Marginolides A-B, polyether macrolide analogues from veined octopus *Amphioctopus marginatus*: anti-hypertensive leads attenuate angiotensin-converting enzyme

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**ABSTRACT**

Angiotensin-I-converting enzyme (ACE) is considered as a major drug target for the treatment of hypertension as it catalyses the production of vasoconstrictor angiotensin II from angiotensin I. ACE inhibitor agents are an effective therapeutic strategy to control high blood pressure. Unprecedented polyether macrolides, marginolide A and B were isolated from the crude extract of marine octopus, *Amphioctopus marginatus* via bioassay-directed sequential chromatographic fractionation. Marginolide A displayed considerably greater ACE attenuation potential (IC\(_{50}\) 0.58 mM) than that exhibited by marginolide B (IC\(_{50}\) 0.72 mM). Higher antioxidant properties of marginolide A against the oxidant species (IC\(_{50}\) \(~ 1\) mM) also supported its potential ACE inhibitory activity. Higher polar characteristics along with acceptable hydrophobic-hydrophilic equilibrium (partition coefficient of octanol-water, log \(P_{ow}\) 2–4) revealed the potential anti-hypertensive activities of marginolides. This study recognized the anti-hypertensive properties of marginolides as promising pharmaceutical leads.

ARTICLE HISTORY

Received 16 October 2021
Accepted 22 November 2021

KEYWORDS

Marine octopus *Amphioctopus marginatus*; polyether macrolides; marginolides A-B; anti-hypertensive; angiotensin-converting enzyme

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2013841.

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1. Introduction

Angiotensin-I-converting enzyme (ACE) plays a critical physiological role in renin-angiotensin-aldosterone assembly for regulating blood pressure and cardiovascular functions (Lee et al. 2004) as it catalyses the biocoversion of angiotensin-I (Ag-I) to angiotensin-II (Ag-II), a powerful vasoconstrictor. The latter promotes the discharge of aldosterone from adrenal cortex and stimulates hypertrophic effects on vascular smooth muscle cells and cardiac myocytes, which eventually leads to hypertension and heart failure (Li et al. 2004). Furthermore, Ag-II promotes the degradation of bradykinin, a vasodilator, which causes the relaxation of arteriolar smooth muscles and increases blood flow (Choi and Hwang 2018). In this context, drugs having ACE inhibitory action are contemplated as an effective therapeutic approach to assuage high blood pressure (Actis-Goretta et al. 2003). Most widely used synthetic ACE inhibitors have been reported to cause adverse health problems, such as skin rashes, dry cough, and other allergies (Wijesekara and Kim 2010). Antioxidant properties of secondary metabolites are reported to have potential effects in preventing cardiovascular diseases, such as hypertension (Safaeian et al. 2018). Unstable reactive oxygen species (ROS) produced as a result of normal metabolic activities can lead to a large number of disease conditions including cardiovascular and neurodegenerative diseases, renal damages, diabetes, arthritis, and cancers due to the presence of unpaired electrons (Manning et al. 2005). For the past few decades, there has been a considerable effort among the scientific community for the search of effective and antioxidant dietary food supplements as alternative medications for hypertension treatment precluding a number of undesirable adverse effects of synthetic agents.

Polyether macrolides of marine origin represent a significant class of natural products with varied structural characteristics and exhibit a broad range pharmaceutical properties, which include cytotoxicity, antibacterial, antifungal, antimitotic, antiviral and other activities (Huryn and Wipf 2014; Zhang et al. 2021). These classes of compounds are biogenetically originated from polyketides that contain usually a ring size of twelve or more members (Zhang et al. 2021). Previous literature reported that the occurrence of even size 14-, 16- 18-membered macrolides is more frequent than the odd-membered rings in which about 50% have oxygenated heterocycles including lactones and cyclic ethers (Frank et al. 2007). This class of compounds was increasingly reported from marine habitat, especially from the molluscan community. Earlier literature reported macrolide derivatives with diverse sub-structural motifs isolated from the marine octopus Amphiocotopus neglectus with potent ACE attenuation properties (Chakraborty et al. 2019). Two sixteen-membered polyether macrocyclic polyketides with anti-lipoxygenase activity were described from gastropods Babylonia spirata and Chicoreus ramosus (Salas and Chakraborty 2018; 2020). Marine-derived sponge Lissodendoryx sp. was reported to possess a series of four polyether macrolides, halichondrins with strong inhibition against the murine leukemia P388 cell lines (Hickford et al. 2009). Another 33-membered polyether macrolide with fused oxygenated heterocyclic rings isolated from a dinoflagellate Dinophysis acuminata was reported to stimulate the activity of skeletal muscle actomyosin ATPase (Hwang et al. 2014). These diverse metabolites were biosynthesized as a part of defense mechanism when subjected to competitive and harsh ecosystem.
The members of cephalopoda belonging to family Octopodidae are recognized as nutrition-rich health food with diverse pharmacological potentials (Chakraborty and Joy 2017; FAO, The State of World Fisheries and Aquaculture 2017). Among the octopus fauna, veined octopus Amphioctopus marginatus is extensively distributed in the Mediterranean and Asian coasts, and occupies the largest portion of annual octopus catches owing to its recognized culinary delicacies. Even though scanty reports on the pharmacological profiles of A. marginatus is available (Krishnan and Chakraborty 2019), elaborated studies on bioactive metabolites in search of prospective pharmacore models to combat lifestyle ailments are not much to the best of our knowledge. As part of our ongoing studies to isolate biopotential secondary metabolites from economically important unexplored cephalopoda, the organic crude extract of A. marginatus collected from the southwestern coast of Indian peninsula was subjected to sequential chromatographic fractionation. Based on this, we have isolated and thereafter characterized two 20-membered polyether macrolides, marginolide A and B based on the detailed spectral studies including NMR (nuclear magnetic resonance), mass spectrometry and FTIR (Fourier transform infrared) analyses. Antioxidant and antihypertensive properties of the isolated metabolites were appraised using in vitro models. Structure-bioactivity correlations were performed by various physico-chemical parameters of the studied compounds. The attenuation potential of the studied marginolides against ACE was substantiated by in silico molecular modeling analyses.

2. Results and discussion

2.1. Bioassay-supported chromatographic purification of marginolides A-B from A. marginatus

Size exclusion chromatographic fractionation of ethyl acetate-methanol (EtOAc-MeOH, 1:1 v/v) extract of A. marginatus (42.5 g) resulted in six combined fractions (AM1–AM6), among which AM3 (11.2 g) revealed considerably greater ACE attenuation potential (IC50 1.12 mg mL\(^{-1}\)) compared to other fractions (IC50 > 1.2 mg mL\(^{-1}\)) and superior antioxidant activities against the oxidants (IC50 ~ 1.3 mg mL\(^{-1}\)). Therefore, AM3 was submitted to flash chromatographic purification to result in four subfractions (AM3-a–AM3-d) (Table S1). Among those, AM3-b (1.37 g) registered greater bioactive potential (Table S1), and therefore, was further sub-fractionated using semi-preparative RP-HPLC to yield two homogenous polyether macrolide analogues, marginolides A and B (Figure 1).

2.2. Spectral analysis

2.2.1. Marginolide A

A furan enclosed 20-membered polyether macrocyclic lactonic compound, marginolide A was isolated as brownish oily liquid by sequential chromatographic fractionation. The molecular formula was ascertained as C\(_{25}\)H\(_{40}\)O\(_6\) \(\{\text{HR(ESI)}\}\) MS \(m/z\) 437.2907 \([M + H]^+\) and its structure was interpreted by spectroscopic analysis encompassing mass, 1D/2D NMR, UV, and FTIR (Figures S1–S15). The purity of marginolide A was proven by a homogenous peak at \(R_t\) of 6.952 min (Figure S16). \(^{13}\)C NMR spectrum
along with distortionless enhancement by polarization transfer (DEPT-135) spectrum (Figures S2 and S3) specified 25 carbon resonances inclusive of twelve $sp^3$ methylenes, three $sp^3$ methines, six $sp^2$ methines, two $sp^3$ methyls and two non-protonated carbons (Table S2). The distinguishing carbon signal at $\delta_C$ 173.2 recognised the manifestation of an ester functionality in marginolide A. Significantly deshielded proton at $\delta_H$ 4.10 at C-12 could correspond to the carbon at $\delta_C$ 75.6 (HSQC, Figure S4), which put forward the attachment of a hydroxyl entity geminal to the C-12 position as corroborated by $^1H-^{13}C$ HMBCs/$^1H-^1H$ COSY correlations (Oguchi et al. 2007). Further, the coupling between the deshielded methine proton at H-12 ($\delta_H$ 4.10) and the $sp^2$ hybridized carbon at C-11 ($\delta_C$ 137.8) attributed that the alkyl hydroxyl OH is contiguous to the olefinic double bond. The attachment of pentenyl structural fragment at C-4 of the 20-membered lactone ring was attributed by the long-range HMBC relationships of the olefinic proton H-2' ($\delta_H$ 5.40) and H-1' ($\delta_H$ 2.02) of alkenyl side chain to the non-protonated carbon C-4 ($\delta_C$ 30.8) of macrocyclic loop. A singlet three-proton signal appeared at $\delta_H$ 1.08 (H-4a), which inferred that the methyl group could remain attached to the non-protonated carbon (Litaudon et al. 1997). Three considerably higher downfield methylene proton signals at $\delta_H$ 3.68 (H-7), 3.99 (H-9) and 3.53 (H-13) corresponding to the carbon peaks at $\delta_C$ 71.7, 73.6 and 74.7 (HSQC, Figure S4) recognized that these protons were adjacent to the electronegative ether functionality in the compound (Salas and Chakraborty 2018). Similarly, significantly downfield methine protons at $\delta_H$ 3.83 (H-15) and $\delta_H$ 4.33 (H-19) attributed to the presence of an oxygen atom in the nearby position. HMBC spectral connections between $\delta_H$ 3.99 (H-9)/$\delta_C$ 71.7 (C-7) and $\delta_H$ 3.83 (H-15)/$\delta_C$ 74.7 (C-13) supported the presence of ether moiety in the macrocyclic ring. The presence of 20-membered macrocyclic ring structure in marginolide A was recognized based on the spectral data for $^1H-^1H$ COSY, TOCSY and $^1H-^{13}C$ HMBC (Figures S5–S7). $^1H-^1H$ COSY spectral experiment identified six isolated
spin systems, I–VI in which five were enclosed within the macrocyclic ring skeleton \{I \&d_\text{H} 2.34 (H-2)/\&d_\text{H} 1.63 (H-3); II \&d_\text{H} 1.37 (H-5)/\&d_\text{H} 1.50 (H-6)/\&d_\text{H} 3.68 (H-7); III \&d_\text{H} 3.99 (H-9)/\&d_\text{H} 5.71 (H-10); IV \&d_\text{H} 5.67 (H-11)/\&d_\text{H} 4.10 (H-12)/\&d_\text{H} 3.53 (H-13); V \&d_\text{H} 3.83 (H-15)/\&d_\text{H} 4.33 (H-19)/\&d_\text{H} 1.74 (H-20)/\&d_\text{H} 1.57 (H-21)/\&d_\text{H} 3.94 (H-22)\} and the remaining one \{VI \&d_\text{H} 5.36 (H-3'/\&d_\text{H} 1.93 (H-4')/\&d_\text{H} 0.89 (H-5')\} was due to the pentenyl sub-structural fragment, which was further substantiated by 2D TOCSY data (Figure S6). Two methylene protons at \&d_\text{H} 2.34 (H-2) and \&d_\text{H} 1.63 (H-3) involved in the spin system I displayed heteronuclear multiple bond correlations with carbonyl carbon \&d_\text{C} 173.2 (C-1) thereby suggesting the I spin system in the neighboring position of the ester moiety. Significantly downfield –CH₂ protons at \&d_\text{H} 3.99 (H-9) recognized that the methylene group could be allocated between the oxygen atom and an olefinic carbon. Greater coupling constants of 15.5 Hz corresponding to the protons at H-10 and H-11 attributed to the trans-disposition of the olefinic group in marginolide A. The alkenic protons H-2₀ and H-3₀ in the pentenyl side-chain exhibited smaller coupling constants of 5.0 Hz thereby inferring that these protons were aligned in cis fashion (Erickson et al. 1997; Chakraborty et al. 2019). Previous literature studies have reported the isolation of marine-derived macrocyclic lactones enclosing furanoid and pyranoid heterocyclic rings (Chakraborty et al. 2019; Francis and Chakraborty 2021), whereas the titled compound enclosed a dihydrofuran ring at the C-15 position. HMBC (Figure S7) connections between \&d_\text{H} 5.93 (H-17)/\&d_\text{C} 79.3 (C-19) and \&d_\text{H} 5.93 (H-17)/\&d_\text{C} 101.5 (C-16) revealed the presence of a fused 16, 17-dihydrofuran moiety. The connection of the furan ring to the basic macrocyclic ring was established by HMBC correlations \&d_\text{H} 3.83 (H-15)/\&d_\text{C} 29.3 (C-20)/\&d_\text{C} 74.7 (C-13) and \&d_\text{H} 5.93 (H-17)/\&d_\text{C} 79.3 (C-19). Further, the ¹H-¹H TOCSY correlation between \&d_\text{H} 3.83 (H-15)/\&d_\text{H} 1.74 (H-20) and \&d_\text{H} 3.83 (H-15)/\&d_\text{H} 4.33 (H-19) in spin system V also supported the attachment of furanoid ring to the former (Figure S6). The elemental composition of the compound submitted the presence of six indices of unsaturation conforming to four double bonds and two cyclic systems. The relative conformation of protons allocated in the stereocenters was assigned by NOESY correlations in conjunction with molecular mechanics MM2 studies (Figure S8(a,b)). NOE correlations at \&d_\text{H} 4.10 (H-12)/\&d_\text{H} 3.83 (H-15)/\&d_\text{H} 4.33 (H-19)/\&d_\text{H} 3.94 (H-22) inferred that these protons were lined up in an identical plane, and were considered as \(\alpha\)-oriented. The NOESY correlations between protons at \&d_\text{H} 1.08 (H-4a)/\&d_\text{H} 4.02 (H-22a) inferred that these protons were directed opposite to that of \(\alpha\)-orientation, thus considered as \(\beta\)-oriented (Salas and Chakraborty 2018). These inferences were further confirmed with the MM2 force-field studies. The base peak at m/z 68 in the gas chromatographic-mass spectrum of the studied compound corresponding to the furan could further corroborate the structural assignments (Figure S12). The base peak at m/z 437 [M + 1]⁺ as deduced by Cl/GC-MS analysis could further confirm the molecular mass of the titled compound (Figure S13). The FTIR spectrum recognized broad and strong (bs) absorption band at 3379 cm⁻¹ indicative of the hydroxyl (O-H) group in marginolide A (Hwang et al. 2014). The presence of ester carbonyl moiety was substantiated by the FTIR stretching vibration band at 1743 cm⁻¹. The strong absorption peak at 1043 cm⁻¹ ascribing to the C-O-C stretching further confirmed the presence of ether bridges in marginolide A (Salas and Chakraborty 2020). Additionally, the olefinic (−C= C stretch) functionalities were suggested from the strong and intense signal at 1624 cm⁻¹ in the IR spectrum (Figure S14).
Another 20-membered polyether macrocyclic lactone, marginolide B with a molecular formula of C$_{30}$H$_{48}$O$_9$ $\{\text{HR-ESI-MS } m/z}$ 553.3381 $[\text{M} + \text{H}]^+$, was isolated by chromatographic fractionation. Exhaustive 1D and 2D NMR spectroscopic techniques coupled with mass, 1D/2D NMR, UV, and FTIR were used for structural depiction (Figures S17–S31, Table S2). The homogeneity of the titled compound marginolide B was confirmed by the appearance of a single peak at $R_t$ of 6.545 min (Figure S32). The spectroscopic data of marginolide B was found to closely relate to marginolide A except for some additional resonance peaks, which could be attributed to the side-chain substitution in the core polyether macrocycle lactone loop. $^{13}$C NMR data along with DEPT-135 exhibited 30 carbon resonances counting those of two carbonyl carbons, one each of $sp^3$ non-protonated and olefinic carbon, five $sp^2$ methine carbons, three each of $sp^3$ methines and $sp^3$ methyl carbons, other than fifteen $sp^3$ methylenes (Table S2; Figures S18 and S19). The titled compound enclosed a 20-membered polyether macrocyclic ring with a fused dihydropyran ring at C-15 and C-20. The non-protonated carbon signals at $\delta_C$ 173.2 and $\delta_C$ 172.5 represented the endocyclic and exocyclic ester functional groups, respectively. Marginolide B included seven spin systems I–VII, in that five could fit into the nucleus macrocyclic ring and two to the substituted side branching. The COSYs, for example \{I $\delta_H$ 2.31 (H-2)/$\delta_H$ 1.61 (H-3); II $\delta_H$ 1.31 (H-5)/$\delta_H$ 1.48 (H-6)/$\delta_H$ 3.67 (H-7); III $\delta_H$ 3.96 (H-9)/$\delta_H$ 5.69 (H-10); IV $\delta_H$ 5.64 (H-11)/$\delta_H$ 4.08 (H-12)/$\delta_H$ 3.51 (H-13); V $\delta_H$ 3.10 (H-15)/$\delta_H$ 4.33 (H-20)/$\delta_H$ 1.67 (H-21)/$\delta_H$ 1.58 (H-22)/$\delta_H$ 3.81 (H-23)\} were accounted for the 20-membered ring and \{VI $\delta_H$ 5.39 (H-2')/$\delta_H$ 3.89 (H-3') and VII ($\delta_H$ 4.02 (H-17')/$\delta_H$ 1.70 (H-17')/$\delta_H$ 0.92 (H-17")\}: COSY off-diagonal peaks corresponded to the methoxy porpenyl and propyl acetate side chains, respectively. The HMBCs between $\delta_H$ 4.33 (H-20)/$\delta_C$ 29.1 (C-21)/$\delta_C$ 82.3 (C-15)/$\delta_C$ 141.1 (C-18) and $\delta_H$ 3.10 (H-15)/$\delta_C$ 76.2 (C-13). It was further corroborated by prominent 2D TOCSYs in spin system V from $\delta_H$ 3.10 (H-15)/$\delta_H$ 4.33 (H-20) and $\delta_H$ 1.58 (H-22)/$\delta_H$ 4.33 (H-20) (Figure S22). A previous report of literature described a 33-membered polyether macrolide, acuminolide A that enclosed two dihydropyrans and two furan moieties (Hwang et al. 2014). Three methylene signals appeared in the downfield positions $\delta_H$ 3.67, 3.96 and 3.51 showed HSQC connections with the carbon resonances at $\delta_C$ 71.0, 73.4 and 76.2 located at C-7, C-9 and C-13, respectively thereby indicating the occurrence of electronegative ether functionality. HMBC pairings between $\delta_H$ 3.96 (H-9)/$\delta_C$ 71.0 (C-7) and $\delta_H$ 3.10 (H-15)/$\delta_C$ 79.2 (C-13) could also reinforce the presence of ether groups within the macrocyclic ring. The propyl acetate side chain and its linking to 2H-dihydropyran ring at C-17 were anticipated by heteronuclear cross-peaks from $\delta_H$ 2.84 (H-17')/$\delta_C$ 110.0 (C-17) and $\delta_H$ 6.15 (H-18)/$\delta_C$ 38.9 (C-17'). The significantly downfield singlet at $\delta_H$ 3.33 (H-5') with three proton integral displayed HSQC with $\delta_C$ 53.4 owing to $sp^3$ methyl group attached with an oxygen atom, and was established by the long-range HMBC of $\delta_H$ 3.33 (H-5') to $\delta_C$ 72.5 (C-3'). $^{13}$C NMR spectrum revealed a downfield resonance signal at $\delta_C$ 75.1 (C-12), which attributed to the attachment of a hydroxyl group. The assignment was validated by the absence of exchangeable hydroxyl in the $^1$H NMR spectrum by deuterium exchange (D$_2$O-$^1$H NMR) analysis. Significantly downfield olefinic proton at $\delta_H$ 6.15 (C-18) put forward the...
presence of oxygenation in the vicinity. One singlet methyl group $\delta_H 1.09$ (H-4a)/$\delta_C 28.1$ (C-4a) was situated at C-4 apportioned by HMBC from $\delta_H 1.09$ (H-4a) to $\delta_C 32.6$ (C-4). Further, the quaternary nature of C-4 (DEPT-135) in consort with the HMBC cross-peaks between $\delta_H 1.09$ (H-4a) to $\delta_C 131.4$ (C-1') implied the additional methoxy propenyl substitution in the former position. Seven degrees of unsaturations were due to five double bonds and two cyclic rings. NOE relations between $\delta_H 4.08$ (H-12)/$\delta_H 3.10$ (H-15)/$\delta_H 4.33$ (H-20)/$\delta_H 3.76$ (H-23) revealed that these protons were aligned in an identical plane of symmetry, and were held as $\alpha$-oriented. NOE couplings between the pair of protons $\delta_H 3.81$ (H-23$\alpha$) and $\delta_H 1.09$ (H-4a) were aligned in the opposite plane and was designated as $\beta$-oriented. These assignments were further validated by MM2 force field mechanics (Figure S24(b)). The major mass fragment in the GC-MS was found to be $m/z$ 84, which could correspond to the dihydro-2H-pyran fragment (Figures S27–S29). The base peak at $m/z$ 553 [M + 1]$^+$ as deduced by CI/GC-MS analysis could further confirm the molecular mass of the titled compound (Figure S29). The IR spectrum of marginolide B displayed absorption bands at 3381 and 1737 cm$^{-1}$ that suggested the presence of hydroxyl and lactonic ester functionalities (Figure S30).

2.3. Bioactivities of marginolides

Bioactive properties including antihypertensive and antioxidant activities of the studied marginolides are described in Table S3, and their bioactivities were compared with those displayed by the standard antioxidant $\alpha$-tocopherol and ACE inhibitor, captopril. Marginolide A showed considerably greater antihypertensive properties against ACE enzyme ($IC_{50}$ 0.58 mM) than that exhibited by marginolide B ($IC_{50}$ 0.72 mM), whereas synthetic ACE inhibitor, captopril registered an $IC_{50}$ value of 0.016 mM. Inhibition of ACE would decelerate the biocatalytic conversion to angiotensin-II, resulting in the control of vasoconstriction. The mechanism of inhibition of ACE by ACE inhibitors was found to involve the binding of various structural moieties to the zinc ions located in the active site of ACE (Shionoiri et al. 1997). In this context, the marginolides with greater number of electron-rich centers might effectively bind with Zn$^{2+}$ ions in the binding site of ACE resulting in its greater inhibition. The ACE inhibitory activities of macrolides were reported previously (Chakraborty et al. 2019; Chakraborty and Francis 2021) that could corroborate the potential antihypertensive activity of marginolides in this study. An earlier report revealed ACE attenuation potential of the organic extract of Amphioctopus marginatus (Krishnan and Chakraborty 2019). Greater antioxidant properties of marginolide A against the oxidant species ($IC_{50}$ $\sim$ 1 mM) also supported its potential ACE inhibitory activity.

2.4. Structure-activity correlation studies

The manifested bioactivities of the studied marginolides A and B were corroborated with structural characteristics-based physiochemical descriptors, such as steric parameters, electronic, and lipophilicity factors. In comparison to the steric bulkiness, marginolide B was found to be bulkier (parachor, $Pr \sim 1271$ cm$^3$ mol$^{-1}$) than marginolide A (parachor, $Pr \sim 1029$ cm$^3$ mol$^{-1}$), which suggested that the latter could aptly fit into
the binding site of ACE enzyme thereby causing better binding interaction than marginolide B. This was further substantiated with the exhibited electronic properties of marginolide A and B (topological polar surface area, tPSA > 70, electric dipole moment, DPOL ∼ 3.0, maximum electrophilic super delocalizability, SEMX ∼ 1.9 and polarizibility Pl, ∼52 × 10⁻²⁴ cm³) when compared to the synthetic ACE inhibitor captopril (tPSA 57.61, DPOL 1.93, SEMX, 1.57 and polarizibility PI, 21.58 × 10⁻²⁴ cm³) (Table S3). Notably, marginolide A with lesser steric aspects demonstrated greater antioxidant potential (IC₅₀ 1.03–1.12 mM) than those recorded for marginolide B (IC₅₀ 1.16–1.28 mM) even though the electronic factor tPSA is comparatively lesser than the former. This indicates that polarity alone could not exclusively bring about all the requirements for greater bioactivity, especially for ACE inhibition where the attenuation potential largely depends on the steric aspects. A measure of lipohilicity, logarithmic coefficient of octanol-water (log P ow) is contemplated to be another important factor in determining the hydrophobicity, one of the major molecular descriptors to explain the pharmacological properties of bioactive leads (Kujawski et al. 2012). It was established that the biological activity exhibited a linear dependence with the hydrophobic character up to a certain limit, beyond that the activity was found to decrease with a further increase in lipophilic character (Kubinyi 1979). The log P ow coefficient of the studied marginolides lies within the boundary of hydrophobic-lipophilic balance (<5.0) consistent with the Lipinski rule of drug-likeness (Lipinski et al. 2001), which further substantiated their cellular permeability. Notably the titled marginolides with significantly greater log P ow than the standard captopril with log P ow 0.24 could appreciably demonstrate the augmented antihypertensive activity of the studied polyether macrolide analogues from A. marginatus.

2.5. Molecular docking analyses of marginolides

Molecular docking analyses of isolated marginolides against ACE enzyme were performed to corroborate their ACE inhibitory activities, and the docking parameters, such as number of hydrogen bonds, docking score, binding energy, intermolecular energy, inhibition constant, and torsional free energy were presented (Figure S33, Table S4). Marginolide A displayed three hydrogen bonds (ARG 522.A, SER 219.A and TRP 220.A) with amino acid remains in the ACE binding site, while marginolide B showed only one hydrogen bond with amino acid GLY 404.A. Lesser binding energy (−10.39 kcal mol⁻¹) and inhibition constant, Ki (24.31 nM) of marginolide A compared to those displayed by marginolide B (binding energy, −10.06 kcal mol⁻¹, inhibition constant, 42.02 nM) put forward the strong interaction of the former in the ACE active site thereby corroborating its greater ACE inhibition potential (IC₅₀ 0.58 mM). Earlier literature reported that the standard ACE inhibitor captopril exhibited an inhibition constant of −1.67 mM and binding energy of −3.79 kcal mol⁻¹ when docked with ACE (Kumar et al. 2018). Correspondingly, intermolecular energy (−11.37 kcal mol⁻¹) and torsional free energy (1.19 kcal mol⁻¹) were found to be lesser for marginolide A than those displayed by marginolide B (−11.16 and 2.68 kcal mol⁻¹, respectively) thereby supported the prospective ACE inhibitory potential of marginolide A.
3. Experimental

3.1. Chemicals, reagents, and instrumentation

Solvents and reagents were of chromatographic, spectroscopic and analytical quality (E-Merck, Darmstadt, Germany and Spectrochem, Mumbai, India). The reagents and solvents were redistilled in the all-glass system. Sephadex LH-20 (lipophilic sephadex) and angiotensin-converting enzyme were procured from Sigma-Aldrich (St. Louis, MO). The homogeneity of the isolated compounds were assessed using analytical high-pressure liquid chromatography (HPLC) apparatus (Shimadzu Corporation, Japan) coupled to a reverse-phase (RP)-C18 column (Phenomenex, USA; Luna 25 cm × 4.6 mm, 5 μ) combined with a binary gradient pump and photodiode array detector. Semi-preparatory RP-HPLC fractionation was performed on a C18 reverse-phase column (25 cm × 4.6 mm, 5 μ) connected with a semi-preparatory HPLC system. One-dimensional and two-dimensional nuclear magnetic resonance (NMR) spectral analyses were carried out on an NMR spectrometer (Bruker AVANCE III 500 MHz, Karlsruhe, Germany). Fourier-transform infrared (FTIR) spectral information was documented at the wavenumber span of 4000 and 400 cm⁻¹ (Perkin-Elmer 2000 FTIR, USA). UV-Vis spectrophotometric analyses were carried out using a Varian Cary 50 UV-visible spectrophotometer (Varian Cary, USA). Gas chromatography-mass spectrometric analysis was recorded through electronic impact (EI/GC-MS) ionization mode (Perkin-Elmer, Clarus 680, Waltham, MA) and chemical ionization mode (CI/GC-MS with isobutene as reagent gas) was carried out in a single quadruple GC-MS instrument (Shimadzu Corporation model QP-2020C NX, Kyoto, Japan). Liquid chromatography-high resolution mass spectrometric (LC-HRMS) analysis was conducted in electron spray ionization (ESI⁺) positive mode on an Agilent 6520 accurate mass Q-TOF LC/MS coupled with Agilent LC 1200 and an C18 column (Extend 1.8 μm, 2.1 × 50 mm).

3.2. Sample collection, pretreatment and extraction

Fresh samples of *A. marginatus* (family Octopodiadea, voucher specimen number of CMFRI/DE.3.1.2.6/MBRM) were brought together from Cochin harbor of the Arabian sea positioned at a latitude of 8°48′ N/longitude 78°9′ E and latitude 9°14′ N/longitude 79°14′ E in the south-west coast of Indian sub-continent. The samples were directly brought to the laboratory in freeze-dried conditions and cleaned properly with distilled water to remove unwanted debris. The samples were unambiguously identified by Dr KK Saji Kumar of Molluscan Fisheries Division in Central for Marine Fisheries Research Institute, Kochi, India. Other than the conventional morphological examination, the octopus species was identified by detailed examination of tentacles, suckers, ligula length and mucous pouches (both dorsal and ventral view). The edible portion (4.6 kg) of the samples was removed carefully and minced to homogeneity. The tissue portion was then subjected to lyophilization by using a laboratory-model lyophilizer (Beta 2-8 LD plus, Martin Christ, Germany) to yield the freeze-dried powder (1253 g, yield 27.23%), which was sonicated with EtOAc-MeOH solvent system (1:1 v/v, 500 × 6) and refluxed for 5-6 h under the nitrogenous atmosphere. The organic extract was dehydrated by passing through anhydrous sodium sulphate (35 g) and concentrated
under reduced pressure (45°C) using a rota-evaporator (Heidolph, Germany) to obtain a gummy material that was regarded as the crude solvent extract of *A. marginatus* (42.5 g).

### 3.3. Bioactivity-directed chromatographic purification

Organic extract of *A. marginatus* (42.5 g) was submitted to bioactivity-directed lipophilic Sephadex® (LH-20; 25–100 μm) chromatography using aqueous MeOH as eluent. Size exclusion chromatographic fractionation resulted in eleven fractions, which were reduced to six groups (AM1–AM6) after thin layer chromatography (dichloromethane/MeOH, 9:1 v/v for fractions AM1–3 and n-hexane/EtOAc, 7:3, v/v for fractions AM4–6) and RP-HPLC (MeOH-acetonitrile ACN, 40:60 v/v). The combined fractions were submitted to bioactivity assessment. Downstream purification of AM3 by flash chromatography over 230–400 meshed silica gel using n-hexane/EtOAc/MeOH afforded four subfractions (AM3-a–AM3-d). The fraction, AM3-b (1.372 g) was subjected to further chromatographic sub-fractionation using semi-preparative RP-HPLC (with MeOH-ACN as mobile phase, 40:60 v/v) to yield two homogenous polyether macrolide analogues, marginolides A and B.

### 3.4. Physicochemical and spectroscopic data

#### 3.4.1. Marginiolide A

Brownish oil; TLC (silica gel GF254; dichloromethane-MeOH, 9:1 v/v) *Rf*: 0.52; ¹H NMR CDCl3 (500 MHz, δH in ppm, J in Hz) (Figure S1); ¹³C NMR CDCl3 (125 MHz, δC in ppm) (Figure S2); ¹³⁵DEPT, ¹H-¹H COSY, TOCSY, HSQC, HMBC, NOESY data (Figures S3–S8a, Table S2); HRMS (ESI): found *m/z* 437.2907 [M+H]+, cal. for C25H41O6 437.2903 (Δ = 0.91 ppm) (Figure S10); GC-MS (El): found *m/z* 436.3 [M]+, cal. for C25H40O6 (Figures S11 and S12); GC-MS (Cl): found *m/z* 437 [M + 1]+ (Figure S13); FT-IR (stretching ν, bending δ, rocking ρ) (νmax, cm⁻¹): 897.15 (alkene C-Hd), 1043.10 (C-O-Cm), 1467.14 (C-Hq), 1743.31 (C = O), 2923.26 (C-Hr), 3373.71 (OHr) (Figure S14); *Rt* (C18-RP; MeOH-ACN, 40:60 v/v): 6.95 min (Figure S16); UV λmax MeOH (log ε): 285 nm (3.52) (Figure S15).

#### 3.4.2. Marginolide B

Brownish oil; TLC (silica gel GF254; dichloromethane-MeOH, 9:1 v/v) *Rf*: 0.57; ¹H NMR CDCl3 (500 MHz, δH in ppm, J in Hz) (Figure S17); ¹³C NMR CDCl3 (125 MHz, δC in ppm) (Figure S18); ¹³⁵DEPT, ¹H-¹H COSY, TOCSY, HSQC, HMBC, NOESY data (Figures S19–S24a, Table S2); HRMS (ESI): found *m/z* 553.3381 [M+H]+, cal. for C30H49O9 553.3377 (Δ = 0.72 ppm) (Figure S26); GC-MS (El): found *m/z* 552.3 [M]+, cal. for C30H48O9 (Figures S27 and S29); GC-MS (Cl): found *m/z* 553 [M + 1]+ (Figure S29); FT-IR (νmax, cm⁻¹): 897.15 (alkene C-Hd), 1063.13 (C-O-Cm), 1400.82 (C-Hq), 1467.14 (C-Hr), 1743.31 (C = O), 2923.26 (C-Hr), 3373.71 (OHr) (Figure S30); *Rt* (C18-RP; MeOH-ACN, 40:60 v/v): 6.54 min (Figure S32); UV λmax MeOH (log ε): 295 nm (3.35) (Figure S31).
3.5. Bioactivity assessment and molecular modeling

ACE attenuation assay of marginolides A and B evaluated their anti-hypertensive potential (Huang et al. 2010), whereas antioxidant activities were measured by DPPH and ABTS$^+$ radical quenching analyses (Joy and Chakraborty 2017). The obtained results were represented as 50% inhibitory concentration (IC$_{50}$) at that compounds/intermediate column fractions scavenge/inhibit 50% of the radical/enzyme activities, and was converted into mM for the isolated compounds and mg mL$^{-1}$ for the fractions obtained from chromatographic purifications. Physiochemical parameters, such as electronic (tPSA, PI), lipohilicity (log P$_{ow}$) and steric factors (MV, MR, and Pr) of marginolides were assessed by using ChemDraw Ultra (ver. 12.0, Cambridge Soft Corp., USA) and ACD ChemSketch (ver. 12.0; Advanced Chemistry Development Inc., Canada).

In silico molecular docking analysis of marginolides A and B was performed using Auto dock 4.0 (ver. 1.5.6). The compounds were drawn with Chemsketch (ver. 3.5). Open Babel GUI (version 2.4.1) software was used to translate MDL molfiles to the format of PDB. The protein structure of ACE I (PDB: 1UZE; resolution 2.0 Å) (Natesh et al., 2004) was downloaded from the protein databank (http://www.pdb.org), and was energetically minimized with Swiss-PdbViewer (SPDBV ver. 4.1) after eliminating the water molecules from the targeted protein. Docking analyses were carried out using this optimised protein. The 3D-grid box was constructed by Auto-Grid calculation, and the grid map set values were selected as $x = 40.623$, $y = 37.523$, $z = 43.456$ (124 × 124 × 120 Å) points. Cygwin-1/Cygwin-2 was used to run the docking algorithm, whereas the results were visualized using USCF chimera (ver. 1.11.2).

3.6. Statistical analysis

Statistical analysis was carried out using SPSS software (Statistical Program for Social Science 13.0, SPSS Inc, USA, ver. 13.0). All the experiments were carried out in triplicate and subjected to ANOVA (analysis of variance) and examined for the level of significance ($p < 0.05$).

4. Conclusions

Bioassay-guided chromatographic purification of the organic extract of veined octopus A. marginatus resulted in the isolation of two undescribed 20-membered polyether macrolide analogues, marginolide A and B. Marginolide A showed greater attenuation potential against angiotensin-converting enzyme. Structure-activity relationship studies revealed that lesser steric aspects with permissible hydrophobic-lipophilic balance favoured the better ligand-receptor interface with the ACE binding site, and also contributes towards greater antioxidant activities that were substantiated by docking parameters. These findings recognized that marginolide A could build up as a potential marine-derived polyether macrolide lead for use against hypertension.

Disclosure statement

The authors declare no competing financial interest.
Funding
The authors gratefully acknowledge the funding by the Indian Council of Agricultural Research (ICAR, New Delhi, India) (grant number MBT/HLT/SUB23). The authors are grateful to the Director, ICAR-CMFRI, and Head, Marine Biotechnology Division of ICAR-CMFRI for facilitating the research activities. The authors are thankful to the Chairman, Department of Chemistry, Mangalore University (Karnataka, India) for providing with the necessary support.

Data availability statement
The chromatographic and spectroscopic spectral data are included as supplementary item.

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