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RESEARCH ARTICLE

Qualitative Detection of Some Secondary Metabolites from Three Turkish Marine Sponges

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Qualitative Detection of Some Secondary Metabolites from Three Turkish Marine Sponges

Summary

During our search for bioactive compounds from Turkish marine sponges, we have detected secondary metabolites from three different marine sponges, which have been collected from Kaş (South Coast of Turkey). Compounds were detected by HPLC method from methanolic extracts of three sponge species, Haliclona cratera (Chalinidae), Axinella damicornis (Axinellidae), Stylissa carteri (Dictyonellidae). Our sponge samples contained bromopyrrole type of alkaloids, brominated alkaloids, pentacyclic alkaloids that showed similarities with the studies carried out by previous researchers.

Key Words: *Secondary metabolites, sponge, alkaloids*

Türkiye Denizlerindeki Süngerlerde Bazı İkincil Metabolitlerin Teşhisi

Özet

Türkiye denizlerindeki süngerlerden biyoaktif maddelerin araştırılması konusunda devam eden çalışmalarımızın bir bölümünde, Kaş'tan (Türkiye'nin Güney Kıyısı) toplanmış üç farklı deniz süngerindeki sekonder metabolitler tespit edilmiştir. Tüm tespit analizleri, Haliclona cratera (Chalinidae), Axinella damicornis (Axinellidae), Stylissa carteri (Dictyonellidae) süngerleri metanollü ekstreleri HPLC de analiz edilmiştir. Süngerlerde tespit edilmiş bromopirol alkaloitler, bromlu alkaloitler ve pentasiklik alkaloitler önceki araştırmacılar tarafından yürütülen çalışmalar ile benzerlik göstermektedir.

Anahtar Kelimeler: *İkincil metabolitler, süner, alkaloitler*

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INTRODUCTION

Oceans cover over 70% of the world's surface and they are rich biodiversitic habitats where life began. Due to their longer evolutionary history, marine organisms have a greater molecular diversity than terrestrial organisms. The lack of natural defence systems (e.g. innate immune system) in the majority of invertebrates leads to development of biologically active secondary metabolites, and these compounds play a role in the defence of the host habitat and adaption to environmental conditions (1).

More than 15.000 marine bioactive compounds have been isolated and tested in the last 20 years until 2012. Sponges are champion producers with large diversity of natural components. They are responsible for more than 5300 different bioactive compounds and every year hundreds of new compounds are being discovered. Marine sponges have provided many examples of novel bioactive compounds that were tested for their anti-inflammatory hypocholesteromeric, antitumor, immunosuppressive, neurosuppressive, muscle relaxants, antiviral, antimalarial, antibiotic,and antifouling potentials. Therefore marine organisms, especially marine sponges are producers of novel natural compounds (2,3). Sponges belong to phylum Porifera. They are primitive and multi-cellular animals that have existed for 700–800 million years. There are approxeimateley 15.000 sponge species, most of them occurring in marine environments (4) .Sponges produce secondary metabolites, deterring predators to compete for space with other sessile species, for communication and for protection against infection. More than 10% of the investigated marine sponge species showed cytotoxic activity, indicating that sponges can be considered as sources of potential medicine such as anticancer agents and immuno-modulators, and some as antifouling agents (4).

During our search for bioactive compounds from Turkish marine sponges, we have analyzed the methanol extracts of three different sponge species (*Haliclona cratera*, *Axinella damicornis, Stylissa carteri* respectively) collected by scuba divers in Kaş, on the South Coast of Turkey.

MATERIALS AND METHODS

Sponge Materials

The samples *Haliclona cratera* (10 m), *Axinella damicornis* (8 m), and *Stylissa carteri* (12 m) were collected by scuba divers in Kaş, on the South Coast of Turkey, in March 2012, and were identified by Dr. Bülent Gözcelioğlu (TUBITAK), and the sponge samples were deposited at Ankara University, Faculty of Pharmacy, Ankara, Turkey.

Methods

Specimens of the sponges were cut into small pieces, then extracted with methanol as described by Ebel (5). The extracts were evaporated under vacuum and lyophilized in a dry freezer. Extracts were investigated for their chemical contents by High Pressure Liquid Chromatography-Diode Array Detector (HPLC-DAD) given in Table 1. Routine detections were realized at 235, 254, 280 and 340 nm. Comparison of online-UV spectra with a spectra library facilitated the compound detections. Samples were solved in 100% HPLC grade methanol, and centrifuged prior to their analysis in order to avoid particles from occluding the HPLC column. Analytical HPLC system specifications are described below:

Table 1. Solvent system and standard gradient employed for analytical HPLC. Flow rate: 1 ml/min.

Time (min)	0.02 % phosphoric acid, pH 2 H_2O (%)	Methanol (%)
0	90	10
5	90	10
35	0	100
45	0	100
50	90	10
60	90	10

RESULTS AND DISCUSSION

Results

HPLC analysis of the crude extracts from three sponge samples, revealed detection of some bromopyrrole alkaloids, a brominated alkaloid, a pentacyclic alkaloid, a quinoline alkaloid and a

Table 2. HPLC detection results of three Turkish marine sponges.

Detected Compound	Classification of Compound	Retention time (min)	Sponge Species
Hymenialdisine	Brominated alkaloid	14.600 12.800	*Stylissa carteri* *Axinella damicornis*
Hymenidin	Bromopyrrole alkaloid	15.460	*Stylissa carteri*
Stevensin	Bromopyrrole alkaloid	17.058	*Stylissa carteri*
Spongiacidin B	Bromopyrrole alkaloid	13.790	*Stylissa carteri*
Oroidin	Bromopyrrole alkaloid	19.356	*Stylissa carteri*
Dehydrocampesterol	Steroid	18.840	*Stylissa carteri*
Spongiacidin F	Bromopyrrole alkaloid	11.200	*Axinella damicornis*
8-Hydroxy-4-quinolone	Quinoline alkaloid	12.423	*Haliclona cratera*
Manzamine A	Pentacyclic alkaloid	17.166	*Haliclona cratera*

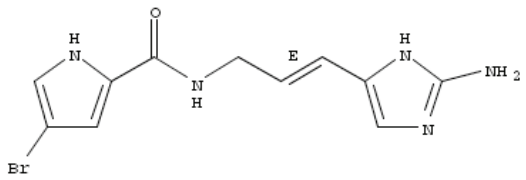
steroid (Table 2, Figure 1). Detections were made by comparing the HPLC chromatograms of crude extracts with Heinrich Heine University local library database. HPLC profile of *Stylissa carteri* is given in Figure 2.

Discussion

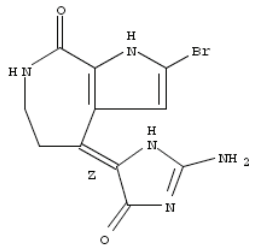
Over the last 25 years, marine secondary products have attracted growing interest due to their unique chemical features and bioactive properties. Thousands of new marine natural products have been reported, proving marine natural organisms to be rich and varied source of new structural classes of secondary metabolites (13). Over the last forty years, sponges (phylum Porifera) have been identified as excellent sources of unique marine natural products, having higher incidences of biologically active compounds than any other single marine phylum. These compounds are interesting candidates for new drugs (Table 3) (14,15).

Table 3. Sponge derived bioactive molecules in clinical and preclinical trials (15)

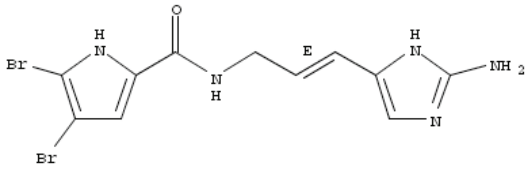
Name	Sponge	Disease	Status
Discodermolide	*Discodermia dissoluta*	Cancer	Phase I
E7389	*Lissodendoryx sp*	Cancer	Phase III
HTI-285 (hemiasterlin derivative)	*Cymbastella sp*	Cancer	Phase II
KRN-7000	*Agelas mauritianus*	Cancer	Phase I
Peloruside A	*Mycale hentscheli*	Cancer	Preclinical
Salicylihalimides A	*Haliclona sp*	Cancer	Preclinical
Laulimalide	*Cacospongia mycofijiensis*	Cancer	Preclinical
Variolins	*Kirkpatrickia variolosa*	Cancer	Preclinical
Dictyodendrins	*Dictyodendrilla verongiformis*	Cancer	Preclinical
Manoalide	*Luffariaella variabilis*	Antipsoriatic	Phase II/discontinued
Bengamide derivative	*Jaspis sp*	Cancer	Phase I/discontinued
Girolline	*Pseudaxinyssa cantharella*	Cancer	Phase I/discontinued



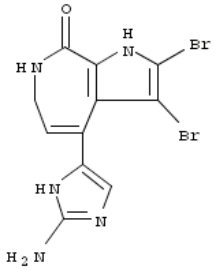
a) Hymenidin (6)



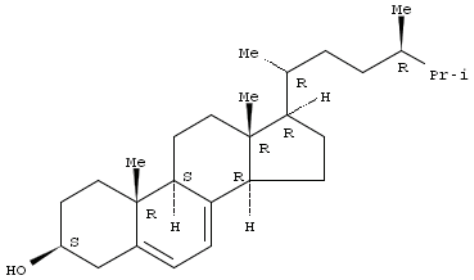
b) Hymenialdisine (7)



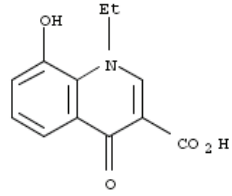
c) Oroidin (8)



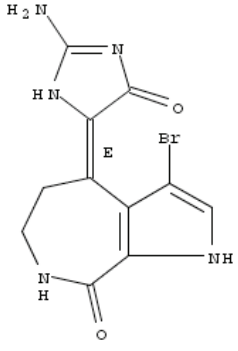
d) Stevensine (9)



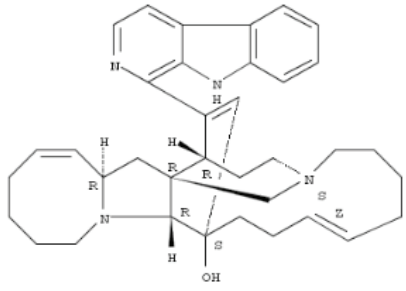
e) Dehydrocampesterol (3)



f) 8-Hydroxy-4-quinolone (10)



g) Spongiacidin B (11)



h) Manzamine A (12)

Figure 1. The chemical structures of detected compounds a-h

It has been reported that bromopyrrole alkaloids are typical secondary metabolites of sponges from the families Agelasidae, Axinellidae, and Hymeniacidonidae (16). More than 30 years, about 140 derivatives have been isolated from more than 20 different sponges of various genera, essentially (but not exclusively) belonging to Agelasidae, Axinellidae, and Halichondridae with various structures and interesting biological activities (15). *Axinella* species have been known to contain terpene derivatives, alkaloids, and cyclopeptides (17,18).

Over the last 40 years sponges belonging to *Haliclona* genus are well-known for producing different kinds of secondary metabolites, most commonly the bioactive alkaloids (19).

The results of this study conform well to the previous reports on *Axinella* and *Haliclona* genera. Bromopyrrole alkaloids and brominated alkaloids were detected in *Axinella damicornis* and *Stylissa carteri* (*Axinella carteri*). Additionally, this was the first study on Turkish marine sponges *Stylissa carteri, Axinella damicornis* and *Haliclona cratera.*

As a conclusion, further studies need to be carried out, in order to isolate the detected compounds. Scientific literature contains numerous examples of bioactive compounds obtained from the samples these sponges. Besides the isolation, several investigations have focused on bioactive effects of the detected compounds. (19-27).

In the light of these findings, we are encouraged to isolate and test bioactivity of our detected compounds.

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REFERENCES

1. Schumacher M, Kelkel M, Dicato M, Diederich M. Gold from the sea: Marine compounds as inhibitors of the hallmarks of cancer. *Biotechnol Adv* 29: 531–547, 2011.
2. Ravichandran S, Kathiresan K, Balaram H. Anti-malarials from marine sponges**.** *Biotechnology and Molecular Biology Review* 2 (2): 33-38, 2007.
3. Marc P, Michaeel P, De Medina P, Poirot S. Process for the preparation of amino sterol derivatives via aminolysis of epoxysteroids PCT. *Int Appl* WO 2013076257 A1 20130530, 2013.
4. Belarbi E, Gomez AC, Chisti Y, Camacho FG, Grima EM. Producing drugs from marine sponges. *Biotechnology Adv* 21: 585–598, 2003.
5. Ebada SS, Edrada-Ebel RA, Lin WH, Proksch P. Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates, *Nat Protoc* 3 (12): 1820-1831, 2008.
6. Rasapalli S, Kumbam V, Dhawane AN, Golen JA, Lovelyb CJ, Rheingoldc AL. Total synthesis of oroidin, hymenidin and clathrodin. Org Biomol Chem {DOI: 10.1039/c3ob40668g] 2013 April-May [cited 2013]; 11 (25): [4133-4137]. Available from: URL: http: www.rsc.org/obc
7. Feng D, Qiu Y, Wang W, Xiang Wang X, Ouyang P, Ke C. Antifouling activities of hymenialdisine and debromohymenialdisine from the sponge *Axinella* sp. *Int Biodeterior. Biodegrad*. 85, November: 359–364, 2013.
8. Takale BS, Desai NV, Siddiki AA, Chaudhari HK, Telvekar VN. Synthesis and biological evaluation of pyrrole-2-carboxamide derivatives: oroidin analogues. Med Chem Res {DOI 10.1007/s00044-013-0743-9], 2013 Aug 17 [cited 2013 sept 1]. Available from: URL: http://link.springer.com/article/10.1007%2Fs00044-013-0743-9
9. Fouad MA, Debbab A, Wray V, Müller WEG, Proksch P. New bioactive alkaloids from the marine sponge *Stylissa* sp. *Tetrahedron* 68:10176-10179, 2012.
10. Rudolf A, Eberhard S, Irmgard B. Quinolinonecarboxylic acid derivatives for pharmaceutical preparations. Ger Offen DE 2856908 A1 19800717, 1980.

11. Scala F, Fattorusso E, Menna M, Taglialatela-Scafati O, Tierney M, Kaiser M, Tasdemir D. Bromopyrrole alkaloids as lead compounds against protozoan parasites. *Mar Drugs* 8: 2162-2174, 2010.
12. Eguchi K, Fujiwara Y, Hayashida A, Horlad H, Kato HH, Rotinsulu H, Losung F, Mangindaan REP, de Voogd NJ,Takeya M, Tsukamoto S. Manzamine A, a marine-derived alkaloid, inhibits accumulation of cholesterol ester in macrophages and suppresses hyperlipidemia and atherosclerosis in vivo. *Bioorg Med Chem* 21: 3831–3838, 2013
13. Qia SH, Wang Y, Zhanga S. Steroids and alkaloids from the South China sea sponge *Axinella* sp. *J Asian Nat Prod Res*. 11 (12):1040–1044, 2009.
14. Eder C, Proksch P, Wray V, Steube K, Bringmann G, Van Soest RWM, Ferdinandus E, Pattisina LA, Wiryowidagdo S, Mokao W. New Alkaloids from the Indopacific Sponge *Stylissa carteri*. *J Nat Prod* 62 (1):184-187, 1999.
15. Sagar S, Kaur M, Radovanovic A, Bajıc VB. Dragon exploration system on marine sponge compounds interactions. *Journal of Cheminformatics* {DOI: 10.1186/1758-2946-5-11] 2013, [cited 2013,]; Available from: URL: http://www.jcheminf.com/content/5/1/11
16. Aiello A, Fattorusso E, Giordano A, Menna M, Müller WEG, Perovic-Ottstadt S, Schröder H. Damipipecolin and damituricin, novel bioactive bromopyrrole alkaloids from the Mediterranean sponge *Axinella damicornis*. *Bioorg Med Chem* 15: 5877–5887, 2007.
17. Cafieri F, Fattorusso E, Taglialatela-Scafati O. Novel bromopyrrole alkaloids from the sponge Agelas dispar. *J Nat Prod* 61:122- 125, 1998.
18. Rudi A, Yosief T, Schleyer M, Kashman Y. Several new isoprenoids from two marine sponges of the family Axinellidae. *Tetrahedron* 55: 5555-5566, 1999.
19. Erickson KL, Beutler JA, Cardellina JH, Boyd MR. Salicylihalamides A and B, Novel Cytotoxic Macrolides from the Marine Sponge. *J Org Chem* 62: 8188-8192, 1997.
20. Kobayashi H, Kitamura K, Nagai K, Nakao Y, Fusetani N, Van Soest RWM, Matsunagaa S. Carteramine A, an inhibitor of neutrophil chemotaxis from the marine sponge *Stylissa carteri. Tetrahedron Letters* 48: 2127–2129, 2007.
21. Mayer AM, Rodríguez AD, Berlinck RG, Fusetani N. Marine pharmacology in 2007–8: Marine compounds with antibacterial,anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal,antituberculosis, and antiviral activities; affecting the immune and nervous system,and other miscellaneous mechanisms of action. *Comp Biochem Physiol C Toxicol Pharmacol* 153 (2): 191–222, 2011.
22. Inbaneson SJ, Ravikumar S. In vitro antiplasmodial activity of marine sponge *Stylissa carteri*. *Asian Pacific Journal of Tropical Disease*, 2 (5): 370-374,2012
23. Pajic I, Kljajic Z, Dogovic N, Sladic D,,Juranic Z, Gasic MJ, A novel lectin from the sponge *Haliclona cratera*: isolation, characterization and biological activity. *Comp Biochem Physiol C Toxicol Pharmacol* 132 (2): 213–221, 2002.
24. Dresch RR, Zanetti GD, Lerner CB, Mothes B, Trindade VMT, Henriques AT, Vozari-Hampe MM. ACL-I, a lectin from the marine sponge *Axinella corrugata*: Isolation, characterization and chemotactic activity. *Comp Biochem Physiol C Toxicol Pharmacol* 148 (1): 23–30, 2008.
25. Aiello A, D'Esposito M, Fattorusso E, Menna M, Müller WEG, Ottstadt SP, Tsuruta H, Gulder V, Bringmann G. Daminin, a bioactive pyrrole alkaloid from the Mediterranean sponge *Axinella damicornis*. *Tetrahedron* 61: 7266–7270, 2005.
26. Aiello A, Fattorusso E, Giordano A, Menna M, Müller V, Ottstadt SP, Schroder HC. Damipipecolin and damituricin, novel bioactive bromopyrrole alkaloids from the Mediterranean sponge *Axinella damicornis*. *Bioorganic & Medicinal Chemistry* 15: 5877–5887, 2005.
27. Erdogan IO, Ozcelik B, Konuklugil B, Putz A, Kaban ÜG, Proksch P. Bioactivity Screening of the Selected Turkish Marine Sponges and Three Compounds from *Agelas oroides. Records of Natural Products,* 6 (4): 356-359, 2012.