetylation of this position leads to a complete loss of activity.

The secondary metabolites from marine invertebrates, especially the soft corals and sponges, have attracted much attention because they possess considerable potential biological activities. The search for new lead compounds from marine invertebrates and investigation of their structures activity relationships are quite interesting and will be getting more important in the future. Accordingly, successful drug discovery will continue to result from isolation and screening programs that make use of natural products. As little of the world biodiversity has been explored, many more useful natural lead compounds are awaiting discovery.

Acknowledgment

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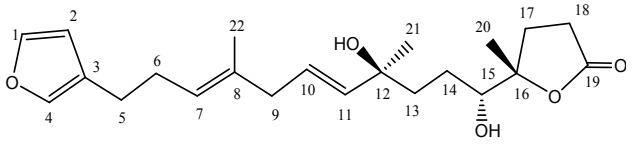

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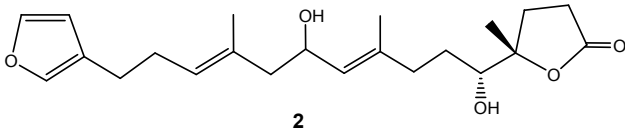

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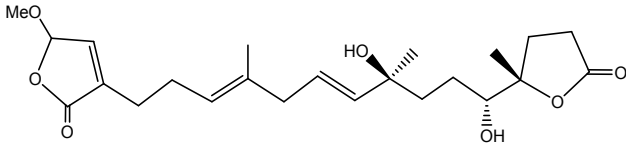

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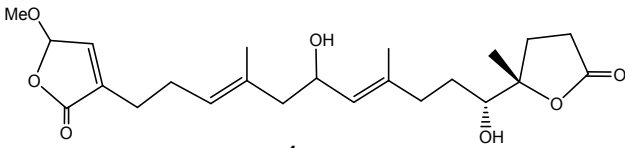

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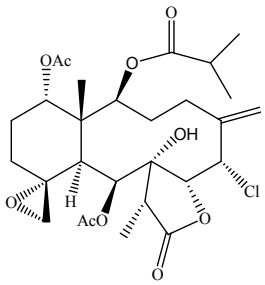

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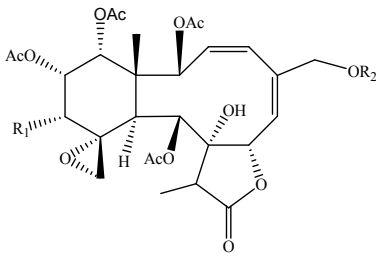
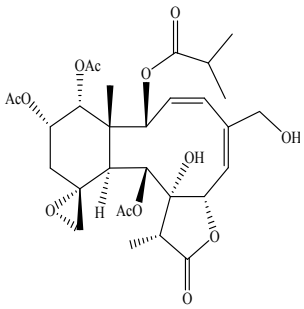


6 R_1=R_2=H
7 R_1=OAc, R_2=H
8 R_1=OAc, R_2=CH_3



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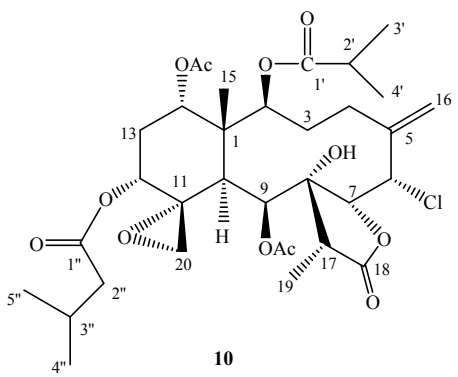

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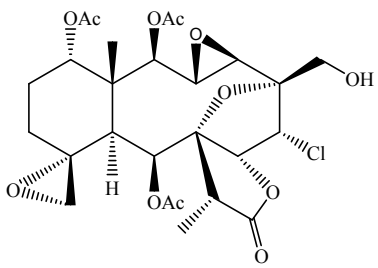

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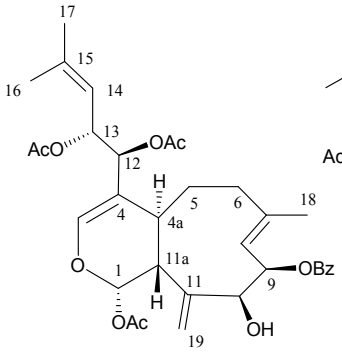

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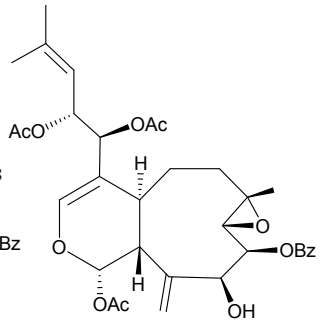

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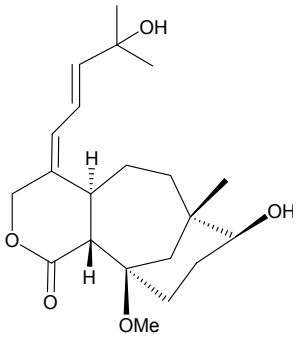

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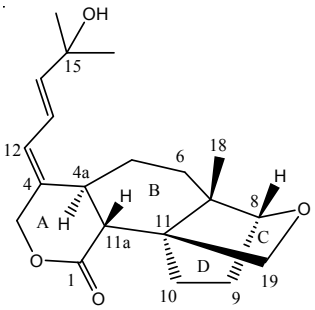

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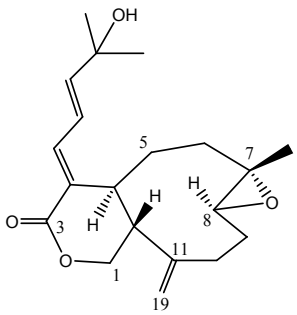

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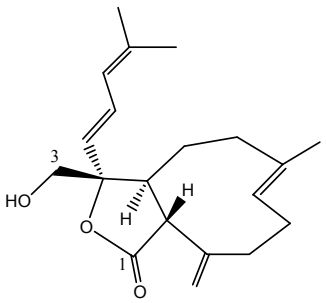

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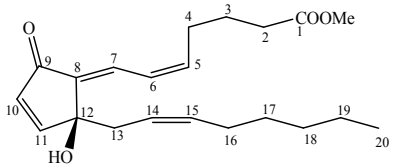

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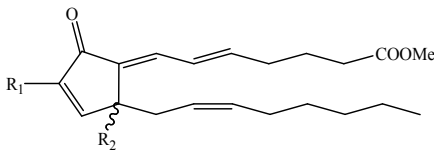
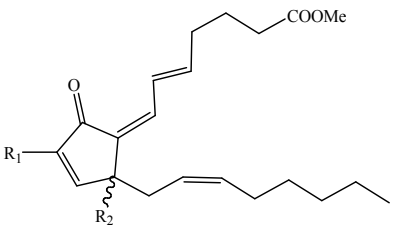
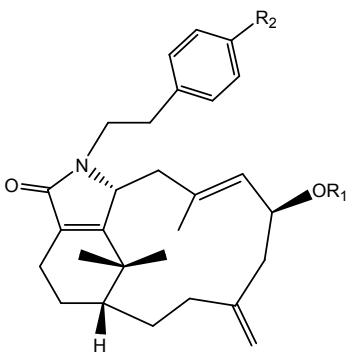


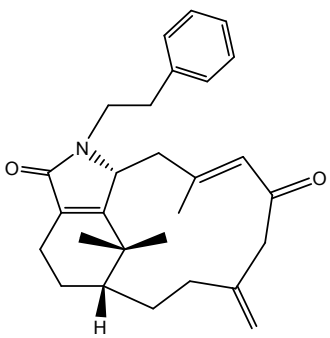
19 R_1=H, R_2= β-OH
20 R_1=Br, R_2=α-OH
21 R_1=I, R_1= α-OH



22 R_1=H, R_2= β-OH
23 R_1=Br, R_2= α-OH
24 R_1=I, R_2= α-OH



26 $R_1 = R_2 = H$
28 $R_1 = H$, R_2 =OH



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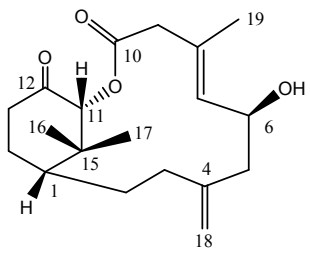

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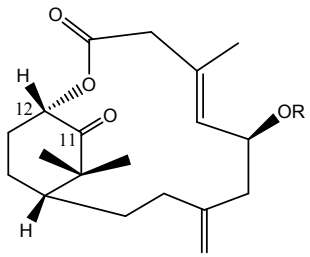
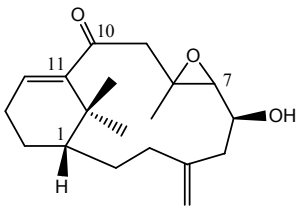


25 R=H
30 R=Ac



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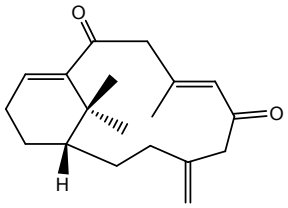

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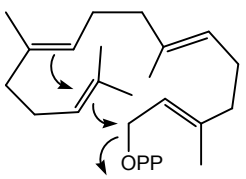
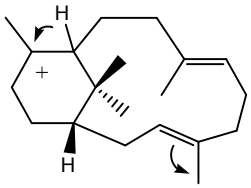
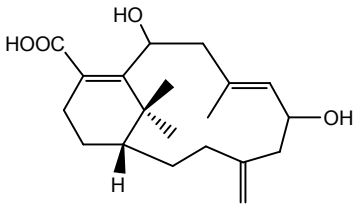


Geranylgeranyl Diphosphate

Cyclase

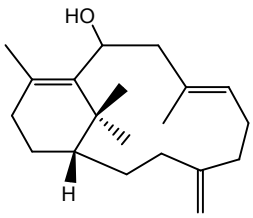


1S-Verticillene



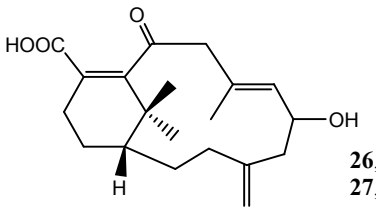
a

Oxidation & Hydroxylation



Cespitularin C

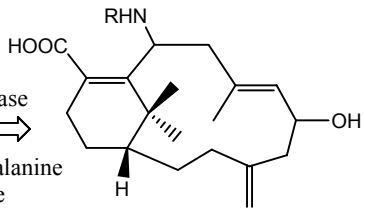
Oxidation



b

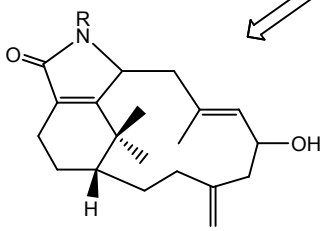
Amino transferase

26, phenylalanine
27, tyrosine



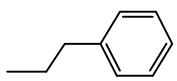
c

$-H_2O$

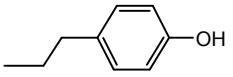


Compounds **26** and **28**

26, R =



28, R =



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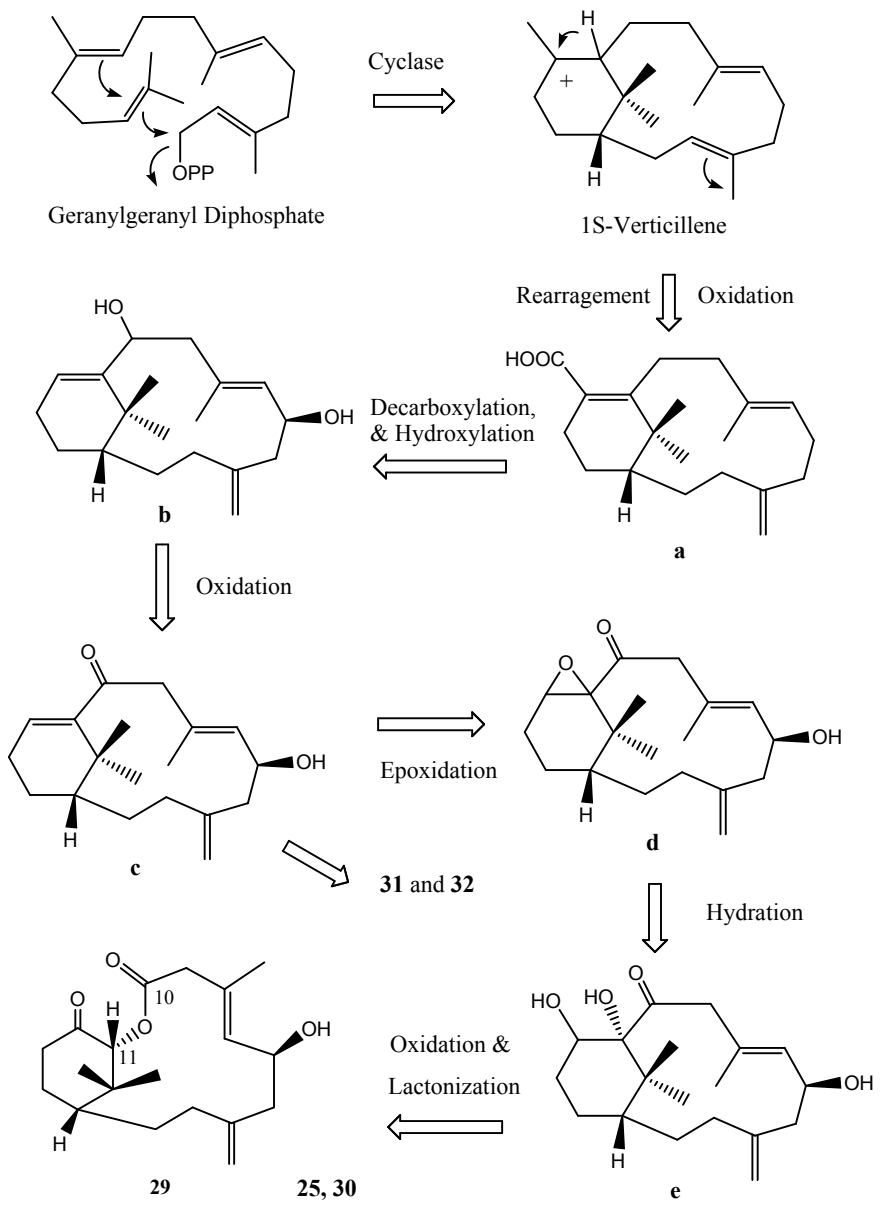
Scheme 1. A plausible biogenetic pathway of **26-28**



Scheme 2. Plausible biogenetic pathway of **29-32**

References

1. Chen, C. Y., Shen, Y. C., Chen, Y. J. and Duh, C. Y. 1999. Bioactive sesquiterpenes from a Taiwanese marine sponge *Parahigginsia* sp. *J. Nat. prod.,* **62,** 573-576.
2. Cheng, Y. B., Jang, J. Y., Khalil, Ashraf Taha, Kuo, Y. H. and Shen, Y. C. 2006. Xenicane-type diterpenes with cytotoxicity from *Xenia florida*. *J. Nat. Prod.*, 69, 675-678.
3. Corey, E. J., Gin, D. Y. and Kania, R. S. 1996. Enantioselective Total Synthesis of Ecteinascidin 743. *J. Am. Chem. Soc.* **118**, 9202-9203.
4. D'Ambrosio, M., Guerriero, A. and Pietra, F. 1987. *Helv. Chim. Acta*. **70**, 2019-2027.
5. Garcia-Fernandez, L. F. and Reyes, F., Sanchez-Puelles. 2002. *Pharmaceutical News* **9**, 495-501.
6. Hale, K. J., Hummersone, M. G., Manaviazar, S. and Frigerio, M. 2002. The chemistry and biology of the bryostatin antitumour macrolides. *Nat. Prod. Rep.* **19**, 413-453.
7. Lindel, T., Jensen, P. R., Fenical, W., Long, B. H., Casazza, A. M., Carboni, J.and Fairchild, C. R. 1997. Eleutherobin, a New Cytotoxin that Mimics Paclitaxel (Taxol) by Stabilizing Microtubules. *J. Am. Chem. Soc.* **119**, 8744-8745.
8. Lin, Y. C., Huang, Y. L., KHALIL, A. T. , Chen, M. H. and Shen, Y. C. 2005. Juncenolides F and G, two new briarane diterpenoids from Taiwanese gorgonian

Junceella juncea. *Chem. Pharm. Bull.* **53**, 128-130.
9. Pettit, G. R., Cherry, L., Herald, Dennis L. Doubek, Delbert L. Herald, Edward Arnold and Jon Clardy. 1982. Isolation and structure of bryostatin 1. *J. Am. Chem. Soc,* **104**, 6846-6848.
10. Proksch, P. R., Edrada, A. and Ebel, R. 2002. Drugs from the seas - current status and microbiological implications. *Appl. Microbiol. Biotechnol*. **59**, 125-134.
11. Rinehart, K. L., Holt, T. G., Fregeau, N. L., Stroh, J. G., Keifer, P. A., Sun, F., Li, L. H. and Martin, D. G. 1990. Ecteinascidins 729, 743, 745, 759A, 759B, and 770: potent antitumor agents from the Caribbean tunicate Ecteinascidia turbinate**.** *J. Org. Chem.* **55**, 4512-4515.
12. Shen, Y. C., Chen, C. Y. and Kuo, Y. H. 2001. New Sesquiterpene Hydroquinones from a Taiwanese Marine Sponge, *Hippospongia metachromia*. *J. Nat. Prod*. **64**, 801-803.
13. Shen, Y. C., Cheng, Y. B., Lin, Y. C., Guh, J. H., Teng, C. M. and Ko, C. L. 2004. New Prostanoids with Cytotoxic Activity from Taiwanese Octocoral *Clavularia viridis*. *J. Nat. Prod*. **67**, 542-546.
14. Shen, Y. C., Ho, C. J., Kuo. Y. H. and Lin Y. S. 2006. Cespitulactones A and B, new diterpenoids from Cespitularia taeniata. *Bioorganic and Medicinal Chemistry Letters* **16**, 2369-2372.
15. Shen, Y. C., Lin, J. J., Wu, Y. R., Chang, J. Y., Duh, C. Y. and Lo, K. L., 2006. New norditerpenoids from Cespitularia hypotentaculata. *Tetrahedron Lett*. **47**, 6651-6655.
16. Shen, Y. C., Lin, Y. C., Ahmed, A. F. and Kuo, Y. H. 2005. New xenicane diterpenoids from Xenia florida. *Tetrahedron Lett.* **46**, 4793-4796.
17. Shen, Y. C., Lin, Y. C. and Huang, Y. L. 2003. *J. Chin. Chem. Soc.*, **50**, 1267-1270.
18. Shen, Y. C., Lin, Y. C., Ko, C. L. and Wang, L. T. 2003. New Briaranes from the Taiwanese Gorgonian *Junceella juncea*. *J. Nat. Prod.* **66**, 302-305.
19. Shen, Y. C., Lin, Y. S., Kuo, Y. H. and Cheng Y. B. 2005. Cespitulactams A, B, and C, three new nitrogen-containing diterpenes from Cespitularia taeniata May. *Tetrahedron Lett.* **46**, 7893-7897.
20. Shen, Y. C., Lo, K. L., Lin, Y. C., Khalil, Ashraf Taha, Kuo, Y. H. and Shih, P. S. 2006. *Tetrahedron Lett*. in press.
21. Shen, Y. C. and Prakash, C. V. S. 2000. Two New Acetylenic Derivatives and a New Meroditerpenoid from a Taiwanese Marine Sponge *Strongylophora durissima*. *J. Nat. Prod*. **63**, 1686-1688.
22. Shen, Y. C., Prakash, C. V. S. and Guh, J. H. 2004. *Tetrahedron Lett.* **45**, 4793-4796.