Erectcyanthins A-C from marine sponge *Hyrtios erectus*: anti-dyslipidemic agents attenuate hydroxymethylglutaryl coenzyme A reductase

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**ABSTRACT**

Bioactivity-steered chromatographic purification of the solvent extract of marine sponge *Hyrtios erectus* (Thorectidae) led to the isolation of three undescribed cyanthiwigin-type diterpenoids, erectcyanthins A-C. Erectcyanthin B exhibited comparable attenuation activity against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (IC\textsubscript{50} 0.07 mM) with that displayed by anti-dyslipidemic agent atorvastatin (IC\textsubscript{50} 0.08 mM). Comparatively greater antioxidant properties of erectcyanthin B (IC\textsubscript{50} \sim 0.4 mM) than that displayed by erectcyanthin A (IC\textsubscript{50} \sim 0.5 mM), erectcyanthin C and the standard \alpha-tocopherol (IC\textsubscript{50} 1.5–1.7 mM) against oxidants also corroborated its promising bioactivity. Erectcyanthin B exhibited considerably greater anti-inflammatory activities (IC\textsubscript{50} 0.88–1.09 mM) than other erectcyanthin analogues in the series. The potential anti-dyslipidemic activity of erectcyanthins was linearly correlated with electronic parameter (topological polar surface area \sim 74.6) along with balanced hydrophilic-lipophilic properties (logarithmic octanol-water partition coefficient 1.76). This study recognized the anti-dyslipidemic property of erectcyanthin B as a promising pharmaceutical lead.

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1. Introduction

The 3-hydroxy-3-methylglutaryl coenzyme A reductase (hMGCR) has a significant role in the biosynthesis of cholesterol and other isoprenoids as it functions as a rate-defining enzyme in the mevalonate pathway (Tabernero et al. 1999; Istvan and Deisenhofer 2000; Istvan et al. 2000). Therefore, hMGCR is deliberated as an important target enzyme to treat dyslipidemia, as it is involved in the first rate-determining step in cholesterol biosynthesis in mammals causing risk factors of atherosclerosis and obesity (Endo 1992). Complications associated with cardiovascular risk management have drawn noticeable attention to develop anti-dyslipidemic medications (Bhatnagar et al. 2008). The hMGCR inhibitors and statins, which are utilized to treat hyperlipidemia, were reported to induce severe side effects comprising liver damage and increased probability of type-2 diabetes (Naci et al. 2013). Therefore, it is of interest to develop newer anti-dyslipidemic pharmacophores from novel sources.

Marine sponges were deliberated as rich sources of bioactive secondary metabolites (Lunder et al. 2012; Blunt et al. 2017; Hayton et al. 2019). An abundance of diterpenes was purified from marine sponges, such as Spongia ceylonensis, Spongia officinalis, Dendrilla rosea, and Aplysillid sponge (Rungprom et al. 2004; El-Desoky et al. 2017; Han et al. 2018; Hayton et al. 2019). Four homoverrucosane-type diterpenes isolated from Halichondria sp. showed cytotoxicity against human multiple myeloma cell line RPMI-8266 (Tian et al. 2020). Cyanthiwigin-type diterpenes were purified from the marine sponge Myrmekioderma styx (Peng et al. 2002). Sponges of the genus Hyrtios (class Demospongiae) were described with various bioactive compounds (Qiu et al. 2004; Shady et al. 2017). One of the predominant marine demosponges, Hyrtios erectus (Keller, 1889) (family Thorectidae) was broadly studied for bioactive metabolites, particularly terpenes. The bioactive compounds belonging to various classes, for example, macrolides, β-carbolines, indole alkaloids, and sesterterpenes were isolated from Hyrtios sp. (Kobayashi et al. 1993; Aoki et al. 2001; Pettit et al. 2005; He et al. 2014).

Thus, in the search for newer pharmacologically important metabolites from marine sponges, we have isolated three cyanthiwigin-type diterpene compounds, erectcyanthins A-C from the crude solvent extract of marine demosponge H. erectus. Those compounds were characterized as 3-isopropyl-6,9,12-trimethylcyclohept[a]indene-1,13-diol (erectcyanthin A), 7,8-dihydroxy-3-isopropyl-6,9,12-trimethylhexahydrocyclohepta[e]indene-1,13 (4H,9H)-dione (erectcyanthin B) and 1-hydroxy-3-isopropyl-6,9,12-trimethylhexahydrocyclohepta[e]inden-13(4H)-one (erectcyanthin C) (Figure 1). Spectroscopic methods encompassing mass, Fourier transform infrared (FTIR), and nuclear magnetic resonance (NMR) spectral experiments were used for their structural interpretation. The bioactivities of erectcyanthins were assessed, and structure-activity correlation analyses were conducted.

2. Results and discussion

2.1. Bioactivity-guided chromatographic purification of erectcyanthins A-C

Organic extract of H. erectus was initially submitted to column chromatographic fractionation over silica gel, and resulted into five major fractions (HE₁-HE₅). Greater
antioxidant (scavenging potential IC$_{50}$ for 2,2-diphenyl-1-picrylhydrazyl DPPH, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS$^+$ $<$ 0.60 mg mL$^{-1}$) and anti-dyslipidemic (IC$_{50}$ hMGCR 0.42 mg mL$^{-1}$) activities together with greater percentage yield (17.2%) of the fraction HE$_2$ could lead us to shortlist the same for further purification by flash chromatography. The flash chromatographic purification of the fraction HE$_2$ resulted in six sub-fractions (HE$_2$-1–HE$_2$-6). The sub-fraction HE$_2$-2 was recorded with greater bioactivities (IC$_{50}$ $<$ 0.55 mg mL$^{-1}$) and yield ($\sim$10%) than the remaining ones (Table S1). Therefore, HE$_2$-2 was fractionated using preparative HPLC separation (acetonitrile (ACN)-methanol (MeOH), 60:40 v/v) leading to the isolation of three cyanthiwigin-type diterpenes, erectcyanthins A-C. The purity of isolated compounds was determined using high-pressure liquid chromatography (HPLC), and detailed spectroscopic analyses were carried out for the structural interpretation of the erectcyanthins.

2.2. Spectral analysis of erectcyanthins

Bioactivity-aided chromatographic purification of crude solvent extract of *H. erectus* ensued in three cyanthiwigin-type compounds, erectcyanthin A through C (Figure 1, Table S2).

2.2.1. Erectcyanthin A

A cyanthiwigin-type diterpene compound, erectcyanthin A, was purified from the crude ethyl acetate (EtOAc): MeOH (1:1 v/v) extract of the marine demosponge *H. erectus* by bioactivity-steered chromatographic fractionation. The molecular formula of erectcyanthin A was established as C$_{20}$H$_{30}$O$_2$ on the basis of pseudo-molecular ion peak {HRESIMS, high-resolution electrospray ionization mass spectrometry m/z 303.2328 [M + H]$^+$} (Figure S1), and the structure was interpreted by NMR analysis (Table S2; Figures S2–S9). The compound exhibited a ultra-violet (UV) wavelength maximum of 240 nm. $^{13}$C NMR and HSQC (heteronuclear single quantum coherence) spectral data along with DEPT-135 (distortionless enhancement by polarization transfer spectroscopy) designated the presence of twenty carbon signals, which were consistent with two $sp^3$ methylenes, five each of $sp^3$ methines and $sp^3$ methyls, together with four both of $sp^2$ methines and non-protonated carbons. Relatively downfield proton signals at $\delta_H$ 5.36, 5.62 and 5.48 could correspond to the olefinic groups in the compound. Similarly, the signals at $\delta_H$ 4.05 and 3.89 were representative of the hydroxyl
groups in erectcyanthin A, and their occurrence was deduced by the deuterium exchange experiment. The FT-IR signals at 3421 cm$^{-1}$ (br) recognized the O-H stretching vibration, whereas those at 1740 and 1637 cm$^{-1}$ revealed the presence of C=O and C=C moieties, respectively in the studied compound (Figure S10). Proton correlation spectroscopy ($^1$H-$^1$H COSY) correlations at $\delta_H$ 2.27 (H-4)/1.66 (H-5)/2.05 (H-10)/5.48 (H-11) along with the heteronuclear multiple bond correlations (HMBC) from $\delta_H$ 4.05 (H-1) to $\delta_C$ 124.9 (H-2), 44.2 (H-4); $\delta_H$ 1.46 (H-5) to $\delta_C$ 38.5 (H-9)/29.3 (C-6)/123.3 (C-11); $\delta_H$ 1.84 (H-15) to $\delta_C$ 69.1 (H-13) attributed a diterpene structure with a novel 7/6/5 tricyclic skeletal backbone (Figure 1) (Tian et al. 2020). NMR data of the isolated compound exhibited close similarity with those of cyanthiwigin Y except for few chemical shift changes (Peng et al. 2002). The absence of non-protonated carbon signal at C-8 compared to cyanthiwigin Y, proposed the absence of ketone functionality at C-8 of erectcyanthin. As opposed to downfield ketone signal, the compound exhibited a relatively upfield signal at $\delta_C$ 134.6 (HSQC with $\delta_H$ 5.62) corresponding to the olefinic group. Additionally, the absence of the ketone group at C-1 was evident from the relatively upfield signal at $\delta_C$ 83.9. This proton signal at $\delta_H$ 4.05 exhibited an HSQC correlation with $\delta_C$ 83.9 (C-1) corresponding to a hydroxyl group at C-1. Attachment of methyl groups at C-6, 9, 12 and C-18 was established by the HMBCs from $\delta_H$ 1.28 (H-16) to $\delta_C$ 57.3 (H-9); $\delta_H$ 1.84 (H-15) to $\delta_C$ 137.9 (H-12); $\delta_H$ 1.28 (H-17) to $\delta_C$ 38.5 (H-9) and $\delta_H$ 1.03 (H-19) to $\delta_C$ 32.5 (H-18) (Figure S9A). Nuclear overhauser effect spectroscopy (NOESY) correlations from $\delta_H$ 4.05 (H-1)/1.66 (H-5)/1.28 (H-16, 17) attributed the equiplaner orientation of these protons, and were $\beta$-oriented. In the same way, the other set of protons $\delta_H$ 2.27 (H-4)/3.89 (H-13) exhibited NOE correlations, and were allocated as $\alpha$-disposed (Figure S9B). NOE correlations were also substantiated by the MM2 force-field calculation (Figure S11). The mass spectrum of the compound was recorded with a molecular ion peak at m/z 302.2, which underwent step-wise elimination of two hydroxyl ions, and resulted in 1-isopropyl-3a,5a,8-trimethyl-octahydrocycloheptaejindene (C) with a molecular weight of m/z 268.2 (Figures S12 and S13). The latter upon elimination of C$_3$H$_7$ and three CH$_3$ radicals could yield octahydrocyclohepta[e]jindene (G) with an m/z of 184.1. Subsequent elimination of C$_3$H$_7^{2\star}$ and C$_4$H$_6^{2\star}$ radicals resulted in cycloheptene radical cation (m/z of 95.1) as the base peak. Based on the comprehensive spectral analysis, the structure of erectcyanthin A was interpreted as 3-isopropyl-6,9,12-trimethyloctahydrocyclohepta[e]jindene-1,13-diol.

2.2.2. Erectcyanthin B
The molecular formula of erectcyanthin B was determined as C$_{20}$H$_{28}$O$_4$ {HRESIMS m/z 333.2069 [M + H]$^+$} (Figure S14). The compound exhibited a UV-wavelength maximum of 242 nm. Detailed NMR analysis was carried out for the structural interpretation of the compound (Table S2; Figures S15–S22). Combined $^{13}$C NMR along with the HSQC data demonstrated the presence of twenty carbon signals that were consistent with two sp$^3$ methylenes, three sp$^2$ methines, along with five each of sp$^3$ methines, sp$^3$ methyls, and non-protonated carbons (Table S2). The IR absorptions at 3437 and 1740 cm$^{-1}$ corresponded to hydroxyl and carbonyl groups, respectively (Figure S23). Non-protonated carbon signals at $\delta_C$ 203.2 and 200.1 were attributed to two ketone functionalities. $^1$H-$^1$H COSY correlations, $\delta_H$ 2.27 (H-4)/1.46 (H-5)/2.00 (H-10)/6.27 (H-11)
along with the HMBC correlations from $\delta_H$ 5.71 (H-2) to $\delta_C$ 203.2 (H-1); $\delta_H$ 2.27 (H-4) to $\delta_C$ 203.2 (H-1); $\delta_H$ 1.46 (H-5) to $\delta_C$ 57.3 (H-9)/29.7 (C-6)/139.3 (C-11); $\delta_H$ 2.93 (H-4) to $\delta_C$ 200.1 (H-13) attributed a diterpene structure with a novel 7/6/5 tricyclic skeletal backbone (Tian et al. 2020). Interpretation of NMR information of the compound exhibited the same structural features with erectcyanthin A and cyanthiwigin Y with few exceptions. In preference to a downfield ketone signal, erectcyanthin B exhibited a relatively upfield signal at $\delta_C$ 71.2 (HSQC with $\delta_H$ 3.51) corresponding to the hydroxyl group. Similarly, the relatively downfield signal at $\delta_H$ 3.24 (H-7) recognized another hydroxyl group at C-7 of the basic tricyclic skeleton in erectcyanthin B. The attachment of methyl groups at C-6, 9, 12 and C-18 was deduced by HMBCs from $\delta_H$ 1.33 (H-17) to $\delta_C$ 57.3 (H-9); $\delta_H$ 0.95 (H-16) to $\delta_C$ 29.8 (H-6); $\delta_H$ 2.44 (H-15) to $\delta_C$ 136.5 (H-12) and $\delta_H$ 1.04 (H-19) to $\delta_C$ 30.7 (H-18) (Figure S22A). NOE correlations between the protons $\delta_H$ 2.27 (H-4)/1.46 (H-5)/3.24 (H-7)/1.33 (H-17) accredited their identical orientation, and were allocated as $\alpha$-oriented (Figure S22B). Correspondingly, the other group of protons exhibited NOE correlations, for example, $\delta_H$ 3.51 (H-8)/0.95 (H-16) signifying $\beta$-orientation of these protons. NOE correlations were also confirmed from the MM2 force-field calculation (Figure S24). The mass spectrum of the compound exhibited molecular ion peak at $m/z$ 332.2, which underwent subsequent elimination of two hydroxyl radicals to yield 1-isopropyl-3a,5a,8-trimethyl-hexahydrocyclohepta[e]indene-3,7(3aH,10bH)-dione (C, $m/z$ 298.2). The latter upon elimination of C$_3$H$_7^+$ radical and followed by removal of three methyl radicals resulted in hexahydrocyclohepta[e]indene-3,7(3aH,10bH)-dione (F, $m/z$ 230.1) (Figures S25 and S26). The step-wise elimination of C$_3$H$_2$O$_2^+$ and C$_4$H$_8^+$ radicals could result in cyclohept-2-enone with $m/z$ 109.1 as the base peak. Based on the comprehensive spectral analysis, the structure of erectcyanthin B was characterized as 7,8-dihydroxy-3-isopropyl-6,9,12-trimethyl-hexahydrocyclohepta[e]indene-1,13 (4$H$/9$H$)-dione.

2.2.3. Erectcyanthin C

The molecular formula of erectcyanthin C was deduced as C$_{20}$H$_{28}$O$_2$ on the basis of mass spectral analysis {HRESIMS, $m/z$ 301.2171 [M + H]$^+$} (Figure S27), and its structure was elucidated by detailed NMR analysis encompassing 2D-NMR experiments (Table S2; Figures S28–S35). $^{13}$C NMR along with the HSQC data revealed the presence of twenty carbon signals that were consistent with two sp$^3$ methylenes, four each of sp$^3$ methines and sp$^2$ methines, in consort with five both of sp$^3$ methyls and non-protonated carbons (Table S2). The compound exhibited a UV-wavelength maximum of 235 nm. Unlike erectcyanthin B, the third diterpene displayed only one ketone signal at $\delta_C$ 201.2. The IR absorptions at 3476 and 1697 cm$^{-1}$ corresponded to hydroxyl and carbonyl groups, respectively (Figure S36). A comprehensive analysis of NMR spectra of erectcyanthin C demonstrated resemblance with erectcyanthin A and B apart from a few chemical shift alterations (Table S2). Erectcyanthin C exhibited an extra olefinic group at C-7/8. The relatively downfield signal at $\delta_H$ 5.62 (H-7, 8) was ascribed to an olefinic group in the compound. HBMC correlation from $\delta_H$ 5.62 (H-8) to $\delta_C$ 26.5 (H-6) deduced the position of olefinic group at C-7/8 position (Figure S35A). Additionally, the absence of hydroxyl groups at C-7 and C-8 positions were evident from the absence of proton signals at $\delta_H$ 3.24 and 3.51. NOE correlations from $\delta_H$ 4.04 (H-1)/
1.65 (H-5)/1.27 (H-16) submitted the identical orientation of these protons, and were allocated as β-oriented (Figure S35B). In the same way, the other set of proton exhibited NOE correlations at $\delta_H$ 2.29 (H-4)/1.33 (H-17), which suggested the α-orientation of these protons. The NOE correlations were also established by the MM2 force-field calculation (Figure S37). The mass spectrum of the compound exhibited a molecular ion peak at $m/z$ 300.2, which underwent removal of hydroxyl radical to yield 1-isopropyl-3a,5a,8-trimethyl-hexahydrocyclohepta[e]inden-7(10bH)-one (B, $m/z$ 28.2). This intermediate ion on the elimination of C$_3$H$_7^+$ and three CH$_3^+$ radicals resulted in the formation of hexahydrocyclohepta[e]inden-7(10bH)-one (F, $m/z$ 199.1). Subsequent elimination of C$_3$H$_4^{2+}$ and C$_4$H$_6^{2+}$ radicals resulted in cyclohept-2-enone (H, $m/z$ 109.1), as the base peak (Figures S38 and S39). Based on the spectral analysis, the structure of erect cyanthin C was elucidated as 1-hydroxy-3-isopropyl-6,9,12-trimethylhexahydrocyclohepta[e]inden-13(4H)-one.

2.3. Bioactive potentials of erectcyanthins A-C

Previous studies have demonstrated the anticancer and antituberculosis activities of cyanthiwigins (Peng et al. 2003). Diterpenoids were previously reported with antioxidant, anti-inflammatory, and antihypertensive activities (González et al. 2015; Tirapelli et al. 2010; Vo et al. 2020). In the present study, we have analysed in vitro antioxidant, anti-inflammatory, antihypertensive, and anti-dyslipidemic activities of the test compounds. Among various bioactivities, the anti-dyslipidemic activity of erectcyanthins against hMGCR was considerably greater ($IC_{50} \sim 0.20$ mM, $p < 0.05$) than other bioactive properties (Table S3). Noticeably, anti-dyslipidemic activity of erectcyanthin B was comparable ($IC_{50}$ 0.07 mM) with that exhibited by the standard, atorvastatin ($IC_{50}$ 0.08 mM), and greater than other isolated analogues ($IC_{50}$ 0.09 mM, erectcyanthin A; $IC_{50}$ 0.18 mM, erectcyanthin C). Antihypertensive activity of erectcyanthins ($IC_{50} \sim 0.61$ mM) was considerably lesser compared to that displayed by captopril ($IC_{50}$ 0.02 mM). The studied erectcyanthins A-C exhibited considerably superior attenuation potential against 5-lipoxygenase (5-LOX, $IC_{50}$ 1 mM) than that displayed by ibuprofen ($IC_{50}$ 4.5 mM) (Table S3). 5-LOX promotes the advancement of inflammatory reactions, such as autoimmune diseases and rheumatoid arthritis in view of its ability to produce inflammatory 5-hydroxyeicosatetraenoic acid and leukotrienes, which could contribute to the upregulation of hMGCR leading to the dyslipidemic condition. Accordingly, 5-LOX is a potential target for biomedical uses in several diseases, comprising dyslipidemia and inflammation (Basil and Levy 2016). Antioxidant properties of erectcyanthins A-C ($IC_{50}$ 0.5–0.7 mM) further supported the observed anti-dyslipidemic potential (Table S3).

2.4. Structure-activity relationship analysis

Bioactivities of erectcyanthins A-C were correlated with molecular attributes, such as hydrophobic (logarithmic octanol-water partition co-efficient, log $P_{OW}$), polar (polarizability PI/topological polar surface area tPSA), and steric {parachor (Pr)/molar volume (MV)/molar refractivity (MR)} factors (Maneesh and Chakraborty 2017) that showed its
structure–activity relationship and pharmacophore–target interaction (Table S3). Erectcyanthin B showed greater electronic properties, encompassing tPSA (74.6 and PI 36.11) than other erectcyanthin analogues recognized the presence of electron-rich centres that could result in increased electronic properties (Table S3). The electronic factors of erectcyanthin B might play a pivotal role in the potential bioactivities against hMGCR. Electronic attributes, such as hydroxyl and carbonyl groups in erectcyanthin B could exhibit greater hydrogen bonding interaction with the active site of hMGCR at its reaction centre, resulting in its promising inhibitory activities. In addition, the significant antioxidant properties of erectcyanthin B (IC$_{50}$ 0.45 mM) could be corroborated by its electronic properties. Conspicuously, the electron-rich centers of erectcyanthin B could be responsible for its inducible polarity, and also towards the favorable interaction with enzyme active site leading to attenuate hMGCR activity. Steric attributes of erectcyanthin B (MR 94 cm$^3$/mol; MV 281 cm$^3$, Pr 737 cm$^3$) were significantly lesser than those in atorvastatin (MR 154 cm$^3$/mol; MV 451 cm$^3$, Pr 1176 cm$^3$), which could lead to favorable interaction of the former with the macromolecular reaction centre. Notably, the hydrophobicity of erectcyanthins (log P$\text{OW}$ 1.8–5.0) was found to reside within the threshold permissible limit of hydrophobic-lipophilic balance (Lipinski and Hopkins 2004) that could be attributed to their promising bioactive properties. Acceptable permeability in cellular network (through the inter-membrane barrier) along with the radical scavenging properties of pharmacophores might result in potential bioactivities.

3. Experimental

3.1. Instrumentation and materials

An ultraviolet-visible spectrophotometer (Varian Cary 50, Agilent Technologies, Inc., Santa Clara, CA) was used to record the UV spectra and infrared (IR) spectra using FT-IR (Perkin-Elmer Series 2000, Waltham, MA). NMR spectra were recorded using an NMR spectrometer (Bruker Avance, 500 MHz Karlsruhe, Germany) using deuterated chloroform (CDCl$_3$) as a solvent. The NMR data was administered using Mest ReNova-7.1.1-9649. Two-dimensional $^1$H–$^1$H COSY, DEPT-135, NOSEY, HSQC, HMBC NMR spectral data were acquired following the standard protocols (Chakraborty and Francis 2020). Purities of compounds were assessed by a reverse phase HPLC system connected with a LC-20AD pump (Shimadzu Corporation, Kyoto, Japan). Flash chromatographic fractionation (Biotage AB SP1-B1A, Uppsala, Sweden) and semi-preparative HPLC separation over a C$_{18}$ reverse-phase column (Phenomenex, Torrance, USA; 25 cm × 1 cm, 5 µm) using a pump (Shimadzu LC 20AD, Nakagyo-Japan) built-in with a photodiode array detector (SPD M20A, Kyoto, Japan) were used for finer downstream purification. A polarimeter (ATAGO AP-300) was used for recording the optical rotations. Gas chromatograph-mass spectrometer (GC-MS) operating with electron-impact (EI) mode (7890A GC; 5975C MS, Agilent Technologies, Inc., Santa Clara, CA) was used for mass spectral analysis, in which the compounds were fractionated over a medium polar partition-liquid stationary phase (HP-5MS 5% phenylmethyl silox; 30 m length x 0.25 µm film thickness x 250 µm internal diameter). HR-ESIMS data were obtained in ESI$^+$ method (QTOF6520LC/MS/MS; Agilent) connected with an HPLC (Agilent LC 1200) connected with a RP C$_{18}$ column (50 × 2.1 mm; 1.8 µm). The solvents and reagents with
analytical grade were purchased from E-Merck (Darmstadt, Germany) and HiMedia (LLC, Kelton, PA). Enzymes used for the assays were obtained from Sigma-Aldrich (St. Louis, MO). Silica gel-coated GF254 plates for thin-layer chromatography (TLC) and silica gel with various mesh sizes (60–120 and 230–400) for column chromatography were procured from E-Merck (Darmstadt, Germany).

3.2. Sample collection, pre-treatment, and extraction

Samples of *H. erectus* (8 kg) (class: Demospongiae, order: Poecilosclerida; family: Microcionidae, voucher specimen number of CMFRI/SUB24/40012) were collected from the Kadiapattanam coast located between 8°12′N and 77°31′S of the Arabian Sea with the help of scuba divers on December 12, 2017. The sponge samples were identified by the marine biologist, Dr. Chandran Retnaraj of the Marine National Park and Sanctuary of Jamnagar in the Gujarata State of India. Microscopic examination of spicules was also carried out for the identification of the species. The cleaned samples were ground, freeze-dried (ScanVac, Labogene, Germany), and powdered (3 kg) before sonication with ethyl acetate-methanol (EtOAc-MeOH 1:1 v/v, 600 mL X 5) solvent system and refluxed at 70–80°C in the presence of nitrogen gas for about 4 h. The extracts were passed through anhydrous Na₂SO₄, and solvents were removed at low temperature by using an IR-coupled rotational vacuum concentrator (RVC/2/33-IR, Martin Christ, Germany) to obtain a gummy residue, which was regarded as the crude extract of *H. erectus* (~68 g, 2.3% yield on dry-weight).

3.3. Bioactivity-directed chromatographic purification

The crude solvent extract (60.23 g) was primarily purified by column chromatography, and resulted in five fractions (HE₁-HE₅). These fractions were assessed for antioxidant potential along with anti-dyslipidemic activities against the hMGCR enzyme. The fraction HE₂ with greater antioxidant and anti-dyslipidemic activities compared to others was further fractionated by flash chromatography (Biotage SP1-B1A, Sweden) using EtOAc/n-hexane (0–25% EtOAc) solvent gradient to afford six sub-fractions (45 mL, HE₂₁–HE₂₆). The sub-fraction HE₂₂ was recorded with greater yield (~10%) as well as higher bioactive potential against various oxidizing agents and hMGCR (IC₅₀ ≤ 0.55 mg mL⁻¹) than those demonstrated by the rest of the fractions. Subsequently, HE₂₂ was purified by RPC₁₈ HPLC (ACN-MeOH, 8:2 v/v) to isolate the erectcyanthin analogues. Erectcyanthin A and C were isolated as brown oil (33.2 and 39.5 mg, respectively), whereas erectcyanthin B (38.2 mg) was separated as a pale yellow oil. The homogeneity of the isolated compounds was assessed by TLC (EtOAc/n-hexane 7:3 v/v) and RP C₁₈ HPLC (MeOH-ACN, 8:2 v/v).

3.4. Physicochemical and spectroscopic data

3.4.1. Erectcyanthin A

Brown oily; UV (MeOH) λ_max: 240 nm (Figure S40); [α]D²⁵ +32.2° (c = 0.34, MeOH); TLC (EtOAc/n-hexane, 7:3 v/v) Rf: 0.60; Rf (HPLC, MeOH:ACN, 8:2 v/v): 2.768 min. (Figure
S41); IR (ν<sub>m</sub>, stretching; δ, bending; ω, wagging; ρ, rocking): 3468, 3421 (br, O-H), 2920 (C-H), 2853 (C-H), 1740 (C=O), 1637 (C=C), 1458 (C-H), 1055 (C-O) (Figure S10); ¹H NMR (in CDCl₃, 500 MHz, J in Hz) (Figure S2): 5.62 (1H, d, 7.1), 5.48 (1H, dd, 14.6, 6.8), 5.36 (1H, d, 10.3), 4.05 (1H/β, d, 10.1), 3.89 (1H, dd, 14.2, 5.6), 2.54 (1H, m), 2.27 (1H, d, 11.3), 2.05 (1H, m), 1.84 (3H, s), 1.72 (1H, m), 1.66 (1H/β, dt, 11.0, 6.7), 1.54 (1H, d, 6.3), 1.34 (1H, d, 11.1), 1.28 (3H/β, s), 1.03 (3H, d, 5.6); ¹³C NMR (in CDCl₃, 125 MHz) (Figure S3): 148.2, 137.9, 134.6, 134.0, 124.9, 123.3, 83.9, 69.1, 46.4, 44.5, 44.2, 38.5, 32.5, 29.3, 27.0, 21.8, 21.2, 18.7, 18.1; DEPT-135, HSQC, ¹H-¹H COSY, HMBC, NOESY data (Figures S4–S8, Table S2); GC-MS (with EI ionization): found m/z 302.2 [M]⁺, cal. for C₂₀H₃₀O₂ (Figures S12 and S13); HRMS (ESI⁺): found m/z 303.2328 [M+H]⁺, cal. for C₂₀H₃₁O₂ 303.2324 (Figure S1).

### 3.4.2. Erectcyanthin B
Pale yellow oily UV (MeOH) λ<sub>max</sub>: 242 nm (Figure S42); [α]<sub>D</sub>⁰⁺ +36.4° (c = 0.34, MeOH); TLC (EtOAc/n-hexane, 7:3 v/v) R<sub>f</sub> 0.59; R<sub>t</sub> (HPLC, MeOH:ACN, 8:2 v/v): 2.278 min. (Figure S43); IR (ν<sub>m</sub>, stretching; δ, bending; ω, wagging; ρ, rocking): 3437 (br, O-H), 2928 (C-H), 2852 (C-H), 1740 (C=O), 1631 (C=C), 1493 (C-H), 1381 (C-H), 1061 (C-O) (Figure S23); ¹H NMR (in CDCl₃, 500 MHz, J in Hz) (Figure S15): 6.62 (1H, t, 8.7), 5.71 (1H, s), 3.51 (1H/β, d, 10.3), 3.51 (1H/β, d, 10.3), 2.93 (1H, m), 2.69 (1H, m), 2.52 (1H, s), 2.44 (3H, s), 2.27 (1H, m), 2.00 (1H, m), 1.70 (1H, m), 1.46 (1H, s), 8.2, 5.3), 1.33 (3H, s), 1.04 (3H, d, 6.8), 1.04 (3H, d, 6.8), 0.95 (3H/β, s); ¹³C NMR (in CDCl₃, 125 MHz) (Figure S16): 203.2, 200.1, 186.2, 129.3, 136.5, 123.2, 75.0, 71.2, 57.3, 51.7, 47.8, 45.3, 30.7, 29.7, 29.2, 20.5, 16.1, 12.3; DEPT-135, HSQC, ¹H-¹H COSY, HMBC, NOESY data (Figures S17–S21, Table S2); GC-MS (EI): found m/z 332.2 [M]⁺, cal. for C₂₀H₂₈O₄ (Figures S25 and S26); HRMS (ESI⁺): found m/z 333.2069 [M+H]⁺, cal. for C₂₀H₂₉O₄ 333.2066 (Figure S14).

### 3.4.3. Erectcyanthin C
Brown oily; UV (MeOH) λ<sub>max</sub>: 235 nm (Figure S44); [α]<sub>D</sub>⁰⁺ +31.2° (c = 0.33, MeOH); TLC (EtOAc/n-hexane, 7:3 v/v) R<sub>f</sub> 0.57; R<sub>t</sub> (HPLC, MeOH:ACN, 8:2 v/v): 2.697 min. (Figure S45); IR (ν<sub>m</sub>, stretching; δ, bending; ω, wagging; ρ, rocking): 3476, 3436 (br, O-H), 2932 (C-H), 2853 (C-H), 1697 (C=O), 1643 (C=C), 1465 (C-H), 1298 (C=O), 1056 (C-O), 941 (C-H) (Figure S36); ¹H NMR (in CDCl₃, 500 MHz, J in Hz) (Figure S16): 6.23 (1H/β, d, 14.1), 5.62 (1H, m, 7.6), 5.62 (1H, d, 7.6), 5.36 (1H, d, 11.1), 4.04 (1H/β, d, 10.8), 3.08 (1H, s), 2.77 (1H, m), 2.41 (3H, s), 2.51 (1H, m), 2.29 (1H, m), 2.03 (1H, m), 1.71 (1H, m), 1.65 (1H/β, dt, 11.1, 6.3), 1.33 (3H, s), 1.27 (3H, s), 1.02 (3H, d, 5.3), 1.02 (3H, d, 5.3); ¹³C NMR (in CDCl₃, 125 MHz) (Figure S29): 201.2, 149.2, 139.9, 136.9, 133.5, 134.3, 83.9, 53.6, 44.8, 44.5, 38.8, 33.6, 29.4, 26.5, 25.5, 22.1, 21.3, 16.3; DEPT-135, ¹H-¹H-COSY, HMBC, NOESY (Figures S30–S34); GC-MS (EI): found m/z 300.2 [M]⁺, cal. for C₂₀H₂₉O₂ (Figures S38–S39); HRMS (ESI⁺): found m/z 301.2171 [M+H]⁺, cal. for C₂₀H₂₉O₂ 301.2168 (Figure S27).

### 3.5. Bioactivity evaluation
The anti-dyslipidemic potential of isolated cyanthiwigin-type diterpenoid derivatives, erectcyanthins A-C were studied by measuring their inhibition capacity against hMGCR.
In short, 50 \(\mu\)g of the erectcyanthins were mixed into a reaction mixture constituting 400 \(\mu\)M HMG-CoA substrate, 400 \(\mu\)M nicotinamide adenine dinucleotide phosphate and potassium phosphate buffer (100 mM, pH 7.4) containing 120 mM potassium chloride, ethylene-diaminetetra-acetic acid (1 mM), and dithiothreitol (5 mM), followed by the addition of hMGCR (2 \(\mu\)L; concentration of hMGCR stock solution 0.5–0.75 mg mL\(^{-1}\)). The reaction mixture was incubated at 37 °C and the absorbance was recorded at 340 nm after 10 min. Atorvastatin was used as the reference control. The hMGCR inhibition (%) was calculated using the formula: \(\left\{\frac{A_C - A_S}{A_C}\right\} \times 100\), where \(A_C\) and \(A_S\) represented the absorbance of control and sample, respectively. Antioxidant activities were assessed using the stable DPPH and ABTS\(^+\) scavenging activities (Anusree et al. 2016). Anti-inflammatory activities were assessed using 5-LOX and isoforms of COX-2/1 (Anusree and Chakraborty 2017). The compounds were also analyzed for anti-hypertensive potential by their attenuation properties against ACE-I (Holmquist et al. 1979). The test compounds and the controls were prepared in five different concentrations (125, 250, 500, 750 and 1000 ppm) for each assay. The steric bulkiness, hydrophobicity, and electronic parameters (Francis and Chakraborty 2020) were determined by ACD ChemSketch (ver. 12.0; Advanced Chemistry Development Inc., Toronto, CA) and ChemDraw Ultra (Cambridge Soft Corp., MA; ver. 12.0) software.

3.6. Statistical analysis

Statistical analysis was performed using the software tool, Statistical Program for Social Sciences 10.0 (SPSS Inc, CA). Triplicate analyses of the independent experiments were performed, and the means were measured for significance differences (\(p \leq 0.05\)) using analysis of variance (ANOVA).

4. Conclusions

Three cyanthiwigin-type diterpenoid derivatives, erectcyanthins A-C were purified from the organic extract of the marine demosponge \(H.\) erectus by bioactivity-guided chromatographic fractionation. Erectcyanthin B exhibited prospective anti-dyslipidemic activity (IC\(_{50}\) 0.07 mM) by attenuating hMGCR, and the activity was comparable with that of the standard atorvastatin (IC\(_{50}\) 0.08 mM). Additionally, the compounds exhibited significant inhibition against 5-LOX other than potential antioxidant properties (IC\(_{50}\) ~ 1 mM), which corroborated the anti-dyslipidemic potential of erectcyanthin. Structure-bioactivity correlation analysis recognized that higher electronic properties along with optimum hydrophobicity of erectcyanthin B (tPSA 74.6) favored the attenuation potential of erectcyanthin, and also predominantly contributed towards greater antioxidant activities. The results further attributed the therapeutic potential of erectcyanthin B as prospective marine-originated cyanthiwigin-type compound against dyslipidemia.

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Disclosure statement
The authors declare no competing financial interest.

Data availability
Chromatographic and spectroscopic spectral data are included as supplementary information.

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