Abstract

Marine soft corals are known to produce a wide array of secondary metabolites, particularly diterpenoids and steroids, and often characterized by uncommon structural features and potent bioactivities. The remarkable abundance and diversity of bioactive small molecule which have been isolated from soft corals have made these organisms an important source of new drug candidates for human diseases, particularly for their anti-inflammatory activity. In this paper, the authors reported anti-inflammatory marine natural products isolated from diverse species of soft corals determined in vitro by their inhibition of lipopolysaccharide-induced expression of inducible NO synthase and cyclooxygenase-2 in murine macrophage cells (RAW 264.7).

Keywords:
- Soft corals
- Lead compounds
- Anti-inflammatory
- Secondary metabolites

1. Introduction

Natural products have been proven to be rich sources of novel compounds exhibiting many different biological activities. Their chemical structures are diverse and complex, and they have often demonstrated selective activities in various biological systems[1]. Compared to the terrestrial environment, which was the focus of the pharmaceutical industry for more than 50 years, marine habitats have remained virtually unexplored for their ability to yield pharmacological metabolites[2]. In the last several decades, researches have expanded from the land to the ocean in order to find new leads for drug candidates and more than 30 000 unique bioactive natural products have been isolated from marine organisms[3]. It has been estimated that the percentage of new metabolites discovered from soft corals represented up to 22% of the total new marine natural products reported from 2010 to 2011[4,5].

Soft corals belonging to the family of Alcyoniidae are characterized by a great variety of colours, shapes, and sizes and they are by far the most dominant reef dwelling octocorals in the Indo-West Pacific. These organisms are known to produce a wide array of secondary metabolites, particularly diterpenoids, sesquiterpenoid and steroids, and often characterized by uncommon structural features. A number of natural products isolated from soft corals have demonstrated that they are of great biomedical interests having antiviral, anti-tumor, anti-inflammatory and anti-fouling properties[4,6].

Inflammation is a pathological condition in which highly reactive species are produced[7]. Increasing evidence suggests a critical link between inflammation and the chronic promotion/progression of various human diseases, including atherosclerosis, diabetes, arthritis, inflammatory bowel disease, cancer and alzheimer[5]. The mechanisms and mediators involved in painful and inflammatory processes have been the targets of several studies in recent years[8]. During the process of inflammation, different cell types are recruited, including monocytes which differentiate locally into macrophages. This leads to the regulated production of various pro- and anti-inflammatory mediators including cytokines, such as tumor necrosis factor-α, chemokines and inducible enzymes [cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS)] which play critical roles in controlling the inflammatory processes[9]. Several natural products from marine organisms, including soft coral, sponges and algae, with anti-inflammatory effects have attracted researchers’ attention in the
past few years.

2. Anti-inflammatory activity

During 2005–2012, 19 studies reported anti-inflammatory marine natural products isolated from diverse species of soft corals such as Sinularia, Paralemnalia, Lobophytum, Nephthea, Klyxum and Sarcophyton. In this paper, we reported anti-inflammatory activities of natural products from soft corals determined in vitro by their inhibition of lipopolysaccharide (LPS)-induced expression of iNOS and COX-2 in murine macrophage cells (RAW 264.7). Information from the year 2005 to 2012 was obtained from existing reviews and relevant articles[5,10-15].

2.1. Sesquiterpenoid

Huang et al. described a novel sesquiterpenoid isoparalemnone (1) from the Formosan soft coral Paralemnalia thyrsoides which significantly inhibited inflammatory iNOS protein expression (70% at 10 μmol/L) in activated RAW 264.7 cells[16]. Cheng et al. isolated a new sesquiterpenoid erectathiol (2) from Nephthea erecta. In vitro anti-inflammatory activity of (2) significantly reduced the levels of the iNOS protein [(58.0 ± 6.5)%] and COX-2 protein [(108.7 ± 4.5)]% (Figure 1)[17].

![Figure 1. Sesquiterpenoid from soft corals.](image)

2.2. Diterpenoid

Chao et al. identified the new cembranoides crassumolides A and C (3, 4) from the soft coral Lobophytum crassum which inhibited the expression of iNOS and COX-2 (apparently IC<sub>50</sub> was less than 10 μmol/L)[18]. Similarly, from the same university, Cheng et al. found that new cembranoides from the soft coral Lobophytum durum, durumolides A–C (5, 6, 7) inhibited both the iNOS and COX-2 proteins in LPS-activated RAW 264.7 cells in vitro (apparently IC<sub>50</sub> was less than 10 μmol/L) suggesting that the α-methylene-γ-lactone moiety of these compounds was necessary for the observed activity[19]. Cheng et al. also isolated durumhemiketalolides A–C (8, 9, 10) and durumolide F (11) from the soft coral Lobophytum durum with anti-inflammatory activity[20,21]. Both compounds 8 and 10 reduced the levels of iNOS to (11.0 ± 1.3)% and (0.0 ± 0.0)%, respectively, and of COX-2 to (66.7 ± 6.4)% and (34.7 ± 4.2)%, respectively. The compound 9 reduced iNOS protein expression (6.4 ± 0.2%), but did not inhibit COX-2 protein expression. The anti-inflammatory activity of the compound 11 (10 μmol/L) significantly reduced the levels of the iNOS protein to (0.8 ± 0.6)% and COX-2 protein to (47.8 ± 9.0)%. Two new Lobophytum crassum diterpenes (12, 13) isolated by Wanzola et al. showed significant inhibitory effect of NO production, and their IC<sub>50</sub> values were less than 10 μmol/L without any cytotoxic effect[22]. The inhibitory mechanism of these cembranoids was confirmed by the inhibition of iNOS expression via the suppression of a transcription factor nuclear factor κB.

Klysimplexin sulfoxide C (14) and simplexin E (15) were isolated from the soft coral Klyxum simplex[23,24]. The compound 14 at a concentration of 10 μmol/L significantly reduced the levels of iNOS protein to (11.3 ± 1.5)% and COX-2 expression [(7.2 ± 2.5)%]. At a concentration of 10 μmol/L, compound 15 was found to significantly reduce the levels of iNOS and COX-2 proteins to (4.8 ± 1.8)% and (37.7 ± 4.7)%, respectively, which was relative to the control cells stimulated with LPS only.

Lin et al. isolated sarcocrassocolides C (16) and D (17) from soft coral Sarcophyton crassocaulis[25]. Compounds 16 and 17 were shown to exert significant in vitro anti-inflammatory activities in LPS-stimulated RAW 264.7. Cheng et al. also found glycosanilides B (18) and C (19) from Sinularia gyrosa[26]. Compounds 18 and 19 at concentration of 10 μmol/L did not inhibit the COX-2 protein expression, but significantly reduced the levels of the iNOS protein (55.2% ± 14.6%, 10.6% ± 4.6%, respectively) by LPS stimulation (Figure 2).

2.3. Steroid

Ahmed et al. reported that the known steroid gibberoketosterol (20) isolated from the Formosan soft coral Sinularia gibberosa significantly reduced pro-inflammatory iNOS and COX-2 proteins in LPS-stimulated murine macrophages at a concentration of 10 μmol/L to 44.5% and 68.3% of control values, respectively[27]. Cheng et al. isolated chabrosterol (21) and nebrosteroid I (22) from the soft coral Nephthea chabroli which significantly reduced the levels of the iNOS protein (12.4% ± 2.9% and 20.2% ± 2.6%) and COX-2 protein (45.2% ± 5.4% and 75.3% ± 3.3%) (Figure 3)[17].

![Figure 3. Steroid from soft corals.](image)

2.4. Glycolipid

Sarcocrenosides A (23) and B (24) were isolated from Sarcophyton ehrenbergi. Compounds 23 and 24 reduced iNOS
protein expression (47.3% ± 7.1%, 46.5% ± 5.3%, respectively), but did not inhibit COX-2 protein expression (Figure 4)\[28\].

![Chemical structures](image)

**Figure 2.** Diterpenoid from soft corals.
3. Anti-inflammatory activity from Indonesian soft corals

3.1. Diterpenoid

Chemical analysis of the less polar fractions of the organic extract obtained from *Sinularia* sp. (order Alcyonacea, family Alcyoniidae) resulted in two known C-4 norcembranoids, named leptocladolide B (25) and scabrolide D (26), and three new ones, named chloroscabrolides A (27) and B (28) and prescabrolide (29) (Figure 5). All the norcembranoids were evaluated for their anti-inflammatory activities. A 15% inhibition of NO₂-production was observed in scabrolide D (26) at a concentration of 10 \( \mu \text{mol/L} \) (Figure 5) [13].

3.2. Glycolipids

A new glycolipid, named sinularioside (30) isolated from *Sinularia* sp. At different concentrations (10, 30, 100 \( \mu \text{mol/L} \)) of sinularioside (30), a significant dose-dependent (\( P < 0.001 \)) inhibition of NO₂-production was observed with 58% inhibition at 30 \( \mu \text{mol/L} \) (Figure 6) [14].

3.3. Alkaloids

Another polar fraction from *Sinularia* sp yielded two new alkaloids, named sinulasulfoxide (31) and sinulasulfone (32). Sinulasulfoxide (31) (Figure 7) was also evaluated for a inhibition of NO production on LPS-stimulated macrophages. The compound was moderately active with about 25% inhibition at 30 \( \mu \text{mol/L} \) [15].

4. Conclusion

Since seven marine natural products or derivatives in different phases of the clinical pipeline have been approved by the Food and Drug Administration or the European Medicines Agency, twelve compounds are in Phase I, II and III of clinical development, and the global marine preclinical pharmaceutical pipeline remains very active. Marine soft corals have been recognized as prolific producers of a wide array of secondary metabolites. Most of secondary
metabolites from soft corals have been studied for their anti-inflammatory activities and have been focused on “screening-like” assays by using COX-2 and iNOS as target markers.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Bernan VS, Greenstein M, Maiese WM. Marine microorganisms as a source of new natural products. Adv Appl Microbiol 1997; 43: 57-90.
[2] Bad MJ, Bedoya LM, Bermejo P. Marine compounds and their antimicrobial activities. In: Mendez-Vilas A, editor. Science against microbial pathogens: communicating current research and technological advances. Badajoz: FORMATEX; 2011. p. 1293-306.
[3] Fattorusso E, Gerwick WH, Taglialatela-Scafati O. Handbook of marine natural products. Berlin: Springer; 2012.
[4] Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. Marine microorganisms as a source of new natural products. Adv Appl Microbiol 1997; 43: 57-90.
[5] Bad MI, Bedoya LM, Bermejo P. Marine compounds and their antimicrobial activities. In: Mendez-Vilas A, editor. Science against microbial pathogens: communicating current research and technological advances. Badajoz: FORMATEX; 2011. p. 1293-306.
[6] El Sayed KA, Hamann MT, Waddling CA, Jensen C, Lee SK, Danstan CA, et al. Structurally novel bioconversion products of the marine natural product sarcophyne effectively inhibit JB6 cell transformation. J Org Chem 1998; 63: 7449-55.
[7] Lopes G, Daletos G, Proksch P, Andrade PB, Valenti P. Anti-inflammatory potential of monoglactosyl diacylglycerols and a monoglycerol from the edible brown seaweed Fucus spiralis Linnaneus. Mar Drugs 2014; 12(3): 1406-18.
[8] Cavalcante-Silva LH, da Motta CB, de Araujo MV, Barbosa-Filho JM, de Lima DP, de Oliveira Santos BV, et al. Antinociceptive and anti-inflammatory activities of crude methanolic extract of red alga Bryothamnion triquetrum. Mar Drugs 2012; 10(9): 1977-92.
[9] Chatter R, Othman RB, Rabhi S, Khaled M, Tahouni S, Vaghasia C, et al. In vivo and in vitro anti-inflammatory activity of neorogioltriol, a new diterpene extracted from the red algae Laurencia glandulifera. Mar Drugs 2011; 9(7): 1293-306.
[10] Aleyan AM, Rodriguez AD, Berlincik RG, Hamman MT. Marine pharmacology in 2005-6: marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Biochim Biophys Acta 2009; 1790: 283-308.
[11] Aleyan AM, Rodriéguez AD, Berlincik RG, Fusetani N. Marine pharmacology in 2007-8: marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol 2011; 153: 191-222.
[12] Mayer AM, Rodríguez AD, Taglialatela-Scafati O, Fusetani N. Marine pharmacology in 2009-2011: marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Mar Drugs 2013; 11: 2510-73.
[13] Fattorusso E, Luciano P, Putra MY, Taglialatela-Scafati O, Ianaro A, Panza E, et al. Chloroscorbolidines, chlorinated norcarbimidines from the Indonesian soft coral Sinularia sp. Tetrahedron 2011; 67: 7983-8.
[14] Putra MY, Ianaro A, Panza E, Bavestrello G, Cerrano C, Fattorusso E, et al. Sinularioside, a triacylated glycolipid from the Indonesian soft coral Sinularia sp., is an inhibitor of NO release. Bioorg Med Chem Lett 2012; 22: 2723-5.
[15] Putra MY, Ianaro A, Panza E, Bavestrello G, Cerrano C, Fattorusso E, et al. Sinulasulfoxide and sinulasulfone, new sulfur-containing alkaloids from the Indonesian soft coral Sinularia sp. Tetrahedron Lett 2012; 53: 3937-9.
[16] Huang HC, Wen ZH, Chao CH, Ahmed AF, Chiang MY, Kuo YH, et al. Novel sesquiterpenoids from the Formosan soft coral Paralemmalia thyrsoides. Tetrahedron Lett 2006; 47: 8751-5.
[17] Cheng SY, Huang YC, Wen ZH, Chio SF, Wang SK, Hsu CH, et al. Novel sesquiterpenes and noresterogsterol from the soft corals Nephthea erecta and Nephthea chabroi. Tetrahedron Lett 2009; 50: 802-6.
[18] Chao CH, Wen ZH, Wu YC, Yeh HC, Sheu JH. Cytotoxic and anti-inflammatory compounds from the soft coral Lobophyllum crassum. J Nat Prod 2008; 71: 1819-24.
[19] Cheng S, Wen Z, Chio S, Hsu C, Wang S, Dai C, et al. Durumolides A-E, anti-inflammatory and antibacterial sesquiterpenoids from the soft coral Lobophyllum durum. Tetrahedron 2008; 64: 9698-704.
[20] Cheng SY, Wen ZH, Wang SK, Chio SF, Hsu CH, Dai CF, et al. Unprecedented hemiketal cembranolides with anti-inflammatory activity from the soft coral Lobophyllum durum. J Nat Prod 2009; 72: 152-5.
[21] Cheng SY, Wen ZH, Wang SK, Chio SF, Hsu CH, Dai CF, et al. Anti-inflammatory compounds from the soft coral Lobophyllum durum. Bioorg Med Chem 2009; 17: 3763-9.
[22] Wanzola M, Furuta T, Kohno Y, Fukumitsu S, Yasukochi S, Watari K, et al. Four new cembrane diterpenes isolated from an okawan soft coral Lobophyllum crassum with inhibitory effects on nitric oxide production. Chem Pharm Bull 2010; 58: 1203-9.
[23] Chen BW, Chao CH, Su JH, Wen ZH, Sung PJ, Sheu JH. Anti-inflammatory eunicellin-based diterpenoids from the cultured soft coral Sinularia gyrosa. Bioorg Med Chem 2010; 8: 2363-6.
[24] Wu SL, Su JH, Wen ZH, Hsu CH, Chen BW, Dai CF, et al. Simplexins A-I, eunicellin-based diterpenoids from the soft coral Sinularia gibberosa. J Nat Prod 2009; 72: 994-1000.
[25] Lin WY, Su JH, Lu Y, Wen ZH, Dai CF, Kuo YH, et al. Cytotoxic and anti-inflammatory compounds from the Dongsha A tolii soft coral Sarcophyton crassocaule. Bioorg Med Chem 2010; 18: 1936-41.
[26] Cheng SY, Chuang CT, Wen ZH, Wang SK, Chio SF, Hsu CH, et al. Bioactive norditerpenoids from the soft coral Sinularia thyrsoides. Bioorg Med Chem 2010; 18: 3379-86.
[27] Ahmed AF, Hsieh YT, Wen ZH, Wu YC, Sheu JH. Polyoxygenated stilbenoids from a marine sponge of the Formosan soft coral Sinularia thyrsoides with inhibitory effects on nitric oxide production. Chem Pharm Bull 2010; 58: 1203-9.