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Antioxidative 2*H*-chromenyls attenuate pro-inflammatory 5-lipoxygenase and carbolytic enzymes: Prospective bioactive agents from Babylonidae gastropod mollusk *Babylonia spirata*

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Abstract

Oxygenated heterocycles are emerging as valuable pharmacophores involved in the prophylaxis and treatment of several diseases elicited by the reactive oxygen species. Bioassay-led chromatographic fractionation of the organic extract of the gastropod mollusk *Babylonia spirata* (family Babylonidae) yielded two unprecedented 2*H*-chromenyl derivatives characterized as 2-(butyryloxy)-5-hydroxy-hexahydro-2*H*-chromene-3-methyl carboxylate (**1**) and (3-hydroxy-hexahydro-2*H*-chromen-2-yl) methyl pentanoate (**2**). The chromenyl derivative (**1**) registered significantly greater attenuation potential against pro-inflammatory 5-lipoxygenase (IC_{50} ~ 2.02 mM) than those exhibited by the compound (**2**) (IC_{50} 2.76 mM) and the non-steroidal anti-inflammatory drug ibuprofen (IC_{50} 4.36 mM, $p < .05$). The compound (**1**) exhibited comparable antioxidant activity (IC_{50} 1.47–1.72 mM) with standard antioxidative agent α-tocopherol (IC_{50} 1.4–1.7 mM). Inhibitory potential of chromenyl derivative (**1**) toward α-glucosidase (IC_{50} 1.18 mM) and α-amylase (IC_{50} 0.92 mM) was greater than those displayed by **2** (IC_{50} 1.16–1.56 mM). Structure–activity relationships revealed that bioactivities of the compounds were determined by the electronic factors and hydrophilic–lipophilic balance.

Practical applications

The marine gastropod *Babylonia spirata* is one of the prominent edible gastropod species harvested from the coastlines along the southwestern region of the Indian peninsula. Two 2*H*-chromenyl derivatives were isolated to homogeneity from the organic extract of the marine buccinid gastropod *B. spirata* by the bioactivity-guided chromatographic fractionation and were found to possess potential antioxidant and attenuation properties against pro-inflammatory 5-lipoxygenase and carbolytic enzymes. The attenuation properties of the 2*H*-chromenyls against pro-inflammatory 5-lipoxygenase showed that 2*H*-chromenyl analogs possessed significantly greater anti-inflammatory potential than the non-steroidal anti-inflammatory drug ibuprofen. In particular, the chromenyl derivative bearing 2*H*-chromene-3-methyl carboxylate framework might constitute a prospective biogenic constituent in functional food and pharmaceutical applications for use against oxidative agents, including inflammation and hyperglycemic pathologies.

Kajal Chakraborty and Soumya Salas have contributed equally to this work.

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KEYWORDS

2*H*-chromenyl derivatives, 2*H*-chromene-3-methyl carboxylate, anti-inflammatory, *Babylonia spirata*, Babylonidae, gastropod mollusk, starch digestive enzymes

1 | INTRODUCTION

Fused ring compounds containing heteroatoms were well-known for their pharmacological abilities to alleviate the pathophysiological conditions leading to the progression of metabolic disorders, such as diabetes, inflammation, and hypertension (Bhadoriya & Jain, 2016). Chromenes are a class of oxygenated heterocycles belonging to 2*H*-1-benzopyrans and their potential pharmacological properties and templates for the synthesis of several drug molecules were previously described (Blunt, Copp, Keyzers, Munro, & Prisep, 2016). These groups of compounds constitute the basic skeleton of several antioxidants including tocopherols, which provide defensive action against lipid peroxidation of membranes and further deleterious effects of free radical stimulated disorders (Kindleysides, Quek, & Miller, 2012). Benzopyran derivatives adopt unique structural features, which impart them the most appropriate hydrophilic–lipophilic balance properties facilitating the trans-bilayer transport within the biological system (Nicolaou et al., 2000). The 2*H*-chromen-2-yl derivatives belonging to sargachromenols, isolated from the colonial ascidian tunicates *Botryllus tuberatus*, were found to be the prospective drug molecules in the treatment of atherosclerosis (Choi et al., 2011). Scabellones possessing particularly rare benzo[*c*]chromene-7,10-dione scaffold showing potential to suppress the generation of superoxide induced human neutrophils in vitro, were isolated from the New Zealand ascidian *Aplidium scabellum* (Chan et al., 2011). Hexahydro chromenyl analogs with anti-inflammatory potentials were isolated from the solvent extract of the mollusk *Sepiella inermis* (Krishnan, Chakraborty, & Joy, 2018).

Previous reports of the literature demonstrated that the benthic marine gastropods, especially those belonging to the family Babylonidae were endowed with nutritional and potential medicinal properties (Chakraborty & Salas, 2020; Govindarajalu, Anand, Chelladurai, & Kumaraguru, 2016; Kuppusamy & Ulagesan, 2016; Salas, Chakraborty, Sarada, & Vijayagopal, 2018). However, they were not subjected to extensive chemical investigation to characterize the bioactive leads (Altena & Gittenberger, 1981; Fraussen & Stratmann, 2013).

The present study reported the purification and characterization of two new oxygenated heterocycles of chromenyl class of compounds from the ethyl acetate–methanol (EtOAc–MeOH) extract of the marine gastropod *Babylonia spirata* harvested from the coastlines along the southwestern region of the Indian peninsula. The studied compounds were designated as 2-(butyryloxy)-5-hydroxy-hexahydro-2*H*-chromene-3-methyl carboxylate (**1**) and (3-hydroxy-hexahydro-2*H*-chromen-2-yl)methyl pentanoate (**2**) (Figure 1) by extensive spectroscopic experiments. The anti-inflammatory and antioxidant potential of the studied compounds were analyzed by different in vitro assays and various physicochemical parameters were utilized to substantiate the structure–activity correlations of the studied chromenyl compounds. The present work also investigated the attenuating potentials of the studied chromenyls on key carbolytic enzymes (α-amylase and α-glucosidase) related to diabetes mellitus.

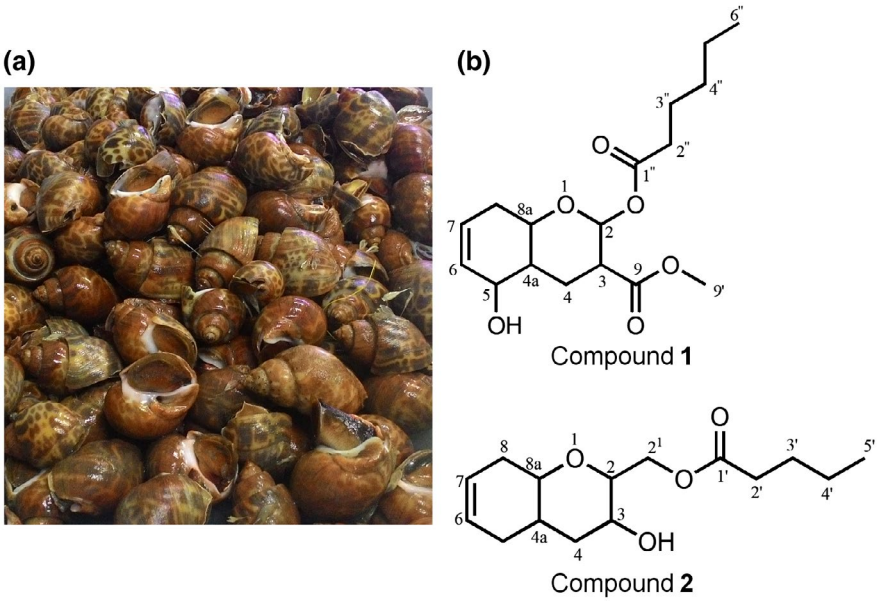


FIGURE 1 (a) Representative shell-on samples of Babylonidae gastropod *Babylonia spirata*. (b) Structural representations of 2-(butyryloxy)-5-hydroxy-hexahydro-2*H*-chromene-3-methyl carboxylate (compound **1**) and (3-hydroxy-hexahydro-2*H*-chromen-2-yl)methyl pentanoate (compound **2**) isolated from the organic extract of *B. spirata*

2 | MATERIALS AND METHODS

2.1 | Chemicals and instrumentation

The chemicals for bioactivity assays were purchased from E-Merck, Sigma-Aldrich (Missouri, USA), Sisco Research Laboratories (Mumbai, India), and HiMedia (HiMedia Laboratories LLC, USA). Silica gel of 60–120 and 230–400 mesh sizes and pre-coated plates of GF254 utilized for column chromatography and thin layer chromatographic separation (TLC), respectively, were purchased from E-Merck, Germany, and Biotage, Sweden. The solvents and reagents of chromatographic or analytical or spectroscopic grade were purchased from E-Merck (Darmstadt, Germany). The Varian Cary 50 conc UV-visible spectrophotometer (Varian Cary, USA) was operated for attaining spectrophotometric measurements. A Bruker AVANCE spectrometer operating at 500 MHz for ^{1}H nuclei and 125 MHz for ^{13}C nuclei was run to conduct the one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectral experiments in the aprotic solvent (deuterated chloroform, $CDCl_3$). Chemical shifts (δ) were denoted in ppm and coupling constants in Hz. Fourier-transform infrared (FTIR) spectra of KBr pellets of the organic extracts were recorded using a PerkinElmer Series 400 FTIR spectrophotometer equipped with an air-cooled DTGS (deuterated triglycine sulfate) detector and the spectral values were expressed in (%) transmittance. An ATAGO AP-300 polarimeter was used to measure the optical rotations. HR-ESI-MS of the isolated compounds was obtained using the Liquid chromatography-mass spectrometry system (Applied Biosystems QTRAP 2000, Germany) operating in the positive ion mode. Analytical high-pressure liquid chromatography (HPLC) instrument (Shimadzu Corporation, Nakagyo-ku, Japan) coupled with RP-C_{18} (bonded reverse-phase; Phenomenex, Torrance, USA; Luna 250 × 4.6 mm, 5 µm) connected with a binary gradient pump (Shimadzu LC-20AD) column and photodiode array detector (SPD-M20A, Kyoto, Japan) was utilized to assess the purity of the isolated compounds.

2.2 | Preparation of organic extract of the buccinid

Live species of the marine gastropod *B. spirata* (Family Babylonidae) (Figure 1) were gathered from the landing centers at Neendakara of Kollam district (Kerala State of India) located along the south-west coast of the Indian subcontinent (lat 8° 56′ N and long 76° 32′ E). The samples were recognized with the specimen accession number DB.24.1.1. by the National Marine Biodiversity Museum at ICAR Central Marine Fisheries Research Institute, Kochi. The fresh samples were directly taken to the laboratory, where the shells were cracked open using a specially devised hammer to recover the edible portions. The tissues (5 kg) were cleaned using distilled water to eliminate the associated dirt and the tissues (5 kg) were thoroughly minced. The shredded tissues were freeze-dried and powdered (1.5 kg) before being sonicated with ethyl acetate–methanol solvent system (EtOAc/MeOH 1:1 v/v, 500 ml × 2, RT, 8 hr) and refluxed at 40–50°C for a duration of 4 hr. The pooled fractions were filtered (using Whatman No.1) through anhydrous Na_2SO_4 (40 g) to remove the water content before evaporating the extract (45°C) to dryness using a rotary vacuum evaporator (Heidolph Instruments GmbH and Co., Schwabach, Germany) under reduced pressure to obtain dark yellow viscous residues, being the crude EtOAc–MeOH extract of *B. spirata*. The extraction processes were repeatedly carried out for three times on the lyophilized powder and the triplicate values were calculated, while estimating the yield (18 ± 0.50 g/kg of wet tissue, yield on dry weight basis 5.8 ± 0.08%).

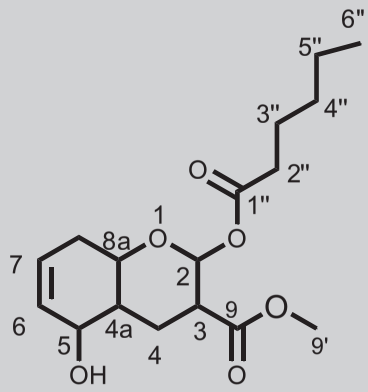
2.3 | Chromatographic purification of the chromenyl compounds from the organic extract of *B. spirata* and spectroscopic characterization

The crude EtOAc–MeOH extract (50 g) of *B. spirata* was slurried with 10 g silica gel (60–120 mesh) and loaded into a glass column (5 × 150 cm) packed with silica gel (60–120 mesh sized, 700 g). The column was initially eluted with the solvent *n*-hexane to eliminate the pigmented and waxy material. The polarity of the eluents (*n*-hexane/EtOAc 9:1 to 2:8, v/v) was progressively increased to attain 13 fractions of 25 ml each, which were minimized to six groups (SB_1–SB_6) based on the TLC (*n*-hexane/EtOAc, 9:1, v/v) analysis. The pooled fractions were hence subjected to bioactivity screening using the anti-oxidant assays, such as 2,2'-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid ($ABTS^{+}$) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical decolorization along with the anti-diabetic and anti-inflammatory assays evaluating the α-amylase/α-glucosidase and 5-LOX inhibitory potentials, respectively. The main fraction SB_3 demonstrated greater α-amylase/α-glucosidase attenuating potential in addition to good antioxidant and anti-inflammatory activities and hence was chosen for further downstream purification. The fraction was slurried with silica gel (100–200 mesh) and loaded on to a silica column. The eluent polarity (*n*-EtOAc/MeOH 9:1 to 2:8, v/v) was gradually increased to provide 12 fractions of 50 ml each, which were further reduced to six groups ($SB_{3\text{-}1}$–$SB_{3\text{-}6}$) based on the TLC patterns (*n*-hexane/EtOAc, 2:8, v/v). The subfraction $SB_{3\text{-}3}$ showing greater inhibitory activity against pro-inflammatory enzyme and carbolytic enzymes was fractionated through RP-HPLC using MeOH–ACN (9:1, v/v) resulting in two major peaks for the purified compounds **1** and **2** (R_t 8.7 and 10.5 min, respectively).

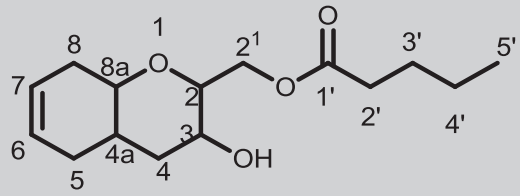
2.3.1 | 2-(Butyryloxy)-5-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromene-3-methyl carboxylate

Pale yellow oily liquid; $[\alpha]^{26}_{D}$ − 24.0 ° ($CHCl_3$, c0.014); UV (MeOH) λ_{max} (log ε): 257 (3.17) nm; TLC {silica-GF(254); EtOAc/*n*-hexane, 3:2 v/v} R_f: 0.40; R_t (HPLC, MeOH: ACN 3:2 v/v): 8.7 min; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (Table 1); ^{1}H-^{1}H-COSY, and HMBC data (Table 1, Figure 2); IR (KBr, cm^{-1}) ν_{max} (ν = stretching, δ = bending, ρ = rocking vibrations) 720.99

TABLE 1 NMR spectroscopic data of compounds **1** and **2** in $CDCl_3$[a]



Compound 1



Compound 2

C. No.	^{13}C	^{1}H NMR (mult, J in Hz)[b]	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	C. No.	^{13}C	^{1}HNMR (mult,J in Hz)[b]	^{1}H-^{1}HCOSY	^{1}H-^{13}C HMBC
1	–		–		1	–			
2	98.2	6.74 (d, *J* = 6.5 Hz, 1H)	H-3	C-3, 2^1	2	70.5	4.14 (dt, *J* = 5.3, 3.4 Hz, 1H)	H-3, 2^1	C-2^1, 3, 8a
3	49.1	2.90 (m, 1H)	H-4	C-9, 4	3	69.0	3.39 (m, 1H)	H-4	C-4, 4a, 2^1
4	24.9	1.60 (m, 2H)	H-4a	C-3, 4a, 8a	4	31.9	1.80 (dd, *J* = 2.9, 5.8 Hz, 1H)	H-4a	C-4a, 5, 2
							1.75 (dd,*J* = 3.6, 2.7 Hz, 1H)		
4a	43.8	2.04 (m, 1H)	H-5		4a	49.5	1.96 (m, 1H)		C-8a, 6
5	68.9	3.50 (dd, *J* = 13.5, 1.5 Hz, 1H)	H-6	C-7	5	30.7	2.04 (dd, *J* = 2.03, 5.6 Hz, 2H)	H-6	C-8a, 6, 4
6	130.0	5.36 (dd, *J* = 5.5, 1.5 Hz, 1H)	H-7	C-4a, 8	6	131.8	5.45 (dt, *J* = 5.8, 1.2 Hz, 1H)	H-7	C-8, 7
7	120.7	5.26 (dt, *J* = 5.5, 1.57 Hz, 1H)	H-8	C-6, 8	7	127.0	5.32 (dt, *J* = 5.9, 1.2 Hz, 1H)	H-8	C-8a
8	31.9	2.21 (dd, *J* = 3.86, 6.7 Hz, 2H)	H-8a	C-7	8	32.4,	2.01 (dd, *J* = 2.8, 9.5 Hz, 1H)	H-8a	C-4a, 7
8a	71.0	3.39 (m, 1H)	–	C-2			1.98 (m, 1H)		
9	173.3	–	–	–	8a	73.6	3.15 (m, 1H)		C-4a, 7
9 ′	52.7	3.66 (s, 3H)		C-C-9	2^1	66.8	3.65 (d, *J* = 6.4 Hz, 2H)		C-1', 2, 3
1′′	175.0	–	–	–	1′	173.3	–		
2′′	34.1	2.31 (t, *J* = 5.6 Hz, 2H)	H-3′′	C-4′′, 1′′	2′	34.2	2.31 (t, *J* = 6.5 Hz, 2H)	H-3′	C-4′, 1′
3′′	29.7	1.64 (p, *J* = 3.2 Hz, 2H)	H-4′′		3′	27.2	1.60 (m, 2H)	H-4′	
4′′	29.4	1.29 (m, 2H)	H-5′′	C-6′′	4′	22.1	1.25 (m, 2H)	H-5′	
5′′	22.7	1.31 (m, 2H)	H-6′′	C-3′′	5′	14.2	0.88 (t, *J* = 6.5 Hz, 3H)		
6′′	14.1	0.89 (t, *J* = 6.8 Hz, 3H)							

[a]NMR spectra were recorded using a Bruker AVANCE III 500 MHz (AV 500) spectrometer (Bruker, Karlsruhe, Germany) in $CDCl_3$ as aprotic solvent at ambient temperature with TMS as the internal standard (δ 0 ppm).

[b]Values in ppm, multiplicity, and coupling constants (*J* = Hz) were indicated in parentheses. Multiplicities were allocated by ^{135}DEPT NMR spectrum. The assignments were made with the aid of the COSY, HSQC, HMBC, and NOESY experiments.

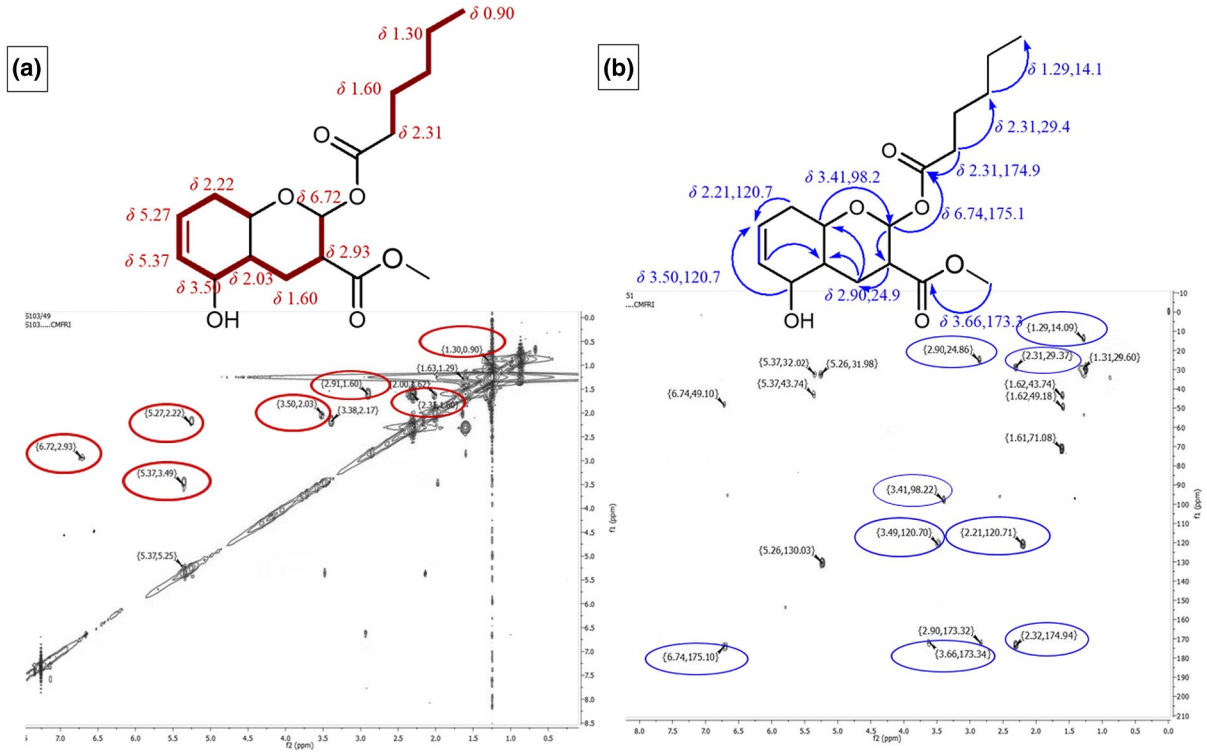


FIGURE 2 (a) ^{1}H-^{1}H COSY (bold-faced bonds) and (b) HMBC spectra (double-barbed arrows) of 2-(butyryloxy)-5-hydroxy-hexahydro-2*H*-chromene-3-methyl carboxylate (compound **1**). The characteristic ^{1}H-^{1}H COSY and HMBC cross-peaks were highlighted in the spectra

(alkene C–H_{δ}), 1,057.28, 1,235.37 (C–O–C_{ν}), 1,459.00 (C–H_{δ}), 1756.00 (C=O_{ν}), 2,925.24 (C–H_{ν}), 3,450.85 (OH_{ν}); (EI) MS *m/z* found 326.1732 $[M]^{+}$ and HR(ESI)MS found 327.1809 $[M + H]^{+}$, calcd. for $C_{17}H_{26}O_6$ 326.1729 (Δ 0.5 ppm).

2.3.2 | (3-Hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromen-2-yl)methyl pentanoate

Pale yellow oily liquid; $[\alpha]^{26}_{D}$ − 19.0° ($CHCl_3$, c0.014); UV (MeOH) λ_{max} (log ε): 231 (3.48) nm; TLC {silica-GF(254); EtOAc/*n*-hexane, 3:2 v/v} R_f: 0.35 (EtOAc/*n*-hexane 3:2, v/v); R_t (HPLC, MeOH:ACN 9:1, v/v): 10.5 min; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (Table 1); ^{1}H-^{1}H-COSY, and HMBC data (Table 1, Figure 3); IR (KBr, cm^{-1}) ν_{max} (ν = stretching, δ = bending, ρ = rocking vibrations) 725.00 (alkene C–H_{δ}), 1,052.00, 1,182.54 (C–O–C_{ν}), 1,376.14 (C–H_{ρ}), 1,463.26 (C–H_{δ}), 1752.16 (C=O_{ν}), 2,932.86 (C–H_{ν}), 3,457.42 (O-H_{ν}); (EI) MS *m/z* found 268.1679 $[M]^{+}$, HR(ESI)MS found 269.1757 $[M + H]^{+}$, calcd. for $C_{15}H_{24}O_4$ 268.1675 (Δ 0.4 ppm).

2.4 | Pharmacological properties

In vitro anti-inflammatory properties of the purified compounds were studied by evaluating their attenuation properties against pro-inflammatory enzyme 5-LOX (Baylac & Racine, 2003). Anti-diabetic properties of the studied compounds and column fractions were evaluated by the inhibitory properties against carbolytic enzymes α-amylase (source, porcine pancreas) and α-glucosidase (source, yeast) (Kim, Kwon, & Son, 2000). The antioxidant properties were assessed by DPPH (Chew, Lim, Omar, & Khoo, 2008) and $ABTS^{+}$ radical decolorization assay (Erel, 2004; Wojdylo, Oszmianski, & Czemerys, 2007). The results were expressed as IC_{50} (the concentration of samples at which 50% of enzyme/radical activities was inhibited/scavenged, presented as mg/mL and/or mM) values. Various physicochemical factors of the isolated chromenyl derivatives were studied in an attempt to compare its exhibited bioactivity with the proposed structure (Ajay, Walters, & Murcko, 1998; Joy & Chakraborty, 2017; Salas & Chakraborty, 2018a). ChemDraw ultra 11 (ver. 8.0; Cambridge Soft Corporation, USA) and ACD ChemSketch (version 12.0) were utilized to generate the structural parameters, such as partition coefficient for octanol–water system (log P_{ow}), categorized as a hydrophobic descriptor (Kujawski, Popielarska, Myka, Drabińska, & Bernard, 2012); topological polar surface area (tPSA) and polarizability (Pl) grouped as electronic descriptors; parachor (P), molar volume (MV), and molar refractivity (MR), classified as steric descriptors.

2.5 | Enzyme kinetic studies

The mechanisms of enzyme inhibition by the isolated 2*H*-chromenyl derivatives were examined by constructing the Lineweaver–Burk plots. The rate of inhibition of the carbolytic enzymes α-amylase and α-glucosidase at varying concentrations (0.0625, 0.125, 0.25, and

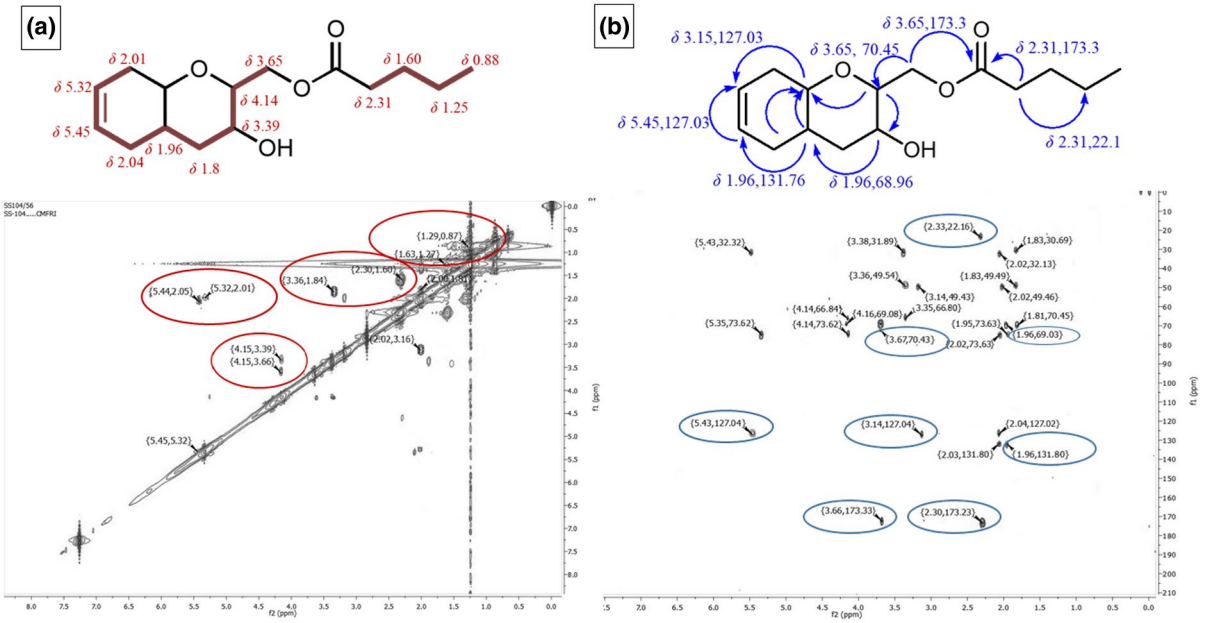


FIGURE 3 (a) ^{1}H-^{1}H COSY (bold-faced bonds) and (b) HMBC spectra (double-barbed arrows) of (3-hydroxy-hexahydro-2*H*-chromen-2-yl) methyl pentanoate (compound **2**). The characteristic ^{1}H-^{1}H COSY and HMBC cross-peaks were highlighted in the spectra

0.5 mM) of substrate soluble starch was represented, in the presence and absence of the inhibitor compounds, in the Lineweaver–Burk plots. Similarly, the double-reciprocal plots were analyzed for the kinetics study of the 5-LOX enzyme against the different concentrations (0.0625, 0.125, 0.25, and 0.5 mM) of natural substrate linoleic acid in the presence and absence of the isolated compounds. The inhibitor compounds used in varying concentrations were expressed in millimolar units (mM).

2.6 | Statistical analysis

Statistical Program for Social Sciences 13.0 (SPSS Inc., Chicago, IL, ver. 13.0) was accessed for calculating significant differences between the means (one-way analysis of variance, ANOVA) of triplicate ± standard deviations of all assays, and was represented as $p < .05$. The analyses were conducted in triplicate and the means of parameters were expressed as mean ± standard deviation. The significance level of the mean values was set at 0.05 and analyzed by one-way analysis of variance (ANOVA) with Scheffe's post hoc analysis. All statistical evaluations were carried out using the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0).

3 | RESULTS AND DISCUSSION

3.1 | Bioassay-directed chromatographic purification of the secondary metabolites of *B. spirata*

Initial chromatography-guided fractionation of EtOAc:MeOH crude extract of the marine gastropod *B. spirata* over the silica gel column ensued six main fractions (SB_1-SB_6) that were evaluated for their antioxidant, anti-inflammatory, and antidiabetic potentials. The fractions SB_1, SB_2, and SB_6 had a solvent volume of 50 ml each; SB_3 and SB_4 measured 75 ml each, and SB_5 of 25 ml. Although all the solvent fractions had good antioxidant potentials, the main fraction SB_3 exhibited the highest antioxidant potential (IC_{50} DPPH/ABTS^{+} scavenging ≤ 0.95 mg/ml) when compared to the remaining fractions. Additionally, the fraction SB_3 demonstrated greater α-amylase/α-glucosidase attenuating potential (IC_{50} α-amylase/α-glucosidase inhibitory activity ≤ 0.98 mg/ml), along with noteworthy anti-inflammatory activities (IC_{50} 5-LOX inhibitory activity of 1.10 mg/ml) (Table S1). Potential attenuating properties against 5-LOX and carbolytic enzymes α-amylase/α-glucosidase displayed the significance of the column fraction, SB_3 for further chromatographic subfractionation, whereas the inhibiting activities of the column fractions $SB_{1\text{-}2}$ and SB_4-SB_6 were significantly lesser (IC_{50} > 1.5 mg/ml, $p < .05$) (Table S1). The main fraction, SB_3 upon flash chromatographic purification, yielded six subfractions ($SB_{3\text{-}1}$–$SB_{3\text{-}6}$), based on TLC (*n*-hexane/EtOAc, 2:8, v/v). The column sub-fraction $SB_{3\text{-}3}$ exhibited antioxidant activity (Table S1) analogous to the standard α-tocopherol itself (DPPH and ABTS radical cation scavenging activities of IC_{50} values 0.60 and 0.73 mg/ml, respectively). The fraction $SB_{3\text{-}3}$ also showed better anti-carbolytic enzyme inhibition activities (IC_{50} ≤ 0.6 mg/ml) and greater inhibitory activity against pro-inflammatory enzyme 5-LOX (IC_{50} ≤ 0.9 mg/ml) (Table S1). Therefore, the fraction $SB_{3\text{-}3}$ was subjected to preparatory reverse-phase (RP)-C_{18} HPLC using MeOH-ACN (9:1, v/v), yielding the purified compounds **1** and **2** (R_t 8.7 and 10.5 min, respectively). The anti-inflammatory potentials of the isolated compounds were determined by in vitro 5-LOX inhibitory assay (IC_{50} 2.02–2.8 mM) and the activities were compared with the non-steroidal anti-inflammatory ibuprofen (IC_{50} 4.36 mM). The antidiabetic activities of the purified compounds

(IC_{50} 0.9–1.6 mM) were estimated using α-amylase and α-glucosidase inhibitory assays, and the results were compared against the activity of the standard drug acarbose (0.4–0.6 mM).

3.1.1 | 2-(Butyryloxy)-5-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromene-3-methyl carboxylate (1)

The purified compound **1** was obtained as a yellow oily liquid. The mass spectrometric analyses [EIMS *m/z* 326.1732 and HRESIMS *m/z* 327.1809 $(M + H)^+$], supported by the one-dimensional (1D) and two-dimensional (2D) NMR data (Figure S1-S9) suggested the elemental composition of the isolated compound as $C_{17}H_{26}O_6$. The ^{13}C NMR analysis proposed the presence of 17 carbon resonances. The downfield signals found at δ 173.3 and 175.0 were confirmed as quaternary carbons due to the absence of these peaks from the DEPT spectrum. The carbon resonances in the range of δ 170–185 belonged to the carbonyl groups, specifically ester functionalities, and hence, the ^{13}C NMR signals at δ 173.3 and 175.0 were attributed to the ester carbons. Further scrutiny of the ^{13}C NMR and DEPT spectral data attributed that compound possessed two methyl groups at δ 14.1 and 52.7, along with six methylene groups at δ 24.9, 31.9, 34.1, 29.7, 29.4, and 22.7. The methine carbon peaks were found at δ 98.2, 49.1, 43.8, 68.9, 130.0, 120.7, and 71.0. The methine carbon at δ 98.2 (C-2) demonstrated significant downfield shifts and marked the presence of electronegative oxygen in the vicinity. The absence of any notable signals in the aromatic region of the proton NMR spectra (δ 6.5–8.5) of the compound ruled out the possibility of an aromatic moiety in the structure. The ^1H-^1H COSY spectra identified two structural fragments of consecutive proton spin systems as follows: (1) δ_H 6.74 (H-2)/2.90 (H-3)/1.60 (H-4)/2.04 (H-4a)/3.50 (H-5)/5.36 (H-6)/5.26 (H-7)/2.21 (H-8)/3.39 (H-8 a); (2) δ_H 2.31 (H-2′′)/1.64 (H-3′′)/1.29 (H-4′′)/1.31 (H-5′′)/0.89 (H-6′′) (Figure 2). The HMBC cross-peaks between the downfield proton δ 6.74 (H-2) and the methine carbon δ71.0 (C-8a) indicated their proximity and plausible linkage via oxygen atoms. The HMBC couplings from δ_H 6.74 (H-2) to δ_C 49.1(C-3); δ_H 2.90 (H-3) to δ_C 24.9 (C-4); δ_H 1.60 (H-4) to δ_C 43.8 (C-4a)/71.0 (C-8a); δ_H 3.50 (H-5) to δ_C 120.7 (C-7); δ_H 5.36 (H-6)/5.26 (H-7) to δ_C 31.9 (C-8) displayed that the first spin system incorporated a closed-loop of two ring systems (Figure 2). The second spin system was found to comprise of a neopentyl carbon chain. The long-range HMBC couplings of the methine proton at δ_H 6.74 (H-2) in the first structural fragment and the methylene protons at δ_H 2.31 (H-2′′) in the second structural fragment to the carbon signal at δ_C 175 ratified that the two partial structures might be joined via the ester carbonyl group. The HMBC couplings from the deshielded methyl protons at δ_H 3.66 (H-9′) and the methine proton at δ_H 2.90 (H-3) to the ester carbonyl at δ_C 173.3 corroborated that the attachment at C-2 ought to be a methyl carboxylate group (Figure 2). The relative stereochemistry of the chiral centers of the molecule was defined by the NOESY spectrum and coupling constants (*J* values). NOE cross-peaks were observed between the following pair of protons: H-2 (δ 6.74) and H-8a (δ 3.39); H-8a (δ 3.39) and H-4a (δ 2.04), which indicated that these protons were inclined at the same plane of reference of the molecule, and therefore, these were arbitrarily assigned as α-disposed. These correlations further confirmed the *cis*-fusion of the pyranose ring to the cyclohexene ring as described in a previous report of literature (Joy & Chakraborty, 2017). The β-disposition of the chiral proton H-3 (δ 2.90) was deduced from the absence of NOE cross-peaks between H-3 (δ 2.90) and the α-assigned protons. The coupling constant of the H-5 proton (δ 3.50) due to its spin interaction with the bridge-head proton (H-4a) at δ 2.04 was about 13.5 Hz, which was attributed to be due to the *trans*-diaxial coupling. Therefore, the chiral proton H-5 was attributed to being β-aligned.

The molecular formula of $C_{17}H_{26}O_6$ established by the parent ion peak at *m/z* 326.1732 and the HRESIMS data *m/z* 327.1809 for the pseudo molecular ion peak $(M + H)^+$, suggested the double bond equivalence of five, which were inclusive of an alkene and two ester carbonyls based on the typical chemical shift values of protons and carbons (^1H and ^{13}C NMR). The remaining degrees of unsaturations had to be accounted by carbocycles within the structure. The parent ion peak at *m/z* at 326 (1a) appeared to eliminate a water molecule to form a prominent peak at *m/z* 308 (1f), which appropriately assigned the incidence of a hydroxyl group in **1**. The fragment peak at *m/z* 308 (1f) appeared to undergo typically McLafferty rearrangement to give rise to the peak at *m/z* 252 (1l). The base peak was recorded at *m/z* 99 (1e), which was attributed to the acylium ion that might form via α-cleavage at the ester carbonyl, thereby attributing that the acid part of the ester might be comprised of a six-carbon chain. The subsequent loss of two neutral CO molecules from the fragment ion at *m/z* 237 (1g) could result in a peak at *m/z* 164 (1h).

The occurrence of the ester carbonyl in **1** was suitably confirmed by an intense band at 1,756 cm^{-1} in the FTIR spectrum (Figure S10). The bands referring to the stretching vibration of the hydroxyl groups and –C=C– were present at 3,450 and 1,646 cm^{-1}, respectively, whereas the absorption bands between 1,250 and 1,054 cm^{-1} (C–O–C stretch) manifested the ether functionality in the isolated compound. The combined interpretation of spectral data attributed the compound **1** as 2-(butyryloxy)-5-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromene-3-methyl carboxylate.

3.1.2 | (3-Hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromen-2-yl)methyl pentanoate (2)

The compound **2** was isolated as a pale yellow oily liquid and its parent ion peak found at *m/z* 268 and HRESIMS m/z found at 269.1757 $(M + H)^+$, which along with extensive NMR studies attributed the elemental composition as $C_{15}H_{24}O_4$ (Figure S11-S19). The studied compound exhibited four double bond equivalences with an alkenic double bond and one ester carbonyl based on the representative chemical shift values of protons and carbons (^1H and ^{13}C NMR), and the remaining degree of unsaturation was due to the carbocycles within the structure. The ^{13}C NMR and DEPT spectroscopic data showed that the compound possessed a total of 15 carbons, including one methyl, seven methylene, four sp^3 methine (including three oxymethines), two

sp^2 methine, and one sp^2 quaternary carbon (Table 1). The ^{1}H NMR spectrum exhibited the resonances of two olefinic methine protons (δ 5.38 and 5.35 Hz). The ^{1}H-^{1}H COSY spectral analysis revealed two structural fragments of uninterrupted proton spin systems, such as (1) δ_H 3.65 (H-2[1])/4.14 (H-2)/3.39 (H-3)/1.8 (H-4)/1.96 (H-4a)/2.04 (H-5)/5.45 (H-6)/5.32 (H-7)/2.01 (H-8)/3.15 (H-8a) and (2) δ_H 2.31 (H-2′)/1.60 (H-3′)/1.25 (H-4′)/0.88 (H-5′) (Figure 3). The key HMBC correlations from δ_H 4.14 (H-2) to 73.6 (C-8a) and 69.0 (C-3); δ_H 3.15 (H-8a) to δ_C 49.5 (C-4a) and 127.0 (C-7); δ_H1.96 (H-4a) to δ_C 73.6 (C-8a) and 131.8 (C-6); δ_H 2.04 (H-5) to δ_C 73.6 (C-8a), 131.8 (C-6), and 31.9 (C-4); δ_H 5.45 (H-6) to δ_C 127.0 (C-7) and 32.4 (C-6); δ_H 5.32 (H-7) to δ_C 73.6 (C-8a); δ_H2.01 (H-8) to δ_C 49.5 (C-4a) led us to conclude the presence of a cyclohexene fused to a pyranose ring within the first spin system (Figure 3). The disposition of carbon atoms in the side chain and their point of attachment to the ring system were explained by the further long-range couplings between δ_H 4.14 (H-2)/δ_C 34.20 (C-2′); δ_H 2.31 (H-2′)/δ_C 70.5 (C-2); δ_H 2.31 (H-2′)/δ_C 173.3 (C-1′); and δ_H 2.31(H-2′)/δ_C 22.1 (C-4′). Thus, the integrated HSQC, HMBC along with ^{1}H-^{1}H COSY correlation analyses attributed the structure of compound **2** as 3-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromen-2-yl)methyl pentanoate. The relative configuration of chiral centers at C-2, C-3, C-4a, and C-8a was deduced by the NOESY correlation analyses. The NOESY cross-peaks were found between the chiral protons at δ 3.15 (H-8a)/δ 4.14 (H-2) and δ 4.14 (H-2)/δ 3.39 (H-3), which appropriately attributed that these protons were positioned at the same plane of reference and assigned as β-protons. The absence of cross-peaks between the chiral proton at δ 1.96 (H-4a) and the β-protons (H-8a, H-2, and H-3) led to the conclusion that the H-4a might be α-disposed. This suggested that the pyranose ring might be *trans*-fused to the cyclohexene ring.

The mass fragmentation pattern of the purified compound showed the parent ion at 268 $[M]^+$. The added confirmation for the presence of the core ring of a hexahydro-2*H*-chromen-2-yl derivative with an –OH group at C-3 was ascribed by the indicative peaks at *m/z* 136 [M^+-side chain-H_2O] (2f), 166 (2g); 148 (2h) and 250 (2b). The elucidation of the side chain was additionally supported by the advent of fragment ion at *m/z* 115 (2e). The abundant fragment ions at *m/z* 166 (2g) and *m/z* 208 (2d) were recognized as the characteristic peaks of the McLafferty rearrangement (MR) ions. The base peak was found at *m/z* 94 (2i), which might be due to the 2-methylene-2*H*-pyran ion fragment, formed by the allylic cleavages of the MR ion at *m/z* 166 (2g).

The FTIR absorptions were observed at around 3,400 cm^{-1}, which attributed the presence of the hydroxyl groups. The distinctive IR absorptions representing the C=C stretching were represented by the peak at 1,643 cm^{-1}, whereas the typical absorption at 1,463 cm^{-1} specified the bending vibrations due to C–H groups. The FTIR absorption bands at 1,182 and 1,052 cm^{-1} substantiated the presence of C–O–C stretching vibrations found in ether linkages, whereas the symmetric stretching of the ester carbonyl was evident at 1,752 cm^{-1} (Figure S20). The integrated spectroscopic experiments attributed the compound **2** as (3-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromen-2-yl)methyl pentanoate (**2**).

3.2 | Bioactive potentials of the 2*H*-chromenyls isolated from *B. spirata*

The in vitro antioxidant properties of the 2*H*-chromenyls isolated from *B. spirata* were appraised according to their ability to quench 1,1-diphenyl-2-picryl-hydrazil (DPPH) and 2,2-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid ($ABTS^{\cdot+}$) radicals, and the target bioactivities were judged against the commercially available standard antioxidants (Table 2). Out of the two isolated 2*H*-chromenyl compounds, the one bearing 2*H*-chromene-3-methyl carboxylate (**1**) exhibited greater antioxidant capacity showing potential scavenging activity against the free radicals in vitro (IC_{50} DPPH 1.47 mM; IC_{50} ABTS 1.72 mM), and the antioxidant activities were comparable to the standard antioxidative agent α-tocopherol (IC_{50}1.39–1.69 mM). The free radical quenching abilities of the chromenyl compounds derived from marine organisms were reported previously (Joy & Chakraborty, 2017). The hypoglycemic properties of the isolated chromenyls were studied through their attenuation potential against carbohydrate digesting enzymes α-amylase and α-glucosidase. The compound (**1**) with substituted 2*H*-chromene-3-methyl carboxylate moiety showed superior α-amylase and α-glucosidase attenuation potentials (IC_{50} 0.92 mM and 1.18 mM, respectively) than those displayed by compound **2** bearing substituted 2*H*-chromen-2-yl-methyl pentanoate moiety (IC_{50} 1.16 mM and 1.56 mM, respectively). The anti-inflammatory activities of the purified compounds as determined by their attenuation properties against pro-inflammatory 5-lipoxygenase also showed that 2*H*-chromenyl analog (**1**) possessed significantly greater anti-inflammatory potential (IC_{50} ~ 2 mM) than that displayed by the compound (**2**) (IC_{50} 2.76 mM) as well as the non-steroidal anti-inflammatory drug ibuprofen (4.36 mM) (Table 2). Earlier reports by Chellaram and Edward (2009a, 2009b) have established marine gastropods *Trochus tentorium* and *Drupa margariticola* were potent inhibitors of exudative and proliferative stages of inflammation. The studies by Santhi, Sivakumar, Thilaka, and Thangathirupathi (2012) vouched that the gastropods of genus *Babylonia* were potent enough to moderate the carrageenan-induced inflammation in albino rats as against the control of diclofenac sodium.

The studied bioactivities of the 2*H*-chromenyls isolated from *B. spirata* were corroborated by different physico-chemical attributes of the studied compounds. The pharmacological properties of the compounds were positively correlated with the polarizability (Pl) and topological polar surface area (tPSA), which defined the electronic properties of the compounds, along with the lipophilic parameter of log P_{ow} (logarithmic value of octanol–water coefficient), instead of the steric attributes. It is of note that the electronic parameters recorded higher values for compound **1** (Pl~33, tPSA 82.06) than those exhibited by compound **2** (Pl~29, tPSA 55.76), Though both the compounds possessed the same basic 2*H*-chromenyl moiety, the ester functionalities attached to appropriate sites of the pyranose ring in compound 1 must have facilitated the easy donation of proton radicals (from H-2 and H-3 positions) through hydrogen atom transfer (HAT) mechanism to stabilize the model-free radicals

TABLE 2 In vitro antioxidant, antidiabetic, and anti-inflammatory activities of the chromenyl derivatives purified from *B. spirata*

	Antioxidant activity[1]		Antidiabetic activity[1]		Anti-inflammatory activity[1]
Compounds	DPPH scavenging	$ABTS^+$ scavenging	α-amylase inhibitory	α-glucoxidase inhibitory	5-LOX attenuation
Compound **1**	$1.47^a \pm 0.01$	$1.72^a \pm 0.01$	$0.92^a \pm 0.02$	$1.18^a \pm 0.01$	$2.02^a \pm 0.02$
Compound **2**	$1.86^c \pm 0.02$	$2.16^c \pm 0.01$	$1.16^c \pm 0.02$	$1.56^c \pm 0.03$	$2.76^b \pm 0.02$
Positive standards	[3]$1.39^b \pm 0.03$	[3]$1.69^a \pm 0.03$	[5]$0.43^b \pm 0.01$	[5]$0.56^b \pm 0.02$	[4]$4.36^c \pm 0.01$
	Molecular descriptors[2]				
	Electronic		Steric		Hydrophobic
	PI ($\times 10^{-24}$ cm^3)	tPSA	*P* (cm^3)	MV (cm^3)	log P_{ow}
Compound **1**	33.08	82.06	717.7	277.2	2.21
Compound **2**	28.53	55.76	619.7	246.8	1.85
α-Tocopherol	51.63	29.46	1,085.3	446.5	9.98
Ibuprofen	24.09	37.31	497.6	200.3	3.75

Note: The samples were analyzed in triplicate ($n = 3$) and expressed as mean ± standard deviation. Means followed by different superscripts (a–c) in a single column indicated significant differences ($p < .05$).

Abbreviations: log P_{ow}, logarithm of the octanol–water partition coefficient; MV, molar volume; *P*, parachor; PI, polarizability index; tPSA, total polar surface area.

[1]The bioactivities were expressed as IC_{50} values (mM).

[2]The structure activity analysis was carried out by various molecular descriptors of the purified compounds as described in the text.

[3]α-Tocopherol.

[4]Ibuprofen.

[5]Acarbose.

employed. Moreover, compound 1 possessed an additional hydroxyl group which could also contribute to the radical scavenging potential (Figure S23). These structural peculiarities might be corroborated by the greater pharmacological properties of the compound possessing substituted 2*H*-chromene-3-methyl carboxylate moiety. The aggregate numbers of electron-localized centers were greater in **1** than those in compound **2** as labeled by the appraisal of electronic variables, thereby resulting in the higher polarizability of the former, than those documented with the compound **2** bearing substituted 2*H*-chromen-2-yl-methyl pentanoate moiety. It is also significant to note that the log P_{ow} of the compound **1** was found to be within the optimum range of 2–5, which has been characteristic of a functional pharmacophore holding appropriate lipophilic–hydrophobic features (Lipinski & Hopkins, 2004). The log P_{ow} of **1** was deduced to be within the acceptable threshold (~2.2) that might result in satisfactory lipophilic–hydrophilic association (Lipinski, 2004) and receptor coherence (Huuskonen, Livingstone, & Tetko, 2000; McNally et al., 2007) resulting in higher pharmacological properties than compound **2**. Notably, the steric bulk of the compound (**1**) (parachor, P~718 cm^3/mol) was greater than compound **2** bearing substituted 2*H*-chromen-2-yl-methyl pentanoate (P~ 620 cm^3/mol) apparently due to the presence of the methyl carboxylate group at the C-3 position of compound **1**. However, compound (**2**) with relatively lower steric parameters demonstrated lesser antioxidant activity, which apparently recognized that the site of the hydroxyl groups and electron-withdrawing ester groups in compound (**1**) has more than compensated its higher steric bulk to impart greater bioactivities. This furthermore established the function of electronic and hydrophobic parameters in attributing the pharmacological potentials of the studied compounds. The Lineweaver–Burk plots (Figure S24-S26) demonstrate that the isolated 2*H*-chromenyl derivatives have a tendency for the non-competitive mode of inhibition toward the enzymes, α-amylase, and α-glucosidase, and against the pro-inflammatory 5-lipoxygenase. The kinetic parameters showed that the studied compounds could cause a decrease in V_{max}, while the K_m value remained constant (Table S2). This suggested that the 2*H*-chromenyls isolated from Babylonidae gastropod mollusk *Babylonia spirata* could compete with the substrates for the active site, but allosterically controlled the enzyme activities.

Marine gastropods have been recognized as potential sources of biomolecules, which could withstand and suppress the pathogenesis of oxidative stress-induced diseases (Chakraborty & Salas, 2019; Chakraborty, Salas, & Joy, 2018; Tamilmuthu & Selvaraj, 2015). Reactive oxygen species (ROS) are major intermediaries that were found to induce inflammatory processes. The discharge of reactive oxygen species from the actuated neutrophils and macrophages could contribute partially to the mechanistic pathway of inflammation resulting in tissue impairment by injuring macromolecules and peroxidation of membranes lipids. Hence, the neutralization of these ROS by antioxidants and radical scavengers could attenuate the inflammation (Hajieva & Behl, 2006). A positive connection between oxidative stress, stimulation of an inflammatory cascade, and many pathogenic conditions, such as cardiovascular diseases, cancer, atherosclerosis, Alzheimer's, diabetes, and aging

was proved in the recent years (Hajieva & Behl, 2006; Paine, Eizvesper, Blasczyk, & Immenschuh, 2010). The oxidative stresses in experimental diabetes have been found to decrease with the supplementation of antioxidants, such as vitamins E and C (Madhu & Devi, 2000). Thus, the chemoprotective role of non-toxic antioxidants in the prophylaxis and treatment of diabetes was established. The enzymes α-glucosidase and α-amylase being the main starch hydrolyzing enzymes, their inhibition is one of the important therapeutic methods to retard the absorbance of carbohydrates in the intestine, resulting in the control of postprandial hyperglycemia. There have been previous reports of antioxidative secondary metabolites, such as a disecosteroid and polyether macrocyclic lactone with potential anti-diabetic and anti-inflammatory properties from the gastropod species *B. spirata* (Chakraborty, Joy, & Salas, 2019; Salas & Chakraborty, 2018b).

4 | CONCLUSIONS

Bioactivity directed chromatographic purification of the organic extract of the Buccinid gastropod mollusk *B. spirata* yielded two 2*H*-chromenyl derivatives. The compounds were structurally characterized as 2-(butyryloxy)-5-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromene-3-methyl carboxylate and (3-hydroxy-3,4,4a,5,8,8a-hexahydro-2*H*-chromen-2-yl)methyl pentanoate with potential bioactivities. The 2*H*-chromene derivative (**1**) possessing substituted 2*H*-chromene-3-methyl carboxylate moiety showed potential antioxidant and anti-inflammatory activities, with greater attenuation potential against pro-inflammatory lipoxygenase-5 than those displayed by its 2*H*-chromen-2-yl)methyl pentanoate chemotype **2**, and ibuprofen. The chromenyl derivative (**1**) exhibited greater attenuation property toward the carbolytic enzymes, α-glucosidase, and α-amylase than those displayed by compound **2** bearing 2*H*-chromen-2-yl-methyl pentanoate moiety. In particular, the chromenyl derivative bearing 2*H*-chromene-3-methyl carboxylate skeleton might constitute a prospective biogenic constituent in functional food and pharmaceutical applications for use against oxidative agents, including inflammation and hyperglycemic pathologies.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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