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Part 2: Occurrence of Racemic Natural Products and Their Biological Activities

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Abstract

Part 2 review is a continuing version of Part 1, providing more examples of racemic natural products isolated from higher plants and microorganisms. The natural products reported here are from the research articles mostly published over the past few years, 2018-2020. This part also provides the information of marine racemic natural products, which had been isolated from marine invertebrates.

Keywords: Natural Products, Racemic mixture, Racemate, Biological activity; Marine natural products

Introduction

Part 1 of the review article covers many examples of natural products isolated as a racemic mixture; these racemates were obtained from higher plants and microorganisms. Herein, racemic natural products are presented in Part 2, which provides more examples of racemic natural compounds isolated from plants and microorganisms. This review also gives examples of racemic mixtures of marine natural products, which were isolated from marine invertebrates.

Occurrence of Racemic Natural Products in Plants

Two racemic mixtures of polyprenylated acylcyclopentanones, evodialones A (**1**) and B (**2**), were obtained from the aerial parts of *Evodia lepta* (Figure 1).[1] Both evodialones A (**1**) and B (**2**) have 3-ethyl-1,1-diisopentyl-4-methylcyclopentane skeleton, however, their specific rotation values are close to zero, suggesting that they are racemates. Separation by chiral HPLC led to the isolation of a racemic mixture of evodialone A (**1**), giving pure enantiomers, (-)-evodialone A (**1a**) and (+)-evodialone A (**1b**) (Figure 1). Effort to separate evodialone B (**2**) by chiral HPLC had been made, but failed to isolate into pure enantiomers. The absolute configurations of (-)-evodialone A (**1a**) and (+)-evodialone A (**1b**) were spiro-established by a combination of computational techniques, i.e., gauge-independent atomic orbital calculation of 1D NMR data and experimental and time dependent density functionnal theory (TDDFT)-calculated electronic circular dichroism (ECD) spectra.[1] Antimicrobial activity of (-)-evodialone A (**1a**), (+)-evodialone A (**1b**), and (±)-evodialone B (**2**) was evaluated. They were inactive toward the bacterial strains tested. However, both compounds **1a** and **1b** exhibited antifungal activity against *Candida parapsilosis*, *C. glabrata*, *C. guilliermondii*, and *C. lusitaniae* with MIC values of 17.1-68.3 µM, while (±)-evodialone B (**2**) did not show the activity at 220 µM.[1]

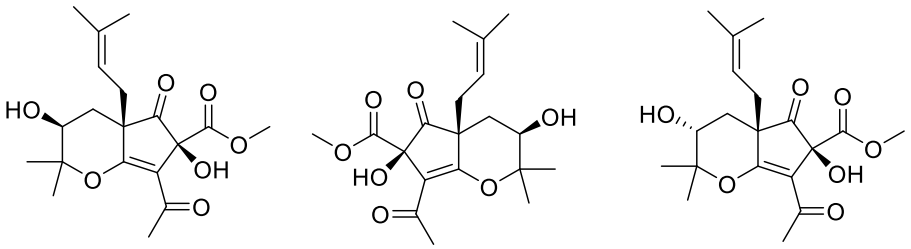
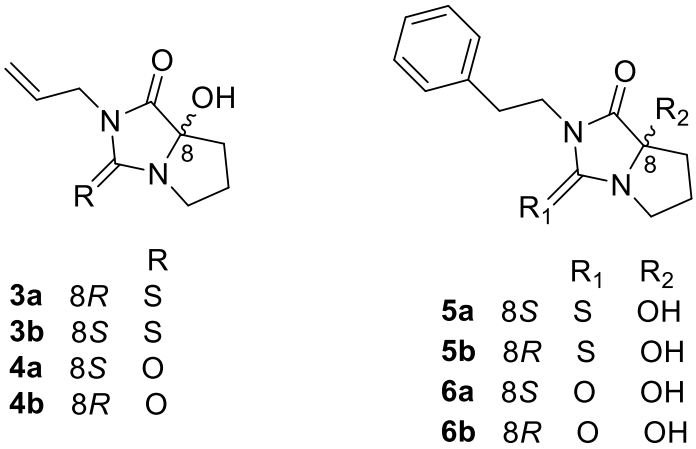


Figure 1: Structures of (-)-evodialone A (**1a**), (+)-evodialone A (**1b**), and (±)-evodialone B (**2**) isolated from *Evodia lepta*.

Armoracia rusticana or horseradish is an edible plant, and its roots are used as condiments and have intense pungency and flavor that is from sulfur-containing compounds. Investigation of a methanol extract of the roots of *A. rusticana* led to the isolation of two classes of compounds, thiohydantoins (**3** and **5**) and hydantoins (**4** and **6**) (Figure 2).[2] Compounds **3**-**6** had specific rotation values close to zero with the absence of Cotton effects in the ECD spectrum, suggesting the racemic property of these natural products. They have different absolute configuration at position 8 in the molecule (Figure 2). Individual enantiomers of compounds **3**-**6** were obtained after separation by chiral-phase semipreparative HPLC, giving enantiomers **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a**, and **6b** (Figure 2). The neuroprotective properties on nerve growth factor (NGF) induction in C6 glioma were evaluated for an individual enantiomer; the enantiomers **3b** and **4a** displayed potent NGF secretion stimulation activities, and they were more potent than 6-shogaol, a standard neuroactive compound of ginger.[3] These biological results indicated that the *N*-2 allylic functional group and the 8*S* absolute configuration in compounds **3b** and **4a** are important for the NGF induction property.



		R
3a	8*R*	S
3b	8*S*	S
4a	8*S*	O
4b	8*R*	O

		R_1	R_2
5a	8*S*	S	OH
5b	8*R*	S	OH
6a	8*S*	O	OH
6b	8*R*	O	OH

Figure 2: Structures of thiohydantoins (**3a**, **3b**, **5a** and **5b**) and hydantoins (**4a**, **4b**, **6a** and **6b**) isolated from the roots of *A. rusticana*.

Morus nigra is a Chinese herbal medicine used for improving immunity and anti-aging property. Two pairs of a flavonoid racemate, namely nigranol C (**7**) and nigragenon E (**8**), were isolated from the twigs of *M. nigra* (Figure 3).[4] Nigranol C (**7**) is an isoprenylated flavonol racemate uniquely featured with an unprecedented 7/6/6 ring system. Both nigranol C (**7**) and nigragenon E (**8**) did not have the Cotton effects in CD spectra, and their optical rotation values were close to zero. Chiral HPLC separation of compounds **7** and **8** provided pure enantiomers of (+)-nigranol C (**7a**), (-)-nigranol C (**7b**), (-)-nigragenon E (**8a**), and (+)-nigragenon E (**8b**) (Figure 3). The absolute configurations of **7a**, **7b**, **8a**, and **8b** were determined by experimental and TDDFT-calculated ECD spectra. Individual enantiomers were evaluated for their α-glucosidase and tyrosinase inhibitory activities. Note that the α-glucosidase inhibitory activity is one of the mechanisms for the design of antidiabetic drugs,[5,6] while tyrosinase inhibitory activity can be applied in cosmetics as whitening agent and in food and agriculture industries as antibrowning agents.[7] Compounds **7a**, **7b**, **8a**, and **8b** were found to be potent α-glucosidase inhibitors with IC_{50} values ranging from 9.79 to 30.21 μM, which were better than a positive control, acarbose (IC_{50} value of 987.90 μM). (-)-Nigragenon E (**8a**) and (+)-nigragenon E (**8b**) displayed tyrosinase inhibitory activity with IC_{50} values of 25.72 and 27.14 μM, respectively, which were comparable to that of kojic acid (IC_{50} value of 27.01 μM), a standard tyrosinase inhibitor.[4] The two pairs of enantiomers of compounds **7** and **8** exhibited comparable α-glucosidase and tyrosinase inhibitory activities, suggesting that the absolute configuration of these compounds did not have the influence on the inhibitory activity toward α-glucosidase and tyrosinase.

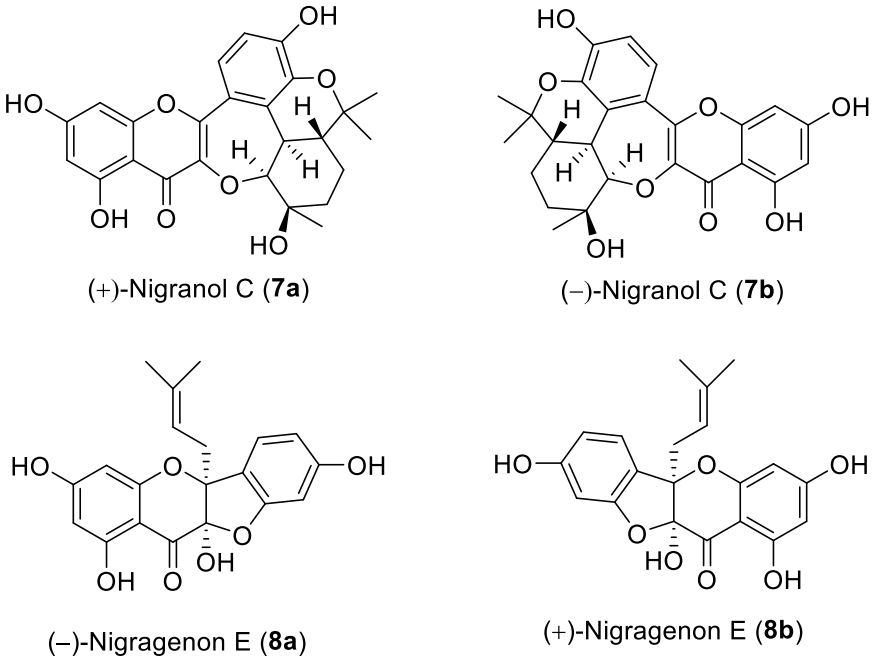


Figure 3: Structures of (+)-nigranol C (**7a**), (-)-nigranol C (**7b**), (-)-nigragenon E (**8a**), and (+)-nigragenon E (**8b**) isolated from the twigs of *M. nigra*.

Fruits of *Xanthium sibiricum* are used in Chinese herbal medicine, which are known as "Cang-Er-Zi" in Chinese Materia Medicinal material.[8] In the traditional Chinese medicine, fruits of *X. sibiricum* are used for the treatment of herpes, fever, leukoderma, sinusitis, headache, and cancer.[9-11] Enantiomers of (±)-xanthiazinones A-C, sulfur-containing natural products, were isolated from the fruits of *X. sibiricum*. The isolated enantiomers were (+)-xanthiazinone A (**9a**), (-)-xanthiazinone A (**9b**), (+)-xanthiazinone B (**10a**), (-)-xanthiazinone B (**10b**), (+)-xanthiazinone C (**11a**), and (-)-xanthiazinone C (**11b**) (Figure 4).[8] Comparison of NMR data of compound **9** with those of xanthiazone previously isolated from *X. sibiricum*[12] revealed a great deal of similarities of NMR data between compound **9** and xanthiazone. Detailed analysis of NMR data suggested that compound **9** contained a thiazinedione core structure attached to a pyrrolidinone moiety. Previous work assigned the thiazinedione core structure attached to a pyrrolidinone moiety via the nitrogen atom (Figure 4),[12] however a single crystal X-ray crystallographic analysis revealed that a linkage was through an oxygen atom of a hydroxyl group of a pyrrolidinone moiety.[8] Therefore, the structure of xanthiazone was revised accordingly as shown in Figure 4. A single crystal X-ray crystallographic analysis of compounds **10** and **11** was also performed, and the X-ray analysis not only confirmed the structures of compounds **10** and **11**, but also indicated the racemic nature of these compounds. Compounds **9**-**11** were separated by chiral HPLC, giving individual enantiomers of (+)-xanthiazinone A (**9a**), (-)-xanthiazinone A (**9b**), (+)-xanthiazinone B (**10a**), (-)-xanthiazinone B (**10b**), (+)-xanthiazinone C (**11a**), and (-)-xanthiazinone C (**11b**) (Figure 4). The absolute configurations of each enantiomer were established by ECD calculations.

The isolated compounds were tested for anti-inflammatory activity by suppressing lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells. (+)-Xanthiazinone B (**10a**) and (-)-xanthiazinone C (**11b**) exhibited anti-inflammatory activity with IC_{50} values of 8.75 and 19.44 μM, respectively. The drug dexamethasone was used as a standard drug, exhibiting an IC_{50} value of 10.50 μM. Therefore, (+)-xanthiazinone B (**10a**) is considered as a potent anti-inflammatory agent, while its corresponding enantiomer **10b** did not have the activity. (-)-Xanthiazinone C (**11b**) exhibited anti-inflammatory activity, however, its corresponding enantiomer **11a** was inactive. Therefore, the absolute configuration of this compound class is essential for anti-inflammatory activity. Compounds **9b**, **10a**, **11a**, and **11b** displayed cytotoxic activity against human cancer cell lines (HepG2, MCF-7, and A549 cancer cells) with IC_{50} values ranging from 24.23 to 87.22 μM.[8] Again, only certain enantiomers of compounds **9** and **10**, i.e., **9b** and **10a**, showed anticancer activity, indicating the importance of the absolute configuration for the activity. However, both enantiomers of compound **11**, i.e., **11a** and **11b**, displayed the activity, suggesting that the absolute configuration is not essential for the activity.

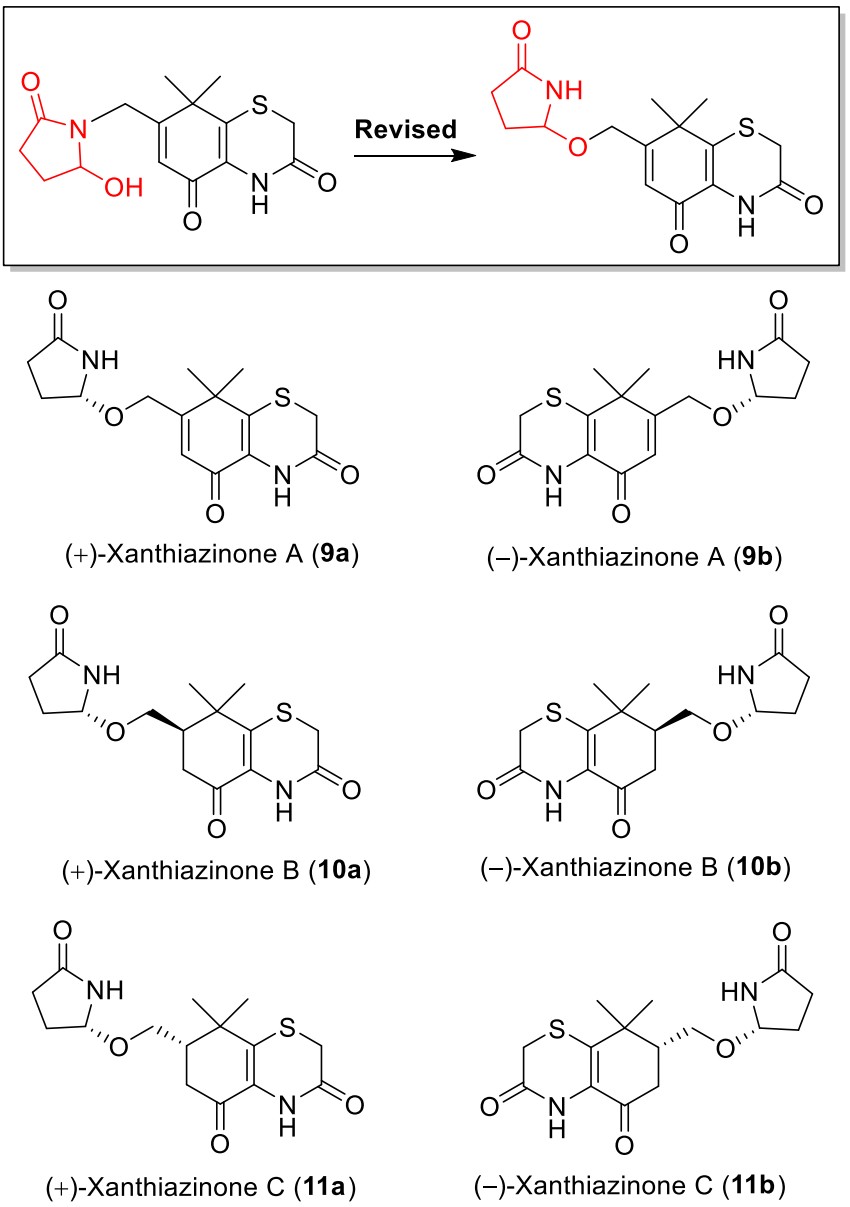
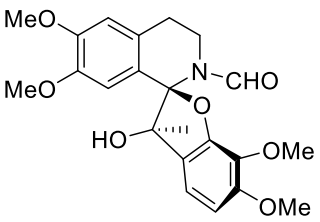
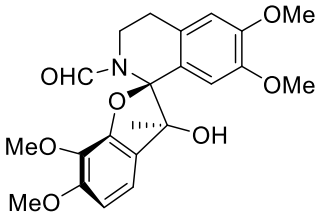


Figure 4: Structure revision of xanthiazinone; and structures of (+)-xanthiazinone A (**9a**), (-)-xanthiazinone A (**9b**), (+)-xanthiazinone B (**10a**), (-)-xanthiazinone B (**10b**), (+)-xanthiazinone C (**11a**), and (-)-xanthiazinone C (**11b**) isolated from the fruits of *X. sibiricum*.

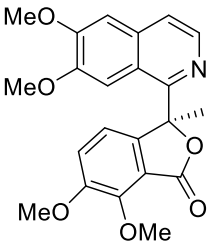
Corydalis yanhusuo is a Chinese herbal medicine, known as "Yan-Hu-Suo" in a traditional Chinese medicine (TCM). Dried tuber of *C. yanhusuo* is well documented in the Chinese Pharmacopoeia, and it is used for the treatment of abdominal pain, spasms and menstrual pain. [13] Six pairs of isoquinoline alkaloid enantiomers including (+)-yuanhusanine A (**12a**), (-)-yuanhusanine A (**12b**), (+)-yanhusanine B (**13a**), (-)-yuanhusanine B (**13b**), (+)-yanhusanine C (**14a**), (-)-yuanhusanine C (**14b**), (+)-yanhusanine D (**15a**), (-)-yuanhusanine D (**15b**), (+)-yanhusanine E (**16a**), (-)-yuanhusanine E (**16b**), (+)-yanhusanine F (**17a**), and (-)-yuanhusanine F (**17b**), were isolated from the aqueous tuber extract of *C. yanhusuo* (Figure 5).[13]



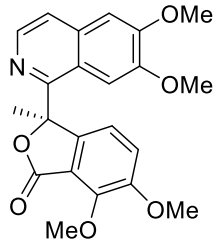
(+)-Yuanhusanine A (**12a**)



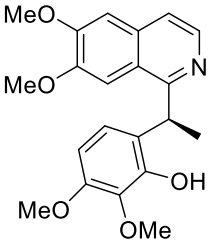
(–)-Yuanhusanine A (**12b**)



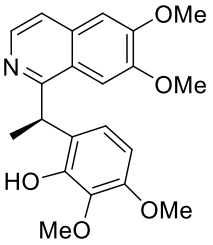
(+)-Yanhusanine B (**13a**)



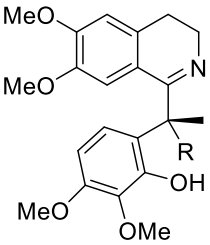
(–)-Yuanhusanine B (**13b**)



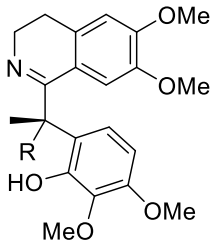
(+)-Yanhusanine C (**14a**)



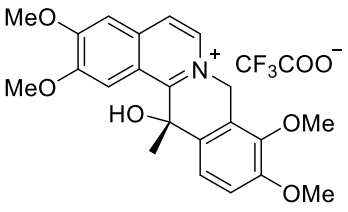
(–)-Yuanhusanine C (**14b**)



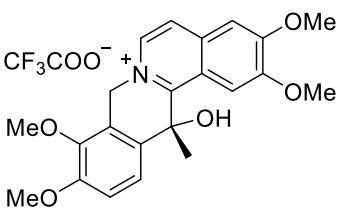
(+)-Yanhusanine D (**15a**), R = H
(+)-Yanhusanine E (**16a**), R = OMe



(–)-Yuanhusanine D (**15b**), R = H
(–)-Yuanhusanine E (**16b**), R = OMe



(+)-Yanhusanine F (**17a**)



(–)-Yuanhusanine F (**17b**)

Figure 5: Structures of (+)-yuanhusanine A (**12a**), (-)-yuanhusanine A (**12b**), (+)-yanhusanine B (**13a**), (-)-yuanhusanine B (**13b**), (+)-yanhusanine C (**14a**), (-)-yuanhusanine C (**14b**), (+)-yanhusanine D (**15a**), (-)-yuanhusanine D (**15b**), (+)-yanhusanine E (**16a**), (-)-yuanhusanine E (**16b**), (+)-yanhusanine F (**17a**), and (-)-yuanhusanine F (**17b**).

The isolated compounds **12**-**17** had the specific rotation close to zero, and lack of Cotton effects in CD spectra indicating that they were racemates. The enantiomer of individual compounds was obtained after the separation by Chiral HPLC, and the configurations of an individual enantiomer were determined by DP4+ NMR calculation methods and by comparison of experimental and calculated ECD spectra. The isolated compounds were evaluated for the inhibition of human carboxylesterases hCE1 and hCE2, the two isoenzymes which are involved in xenobiotic metabolism, and thus playing a role in drug metabolism and insecticide detoxication. [14] Except for compounds (+)-**12a**, (−)-**12b**, (+)-**14a**, and (−)-**14b**, the remaining enantiomers, selectively exhibited hCE2-mediated fluorescein diacetate hydrolysis. [13] The activity of (+)-yanhusanine B (**13a**) and (-)-yuanhusanine B (**13b**) was more active than that of the reference drug, loperamide. It is worth mentioning that each pair of this isoquinoline alkaloid enantiomer did not have different activity toward carboxylesterase enzyme, implying that the absolute configuration of this class of alkaloid is not important for carboxylesterase inhibitory activity.

Melicope patulinervia is in the Rutaceae family, which is comprised of many species, but *Melicope patulinervia* is the major species of this plant family. [15] *M. patulinervia* is found mainly in Hainan province, China, not in other areas. Previous investigations on this plant revealed the presence of flavonoids. [16] Three pairs of enantiomers of (±)-melipatulinones A-C (**18**-**20**) were isolated from *M. patulinervia*, and they are lignin-phloroglucinol hybrids decorated with a novel spiro[hydrobenzofuran-2,3'-furan] 5/5/6 tricyclic ring system, as well as spiro[cyclopenta[*b*]hydrofuran-2,3'-furan] 5/5/5 tricyclic framework (Figure 6). [17] The structure of melipatulinone A (**18**) was confirmed by X-ray analysis with Cu Kα radiation, but showing absence of a Flack parameter; this suggested that melipatulinone A (**18**) was a racemic mixture. Moreover, the value of optical rotation of melipatulinone A (**18**) was close to zero, and thus confirming the racemic nature of this compound. [17] Chiral HPLC separation led to the isolation of the two enantiomers, (+)-melipatulinone A (**18a**) and (-)-melipatulinone A (**18b**), whose absolute configurations were determined by ECD calculation and TDDFT. Optical rotation values of (±)-melipatulinones B and C (**19** and **20**) were also close to zero, implying that they are racemates. Individual enantiomers of (±)-melipatulinones B and C (**19** and **20**) were obtained after chiral HPLC separation, yielding (+)-melipatulinone B (**19a**), (-)-melipatulinone B (**19b**), (-)-melipatulinone C (**20a**), and (+)-melipatulinone C (**20b**), respectively (Figure 6). The absolute configurations of these enantiomers were determined by ECD calculation and TDDFT. The isolated compounds were evaluated for the inhibition of pancreatic lipase, which is one of the mechanisms for the drug design for controlling of obesity. [18] The racemic (±)-melipatulinones A-C (**18**-**20**) were found to inhibit pancreatic lipase with IC_{50} values of 6.14, 10.66, and 3.48 μM, respectively. [17] However, individual enantiomers of compounds **18**-**20** exhibited 2 to 5 folds less inhibitory activity than its corresponding racemate; this implied that the pair of enantiomer may have synergistic effect toward the inhibition of pancreatic lipase.

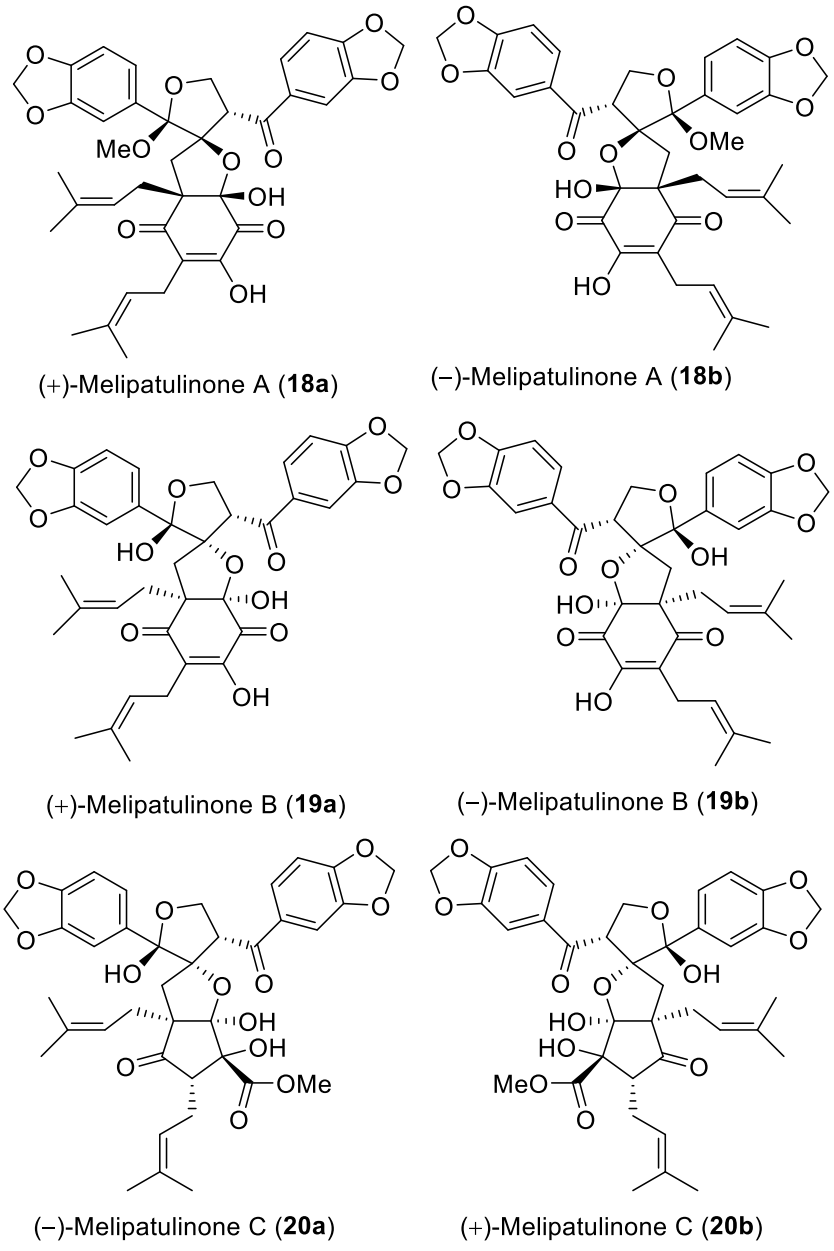


Figure 6: Structures of (+)-melipatulinone A (**18a**), (-)-melipatulinone A (**18b**), (+)-melipatulinone B (**19a**), (-)-melipatulinone B (**19b**), (-)-melipatulinone C (**20a**), and (+)-melipatulinone C (**20b**).

Occurrence of Racemic Natural Products in Microorganisms

Recent investigations of bioactive compounds from microorganisms revealed the presence of racemic mixtures of microbial metabolites. Three pairs of enantiomers of aromatic polyketide dimers, (±)-canescones A-C (**21**-**23**), were isolated from the fungus *Penicillium canescens* (Figure 7).[19] (±)-Canescones A-C (**21**-**23**) have an unprecedented 5/6/6/6/5 heterocyclic chemical structure, and they have a rare pentacyclic dihydrobenzo[1,4]dioxine core in their molecules. Canescone A (**21**) existed as a racemic mixture as revealed by a single crystal X-ray analysis with Cu Kα radiation,

showing the crystallization space group *P*21/*c*, which was centrosymmetric. X-ray analysis also revealed that canescones B and C (**22** and **23**) were racemic mixtures. Chiral HPLC separation of (±)-canescones A-C (**21**-**23**) gave pure enantiomers, (+)-canescone A (**21a**), (-)-canescone A (**21b**), (+)-canescone B (**22a**), (-)-canescone B (**22b**), (+)-canescone C (**23a**), and (-)-canescone C (**23b**), respectively (Figure 7). The absolute configurations of an individual enantiomer were determined by the experimental and simulated spectra generated by TDDFT at the B3LYP/6-311++G(d,p) level (Gaussian 09 W).[19] The isolated compounds were assessed for their protein tyrosine phosphatase 1B inhibitory activity, which is a drug target for the treatment of type 2 diabetes and obesity.[20,21] (+)-Canescone C (**23a**) and (-)-canescone C (**23b**) exhibited potent protein tyrosine phosphatase 1B inhibitory activity with respective IC_{50} values of 11.39 and 9.42 µM, while the positive control, oleanolic acid, had the IC_{50} value of 7.85 µM.[19] Both enantiomers **23a** and **23b** displayed relatively the same level of activity, implying that the absolute configuration is not essential for the inhibition of protein tyrosine phosphatase 1B enzyme.

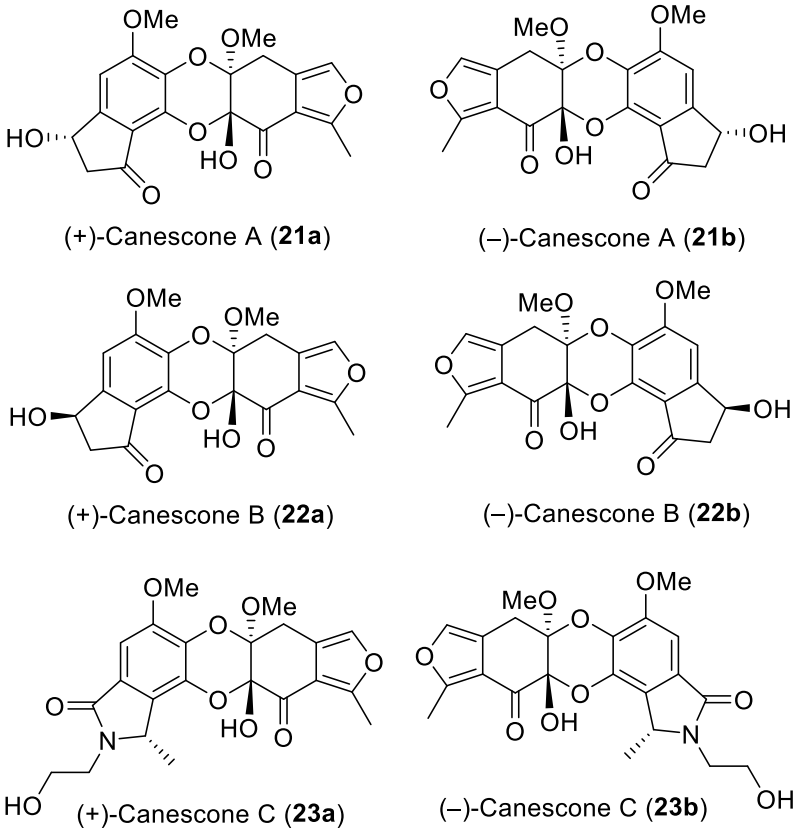


Figure 7: Structures of (+)-canescone A (**21a**), (-)-canescone A (**21b**), (+)-canescone B (**22a**), (-)-canescone B (**22b**), (+)-canescone C (**23a**), and (-)-canescone C (**23b**).

Three pairs of enantiomeric alkaloids, (+)-(*S*)-pratensilin A (**24a**), (−)-(*R*)-pratensilin A (**24b**), (+)-(*S*)-pratensilin B (**25a**), (-)-(*R*)-pratensilin B (**25b**), (+)-(*S*)-pratensilin C (**26a**), and (-)-(*R*)-pratensilin C (**26b**) (Figure 8), were isolated from a marine bacterium *Streptomyces* sp., which was isolated from a marine sediment in China.[22] These alkaloids have a novel algae indolinone-naphthofuran skeleton. The structures of pratensilin A (**24**) and pratensilin B (**25**) were elucidated by analysis of spectroscopic data, as well as by X-ray diffraction analysis. The centrosym-metric space obtained from X-ray analysis revealed that pratensilin A (**24**) was racemate. Chiral HPLC separation of compounds **24** and **25** successfully yielded pure enantiomers, (+)-(*S*)-pratensilin A (**24a**), (−)-(*R*)-pratensilin A (**24b**), (+)-(*S*)-pratensilin B (**25a**), and (-)-(*R*)-pratensilin B (**25b**). Pratensilin C (**26**) was a derivative of pratensilin B (**25**), and it also existed as a racemic mixture. Chiral HPLC separation led to the isolation of the two enantiomers, (+)-(*S*)-pratensilin C (**26a**) and (-)-(*R*)-pratensilin C (**26b**) (Figure 8).[22] The absolute configuration of each enantiomer was addressed by DFT and TDDFT calculations. Interestingly, a racemization of (+)-(*S*)-pratensilin A (**24a**) and (−)-(*R*)-pratensilin A (**24b**) was observed either in aprotic or in protic solvents, while that of (+)-(*S*)-pratensilin B (**25a**) and (-)-(*R*)-pratensilin B (**25b**) occurred in protic solvents. The racemization of these alkaloids might be *via* a keto-enol-type tautomerism. To prove the mechanism of the racemization, the hydroxyl group of (±)-pratensilin B (**25**) was protected by methylation to give a methylated product (**27**), which was then isolated by chiral HPLC to give the enantiomers, Me-(+)-(*S*)-pratensilin B (**27a**) and Me-(-)-(*R*)-pratensilin B (**27b**) (Figure 8). Indeed, the enantiomers of the methylated products **27a** and **27b** could not undergo racemization, confirming that the racemization of the alkaloids **24a** and **24b** or **25a** and **25b** occurs through a keto-enol-type tautomerism. Racemic mixture of pratensilin A (**24**) displayed moderate cytotoxic activity toward cancer cell lines, while that of pratensilin B (**25**) exhibited only weak activity. The enantiomers (+)-(*S*)-pratensilin C (**26a**) and (-)-(*R*)-pratensilin C (**26b**) showed weak cytotoxic activity, and both enantiomers displayed similar levels of cytotoxicity. The enantiomer Me-(+)-(*S*)-pratensilin B (**27a**) showed better cytotoxic activity than Me-(-)-(*R*)-pratensilin B (**27b**).[22]

Two pairs of alkaloids, (+)-farinomalein F (**28a**), (-)-farinomalein F (**28b**), (+)-farinomalein G (**29a**), and (-)-farinomalein G (**29b**) were isolated from the endophytic fungus *Phomopsis* sp. (Figure 9), which was isolated from a mangrove tree, *Kandelia candel*.[23] Optical rotation and CD spectra suggested that both farinomaleins F and G (**28** and **29**) existed as racemic mixtures. Individual enantiomers of farinomaleins F and G (**28** and **29**) were obtained after chiral HPLC separation, and the absolute configuration of each isomer was established by the calculated and experimental ECD data. Accordingly, the absolute configurations of (+)-farinomalein F (**28a**) and (-)-farinomalein F (**28b**) were assigned to be *2R* and *2S*, respectively. Similarly, the absolute configurations of (+)-farinomalein G (**29a**) and (-)-farinomalein G (**29b**) were also established by ECD calculation technique. Each enantiomer was evaluated for its anti-inflammatory activity through the inhibition of nitric oxide production in the lipopolysaccharide-induced mouse macrophages RAW 264.7 cells. The enantiomers of farinomaleins F and G (**28** and **29**) exhibited weak anti-inflammatory activity with IC_{50} values of 48.5-50.0 μM.[23]

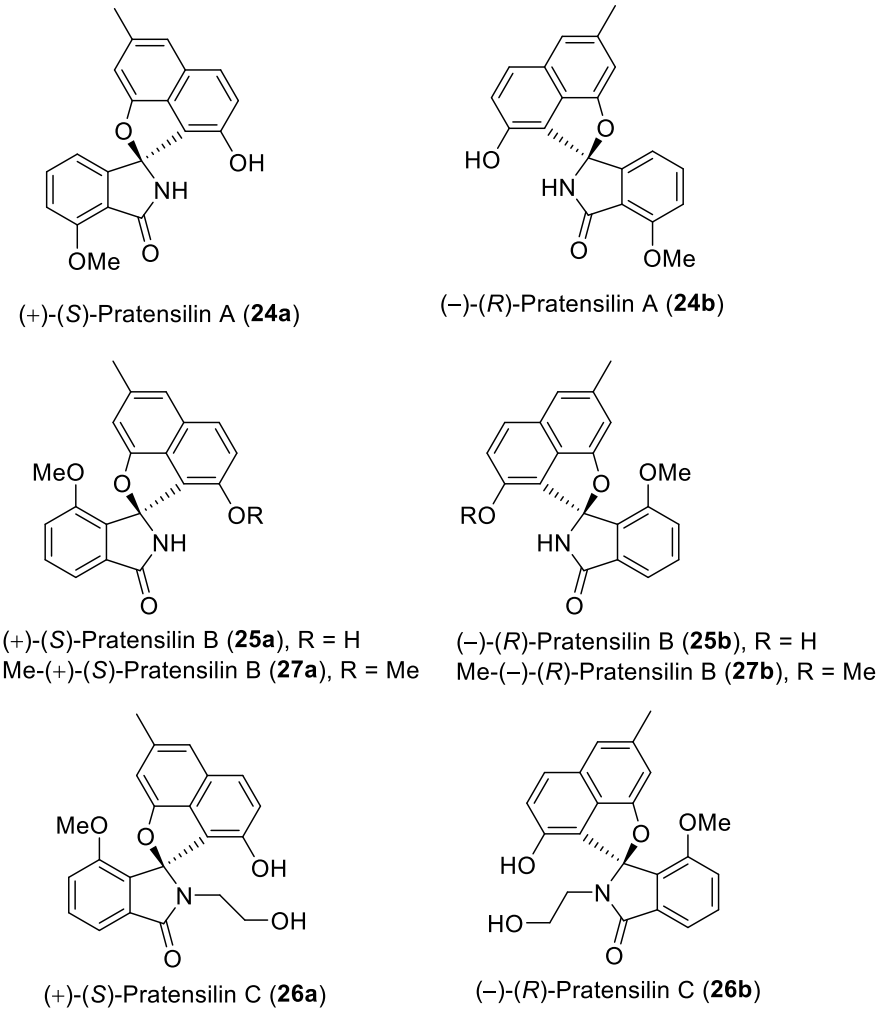
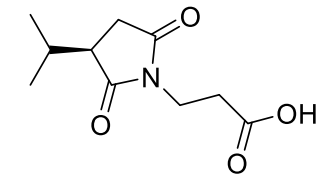
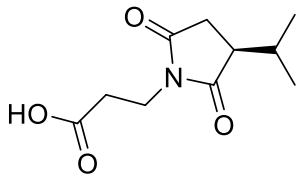


Figure 8: Structures of (+)-(*S*)-pratensilin A (**24a**), (–)-(*R*)-pratensilin A (**24b**), (+)-(*S*)-pratensilin B (**25a**), (-)-(*R*)-pratensilin B (**25b**), (+)-(*S*)-pratensilin C (**26a**), (-)-(*R*)-pratensilin C (**26b**), Me-(+)-(*S*)-pratensilin B (**27a**), and Me-(-)-(*R*)-pratensilin B (**27b**).

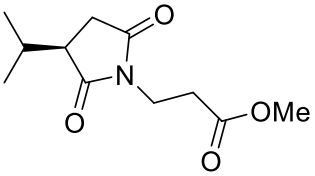
A pair of an enantiomeric benzo-phenone-hemiterpene hybrid, (+)-cytorhizophin A (**30a**) and (-)-cytorhizophin A (**30b**), was isolated from the endophytic fungus *Cytospora rhizophorae* (Figure 9), which was isolated from a Chinese herb, *Morinda officinalis*.[24] Cytorhizophin A (**30**) had an unprecedented 6/ 7/ 6/ 7 tetracyclic fused ring system, and its structure was confirmed by a single crystal X-ray analysis, which also indicated that cytorhizophin A (**30**) existed as a racemic mixture. The enantiomers, (+)-cytorhizophin A (**30a**) and (-)-cytorhizophin A (**30b**), were isolated by chiral HPLC separation, and ECD calculations were used to address the absolute configurations of the two enantiomers. Both enantiomers were evaluated for anti-microbial activity, but unfortunately they did not show the activity at a concentration of 100 μg/mL.[24] alga



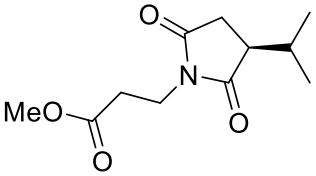
(+)-Farinomalein F (**28a**)



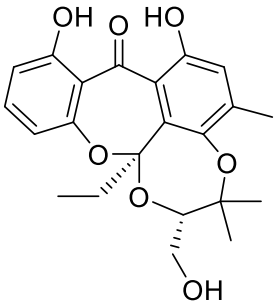
(–)-Farinomalein F (**28b**)



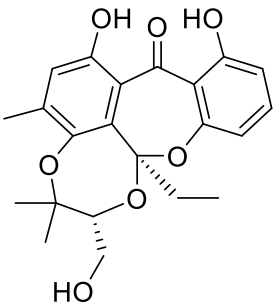
(+)-Farinomalein G (**29a**)



(–)-Farinomalein G (**29b**)



(+)-Cytorhizophin A (**30a**)



(–)-Cytorhizophin A (**30b**)

Figure 9: Structures of (+)-farinomalein F (**28a**), (-)-farinomalein F (**28b**), (+)-farinomalein G (**29a**), (-)-farinomalein G (**29b**), (+)-cytorhizophin A (**30a**), and (-)-cytorhizophin A (**30b**).

Occurrence of Racemic Natural Products in Marine Invertebrates

Marine natural products are important sources of bioactive compounds in drug discovery because many drugs are inspired or derived from marine natural products. [25-27] Marine organisms, i.e., marine sponges, algae, and tunicates, are rich sources of natural products with a variety of chemical skeletons and biological activities. [28-30] A few racemic mixtures of marine natural products have been recently reported. Here are examples of racemic natural products isolated from marine bio-resources.

Screening of antifungal activity of crude extracts of marine invertebrates revealed that an extract of a marine sponge *Hippospongia lachne* had antifungal property. A pair of enantiomeric sesterterpenoid, hippolide J (**31**) (Figure 10), was isolated from the sponge *H. lachne*. [31] The specific rotation value of + 20 for hippolide J (**31**) suggested that it might be a racemic mixture, and chiral HPLC analysis revealed the enantiomeric ratio of 47:53 for the two enantiomers, (-)-hippolide J (**31a**) and (+)-hippolide J (**31b**) (Figure 10). The absolute configurations of the two enantiomers were addressed by the calculated ECD spectra through TDDFT method. Both (-)-hippolide J (**31a**) and (+)-hippolide J (**31b**) showed potent antifungal activity against *Trichophyton rubrum*, *Candida albicans*, and *C. glabrata* with the MIC values of 0.125–0.25 μg/mL, which were more potent than antifungal drugs, itraconazole

(MIC values of 2-32 μg/mL) and terbinafine (MIC values of 0.5-64 μg/mL). Moreover, both enantiomers (**31a** and **31b**) displayed only weak cytotoxic activity, suggesting that both compounds have potential in further antifungal drug development.[31] However, the two enantiomers (**31a** and **31b**) had the same magnitude of activity, indicating that the stereochemistry of this compound type does not play a significant role for the antifungal activity.

Two pairs of enantiomers of furan butanolides, sponalisolides A (**32**) and B (**33**), were isolated from a marine sponge *Spongia officinalis* (Figure 10).[32] Sponalisolide A (**32**) had a butanolide moiety, while sponalisolide B (**33**) was decorated with an *N*-acyl homoserine lactone moiety. Both sponalisolides A (**32**) and B (**33**) were optically inactive, indicating the racemic nature of these sponge metabolites. Enantiomers of the two compounds, (+)-sponalisolide A (**32a**), (-)-sponalisolide A (**32b**), (+)-sponalisolide B (**33a**), and (-)-sponalisolide B (**33b**) were obtained after chiral HPLC separation. The total synthesis of sponalisolides A (**32**) and B (**33**) was performed in order to assign the absolute configuration of an individual enantiomer. Individual enantiomer of each compound was synthesized, and finally the absolute configuration of each natural enantiomer was assigned as shown in Figure 10.[32] The enantiomers, (**32a**), (**32b**), (**33a**), and (**33b**), displayed inhibitory activity against LasR and functioned as *Pseudomonas aeruginosa* quorum sensing inhibitors. LasR is a transcriptional activator of *P. aeruginosa* virulence genes.[33]

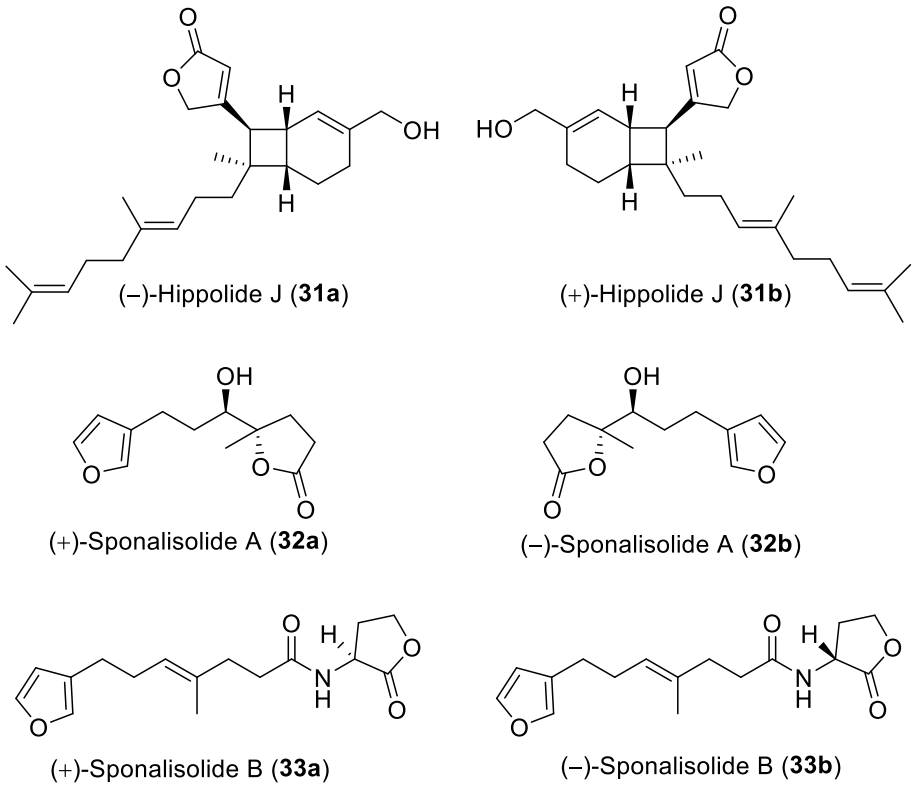


Figure 10: Structures of (-)-hippolide J (**31a**), (+)-hippolide J (**31b**), (+)-sponalisolide A (**32a**), (-)-sponalisolide A (**32b**), (+)-sponalisolide B (**33a**), and (-)-sponalisolide B (**33b**).

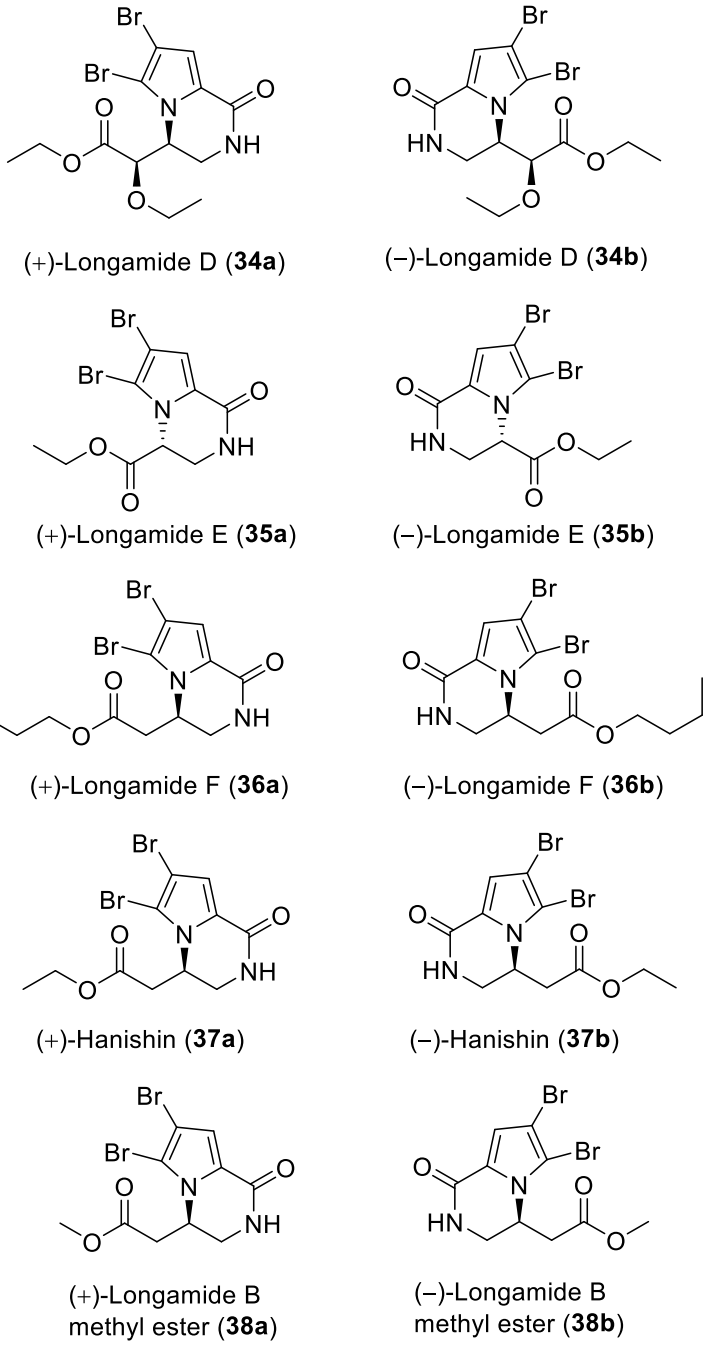


Figure 11: Structures of (+)-longamide D (**34a**), (-)-longamide D (**34b**), (+)-longamide E (**35a**), (-)-longamide E (**35b**), (+)-longamide F (**36a**), (-)-longamide F (**36b**), (+)-hanishin (**37a**), (-)-hanishin (**37b**), (+)-longamide B methyl ester (**38a**), and (-)-longamide B methyl ester (**38b**).

Screening of extracts of marine organisms showed that an extract of a marine sponge *Agelas* sp. collected from the Xisha Islands, South China Sea, exhibited antifungal activity. Bromopyrrole alkaloids including (+)-longamide D (**34a**), (-)-longamide D (**34b**), (+)-longamide E (**35a**), (-)-longamide E (**35b**), (+)-longamide F (**36a**), (-)-longamide F (**36b**), (+)-hanishin (**37a**), (-)-hanishin (**37b**), (+)-longamide B methyl ester (**38a**), and (-)-longamide B

methyl ester (**38b**) were isolated from the sponge *Agelas* sp. (Figure 11).[34] Among these alkaloids, hanishin (**37**) and longamide B methyl ester (**38**) were previously obtained from the marine sponges, *Acanthella carteri* and *Agelas ceylonica*, respectively.[35,36] Optical rotation values of these bromopyrrole alkaloids were close to zero, suggesting that they were racemates. Chiral HPLC separation led to the isolation of an individual enantiomer with the structure shown in Figure 11. The absolute configurations of these enantiomers were established by ECD calculations, as well as by comparison of CD spectra. Among the enantiomers isolated, (+)-longamide D (**34a**), (-)-longamide E (**35b**), (+)-longamide F (**36a**), (+)-hanishin (**37a**), and (+)-longamide B methyl ester (**38a**) exhibited antifungal activity. However, the opposite configuration of the corresponding enantiomer did not show the activity. These results indicate that the absolute configuration significantly contributes to the effects toward antifungal activity of bromopyrrole alkaloids.[34]

In summary, several natural products existing as racemic mixtures have been isolated from higher plants, microorganisms, and marine invertebrates. Natural racemates have a variety of chemical skeletons with diverse biological activities.

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