Ulvapyrone, a pyrone-linked benzochromene from sea lettuce *Ulva lactuca* Linnaeus (family Ulvaceae): newly described anti-inflammatory agent attenuates arachidonate 5-lipoxygenase

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**ABSTRACT**

Green marine macroalgae, particularly *Ulva lactuca*, is an essential constituent of the cuisines in many Asian countries. The present work aims to separate a bioactive pyrone attached benzochromene analogue, named as ulvapyrone from the organic extract of *U. lactuca*, followed by its structural characterisation as 2-\{(6a'-hydroxyethyl-4'-methyltetrahydro-2H-pyran-2'-one)-6'-yl\}-4'-methyl-7-ethylacetate-8-hydroxy-7, 8-dihydrobenzo [de]chromene. Ulvapyrone exhibited prospective inhibition property against arachidonate 5-lipoxygenase (IC\(_{50}\) \(\approx\)1 mg mL\(^{-1}\)) comparable to that demonstrated by ibuprofen (IC\(_{50}\) 0.9 mg mL\(^{-1}\)), which connoted its anti-inflammatory activity. The studied benzochromene exhibited promising antioxidant potential (IC\(_{50}\) 0.5–0.6 mg mL\(^{-1}\)), which further reinforced its attenuation property against 5-lipoxygenase. Bioactivities of ulvapyrone were linearly correlated with electronic parameter (topological polar surface area \(\sim 102\)) along with less binding energy (\(-8.22\) kcal mol\(^{-1}\)) with the allosteric site of 5-lipoxygenase. *In silico* predictions of physicochemical parameters along with absorption, distribution, metabolism and excretion could recognise the acceptable oral bioavailability of ulvapyrone.
1. Introduction

Green marine macroalgae are common ingredients in the cuisine of south-east Asian countries like Japan, Korea and China primarily for their captivating flavour, and also been developed as functional foods in the western countries (Mani et al. 2021). Green marine macroalgae belonging to the family Ulvaceae (142 species) were recognised to constitute a significant share among the division Chlorophyta (Guiry and Guiry 2013). Consumption of green marine macroalgae, such as *Ulva* sp. is notably known for its desirable flavor and bioactivities, which were corroborated with higher life expectancy in humans. For instance, *Ulva pertusa* contain high levels of protein (20–26%) and are consumed by Japanese people as ‘ao-nori’ (Fujiwara-Arasaki et al. 1984). They were reported to contain amino acids, fatty acids, fiber, vitamins, and minerals, which have been utilised in animal feed and human food (Rohani-Ghadikolaei et al. 2012; Santos et al. 2015; Bikker et al. 2016). Conspicuously, the bioavailability of functional metabolites was influenced by their microbiota, and their metabolising capacity is the key factor for biotransformation of ingested food components (Vamanu and Gatea 2020). Noticeably, the infection of COVID-19 in Hokkaido was very low, and it was attributed to consumption of green marine macroalgae, which is predominant traditionally in Japan. Antioxidant property of marine macroalgae derived compounds could able to maintain numerous immune systems, act against oxidative impairment, and hasten cell signaling (Kavitha 2020).

Pyrone-containing natural products isolated from marine sources, with several pharmacological activities represent a distinctive class of secondary metabolites (Singh 2020). Also, two isomers of this class (α and γ pyrone) exhibited as important motifs in synthetic chemistry (Lee 2015). Two novel pyrone metabolites derived from alga-associated fungus *Aspergillus flavus* were tested against cyclic AMP assay on HEK293 and CHO cells (Lin et al. 2008). Pyrone ring comprising macrocyclic enol ethers were isolated from marine macroalga *Phacelocarpus labillardieri* (Kazlauskas et al. 1982; Shin et al. 1986). A novel macrocyclic pyrone metabolite was isolated from the Australian
red alga *Phacelocarpus peperocarpos* (Murray et al. 1995). Anti-inflammatory pyranoid pharmacophores were isolated from an intertidal macroalga *Turbinaria conoides* (Chakraborty and Dhara 2020). A nitric oxide inhibitor of pyrone class (marinopyrone D) was isolated from marine alga-associated actinomycetes (Lee et al. 2016). Labdane diterpenoids isolated from green marine macroalga *Ulva fasciata* showed promising antibacterial properties (Chakraborty et al. 2010a). Earlier investigations on the chemical compounds from *U. fasciata* identified the occurrence of guaiane sesquiterpene and sphingosine with free radical scavenging and antiviral properties (Garg et al. 1992; Chakraborty et al. 2010b; Chakraborty and Paulraj 2010).

Green marine macroalgae belonging to the family Ulvaceae were considered as essential food components in the Asian countries, and among which *Ulva lactuca* (formerly *Ulva fasciata*), commonly known as sea lettuce, is a prominent edible species. Green marine macroalgae are available in large quantities all over the Indian coastline, and the existing reports (Blunt et al. 2018) instituted these species as valuable marine sources of bioactive compounds. *U. lactuca* (phylum Chlorophyta) is an abundantly available species of Ulvaceae family, in the Southern coastal area of India. *U. lactuca* is an edible marine macroalga used in salads and soups, and has reported antibacterial, antioxidant, and antiviral activities (Beach et al. 1995, Rouxel et al. 2001; Chakraborty et al. 2010b). There were limited reports related to its chemical constituents, recognising the exploration of *U. lactuca* for bioactive secondary metabolites. During the chemical investigation to isolate bioactive metabolites from *U. lactuca*, the present work aims to separate a bioactive pyrone attached benzochromene analogue, named as ulvapyrone from the ethyl acetate-methanol (EtOAc-MeOH, 1:1 v/v) extract of the alga harvested from the south west coast of Indian peninsular expanse, followed by its structural interpretation (Figure 1). The purified compound was analysed for their potential to attenuate 5-lipoxygenase (5-LO). Comprehensive spectroscopic techniques encompassing nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), and mass spectral experiments were used to unequivocally characterise the isolated compound. The targeted bioactivities of ulvapyrone were structurally correlated with various physicochemical parameters, whereas molecular docking study described the interaction with the active site of 5-LO. In addition, oral bioavailability of ulvapyrone
was evaluated with Swiss ADME (absorption, distribution, metabolism, and excretion) predictive tools.

2. Results and discussion

2.1. Chromatographic fractionation and spectral analysis of ulvapyrone

Organic extract of the thallus parts of *U. lactuca* was subjected to repeated chromatographic purification to isolate ulvapyrone, which was characterised as 2-{(6α′-hydroxyethyl-4′-methyltetrahydro-2H-pyran-2′-one)-6′-yl}-4-methyl-7-ethylacetate-8-hydroxy-7, 8-dihydrobenzo[de]chromene.

Molecular formula of ulvapyrone was determined as C_{24}H_{28}O_{7} on the basis of mass spectral experiment {high resolution electrospray ionisation mass spectroscopy, HRESIMS, m/z 429.1923 [M + H]^+}, and the structure was elucidated by detailed NMR analysis (Figures S1–S10). ^{13}C NMR spectrum (Figure S2) along with DEPT-135 (distortion-less enhancement by polarisation transfer spectroscopy) (Figure S3) showed resonances for twenty four carbon atoms with four methyl sp^3, two methylene sp^2, ten methine sp^2 and eight non-protonated carbons (Table S2). Characteristic ^{13}C NMR signals recorded at δ_C 168.4 and 173.3 were due to the ester functionalities, which were attributed to the cyclic or straight chain ester groups (Chakraborty and Dhara 2020). Presence of highly deshielded protons at δ_H 4.08 and δ_H 3.97 (due to the attachment with an electronegative atom, might be oxygen) was observed in the ^{1}H NMR spectrum. ^{1}H NMR spectrum (Figure S1) of the titled compound displayed a doublet olefinic proton at δ_H 5.23 (doublet, coupling constant J = 7.48 Hz) along with a singlet at δ_H 5.69 (H-3), which demonstrated the presence of olefinic groups. Occurrences of other two olefinic protons were confirmed by its characteristic peaks at δ_H 5.95 and 5.82 in ^{1}H NMR (Figure S1) and their HSQC (heteronuclear single quantum coherence spectroscopy) correlations (Figure S5) with the signals at δ_C 128.1 and 130.9, respectively. Deshielded methine (-CH-) protons at δ_H 4.21 (correlated with δ_C 65.1, from HSQC) and δ_H 4.25 (conforming to δ_C 69.1, from HSQC) were geminal to the hydroxyl groups. The titled compound was deduced to contain eleven degrees of hydrogen deficiencies, which included two carbonyls, four rings (including one aromatic ring) and five olefinic bonds. Occurrence of hydroxyl group was deduced by deuterium exchange experiment, and also by the O-H stretching frequency in IR spectrum at 3438 cm\(^{-1}\) (Figure S10). ^{13}C NMR signals at δ_C 133.2 (C-6a) and 136.6 (C-4) were assigned as non-protonated carbons, which were confirmed by the disappearance of these peaks in DEPT spectrum (Figure S3). Two non-protonated carbons at δ_C 156.3 (C-9a) and 139.9 (C-2) exhibited deshielding shift in ^{13}C NMR attributable to the attachment with electronegative oxygen functionality. Other two non-protonated carbons at δ_C 116.4 (C-9b) and 129.0 (C-3a) were comparable with the earlier reported phenolic polyketides (Liu et al. 2017). ^{1}H–^{1}H COSY cross-peaks (Figure S4) of ulvapyrone exhibited spin systems between δ_H 1.24 (H-12)/4.08 (H-11); δ_H 2.65 (H-7)/4.21 (H-8); δ_H 5.82 (H-5)/5.95 (H-6), and δ_H 4.21 (H-8)/5.23 (H-9). Presence of 4-methyl-7-ethyl acetate-8-hydroxy-7,8-dihydrobenzo[de] chromene framework was established by ^{1}H/^\(^{13}\)C HMBC correlations. HMBC correlations (Figure S6) were observed at δ_H 5.69 (H-3) to δ_C 116.4 (C-9b)/129.0 (C-3a); δ_H 5.82 (H-5) to δ_C 136.6 (C-4)/133.2 (C-6a); δ_H 5.95
(H-6) to δC 130.9 (C-5)/136.6 (C-4); δH 2.65 (H-7) to δC 133.2 (C-6a)/65.1 (C-8)/98.6 (C-9); δH 4.21 (H-8) to δC 41.9 (C-7)/156.3 (C-9a); δH 5.23 (H-9) to δC 65.1 (C-8)/116.4 (C-9b); δH 1.24 (H-12) to δC 71.6 (C-11); and δH 4.08 (H-11) to δC 168.5 (C-10). Attachment of ethyl acetate group with 4-methyl-8-hydroxy-7, 8-dihydrobenzo [de]chromene was established by the HMBCs from δH 2.65 (H-7) and 4.21 (H-8) with the non-protonated carbon at δC 168.5 (C-10). One methyl group was appeared at δH 1.89 (H-13) with singlet, which exhibited strong HMBC with δC 136.6 (C-4). Another spin system exhibited between δH 0.88 (H-4a′)/2.11 (H-4′)/2.70 (H-3′), 1.41 (Hb-5′), δH 1.41 (Hb-5′)/3.97 (H-6′)/4.25 (H-6a′). Strong HMBC correlations were observed at δH 1.15 (H-5a′) to δC 69.1 (C-6a′); δH 4.25 (H-6a′) to δC 74.9 (C-6′); δH 3.97 (H-6′) to δC 24.9 (C-5′)/38.2 (C-4′); δH 2.11 (H-4′) to δC 24.9 (C-5′)/173.3 (C-2′); δH 0.88 (H-4a′) to δC 38.2 (C-4′); and δH 2.70 (H-3′) to δC 173.3 (C-2′), could attribute the presence of 6a′-hydroxyethyl-4′-methyltetrahydro-2H-pyran-2′-one moiety in the titled compound. Connectivity of 6a′-hydroxyethyl-4′-methyltetrahydro-2H-pyran-2′-one moiety with 4-methyl-7-ethyl acetate-8-hydroxy-7,8-dihydrobenzo[de]chromene was affirmed by HMBC at δH 3.97 (H-3′) to δC 139.9 (C-2) and 114.8 (C-3). IR spectrum (Figure S10) of ulvapyrone also supported the presence of olefinic (C= C) functionalities, which were represented by the signal at 1632 cm\(^{-1}\). IR absorption bands at 1748 cm\(^{-1}\) recognised the carbonyl stretching frequencies in this compound. Relative stereochemistry at C-8, C-7, C-3′, C-4′, C-6′, C-6a′ were established from nuclear overhauser effect spectroscopy (NOESY) cross-peaks. NOE correlations (Figures S7 and S12) were obvious at δH 2.65 (H-7)/4.21 (H-8), which described that these two protons were disposed at the equal plane of reference, and were \(\alpha\)-oriented. The \(\alpha\)-orientation of H-7 proton designated the opposite orientation (assigned as \(\beta\)-oriented) of ethyl acetate side chain at C-7 position. Other NOE correlations were observed at δH 4.25 (H-6a′)/2.70 (H-3′) and δH 0.88 (H-4a′)/4.25 (H-6a′), which represented the alignment of methyl group at C-4′ and the proton at C-6a′ were inclined on the same plane, and they were assigned as \(\alpha\)-oriented. Besides, the hydroxyl groups at C-8 and C-6a′ were aligned at the reverse plane of the proton at δH 4.21 (H-8) and 4.25 (H-6a′), respectively. NOE correlation was predicted between δH 2.11 (H-4′) and 3.97 (H-6′), and their alignment were assigned as \(\beta\)-oriented (Figure S7).

### 2.2. Bioactivities of ulvapyrone

Ulvapyrone was primarily assessed for their bioactivities against the enzyme 5-lipoxygenase (5-LO), which could cause the pathophysiologic conditions leading to inflammation (Table S3). The studied compound exhibited comparable inhibitory potential against 5-LO (IC\(_{50}\) 1 mg mL\(^{-1}\)) with anti-inflammatory agent ibuprofen (IC\(_{50}\) 0.93 mg mL\(^{-1}\)). 5-LO fosters the progression of inflammatory reactions, for example rheumatoid arthritis and autoimmune diseases on account of its ability to produce inflammatory 5-hydroxyeicosatetraenoic acid and leukotrienes. Consequently, 5-LO is a favorable target for pharmaceutical applications in several diseases, comprising inflammation (Basil and Levy 2016). Antioxidant properties of ulvapyrone (IC\(_{50}\) 0.5–0.6 mg mL\(^{-1}\)) further supported its anti-inflammatory potential. Occurrence of electron-rich side chains as well as the 2H-pyranone unit might play a pivotal role towards potential bioactivities of the studied compound by electron delocalisation process.
2.3. Structure-activity relationship analysis

Bioactivities of ulvapyrone were correlated with several molecular descriptors, such as hydrophobic receptor (logarithmic octanol-water partition co-efficient, log \( \text{P}_{\text{OW}} \)), polar (polarisability PI/topological polar surface area tPSA), and steric \{parachor (P)/molar volume (MV)/molar refractivity (MR)\} factors (Maneesh and Chakraborty 2017a), which demonstrated its structure-activity relationship and pharmacophore-target interaction (Table S3). Noticeably higher tPSA value of titled compound (102.29), compared to standards (tPSA < 35) recognised the presence of electron-rich centres in ulvapyrone, which could be the cause of the increased electronic properties (Table S3). Electronic factors might display greater interaction with active site of 5-LO at their reaction centres, resulting in higher inhibitory activities of ulvapyrone. As well, the significant antioxidant properties of ulvapyrone (IC\(_{50} \approx 0.6 \text{ mg mL}^{-1}\)) could be properly justified by its greater electronic properties. Hydrophobicity of ulvapyrone (log \( \text{P}_{\text{OW}} \) 1.8) were recorded within the admissible boundary of hydrophobic-lipophilic balance (Lipinski and Hopkins 2004), which could be ascribed to their potential bioactive properties. An adequate permeability in cellular system in conjunction with antioxidant properties of the studied compound might result in its potential bioactivities.

2.4. Physicochemical properties and ADME parameters

Swiss ADME web based tools were used (Daina et al. 2017) for prediction of various physicochemical properties, lipophilicities, solubilities, and drug-likeness of ulvapyrone with commercially available standard ibuprofen. Based on some physicochemical properties the qualitative prediction was performed, and as a result ulvapyrone conceded the filter of Lipinski’s rule with zero contravention (Table S4). Consequently, as result of these drug-likeness predictive parameters, ulvapyrone showed an acceptable oral bioavailability, and could conceivably be adopted as oral drugs, besides anticipated bioavailability score for ulvapyrone was similar to ibuprofen (0.55) (Table S4). For rapid estimation of drug-likeness, a bioavailability radar plot was effected with lipophilicity, size, polarity, solubility, flexibility and saturation (Figure S13) (Daina et al. 2017). The range of optimum values for each parameter was displayed by a pink area and evidently, no aberration was observed for ulvapyrone resulting in optimal physicochemical properties for oral bioavailability. Estimated aqueous solubility (SILICOS-IT, ESOL and log S) for ulvapyrone based on molecular structure was in soluble range (Table S4). Skin permeability coefficient \( K_p \) (pharmacokinetics) for ulvapyrone was observed a closer value \((-7.41 \text{ cm/s})\) with respect to ibuprofen \((-5.07 \text{ cm/s})\) (Table S4). The isolated compound exhibited as non-permeability glycoprotein substrates, which could be related with its high human gastrointestinal absorption (TPSA < 140 Å\(^2\)), resembling ibuprofen.

2.5. In silico molecular docking studies of ulvapyrone

Molecular docking analysis study of ulvapyrone was acted upon against pro-inflammatory enzyme 5-LO. \textit{In-silico} molecular docking analysis suggested the interaction between active site of targeted enzyme with the docked ligand. Ulvapyrone on molecular modeling exhibited two hydrogen bonding interactions with PRO 242.B
(molecular distance 2.34 Å) and PHE 359.B (molecular distance 3.58 Å) aminoacyl residues in the active site of 5-LO. Close interface between 5-LO and ulvapyrone was represented by Figure S14. The docking score, binding energy, intermolecular energy, and inhibition constant along with torsional free energy of the compound was tabulated (Table S5). The titled compound was recorded a minimum binding energy of $-8.22 \text{kcal mol}^{-1}$ with less docking score ($-8.46 \text{kcal mol}^{-1}$), enzyme inhibition constant (1.94 μM) and intermolecular energy ($-8.83 \text{kcal mol}^{-1}$). The minimum docking score and binding energy of ulvapyrone could appropriately define its enzyme attenuation potential against 5-LO.

3. Experimental

3.1. Chemicals and instrumentation

Analytical and high pressure liquid chromatography (HPLC)-grade solvents, chemicals and reagents were obtained from E-Merck (Darmstadt, Germany). FTIR absorption spectra and optical rotations of the isolated compound were measured by using an FT-IR spectrophotometer scanning from 4000 to 400 cm$^{-1}$ (Perkin-Elmer-2000, Waltham, MA) and a polarimeter (ATAGO/AP-300, Atago USA, Inc., Bellevue, WA), respectively. Gas chromatograph-mass spectrometer (GC-MS) operating with the electron-impact (EI) mode (7890 A GC; 5975 C 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 $\times$ 0.25 μm film thickness $\times$ 250 μm internal diameter). HR-ESIMS data were obtained in ESI$^{+}$ method (Q$^{\text{TOF}}$6520LC/MS/MS; Agilent) connected with an HPLC (Agilent LC 1200) connected with a RP C$_{18}$ column (50 $\times$ 2.1 mm; 1.8 μm). NMR spectroscopic study was carried out on a Bruker Avance DPX 500 MHz spectrometer (Bruker, Rheinstetten, Germany). The NMR data was administered using Mest ReNova-7.1.1-9649. Exhaustive analysis of compound was achieved by using two dimensional $^1$H-$^1$H COSY, DEPT, NOSEY, HSQC, and HMBC experiments following the standard protocols (Chakraborty et al. 2010a ). UV spectral analysis was performed by using a UV visible spectrophotometer (Varian Cary 50, Walnut creek, USA). Reverse-phase HPLC was carried out using a reverse phase C18 column (Luna 250 $\times$ 4.6 mm, 5 mm, Phenomenx, Torrance, USA) connected with a pump (Shimadzu LC 20AD, Nakagyo-Japan) and a photodiode array detector (SPD M20A, Kyoto, Japan).

3.2. Collection of marine macroalga and preparation of crude extract

Samples of *U. lactuca* Linnaeus (family Ulvaceae) (~10 kg, wet weight) (voucher specimen number of CMFRI/AB. 2.6.2.3) were collected from the Mandapam region (8°48′ N, 78°9′ E and 9°14′ N, 79°14′E) in the Gulf of Mannar region of the Indian subcontinent. The algal samples were unambiguously identified by marine algologist Dr. Chellaiah Periyasamy of the Aquaculture Foundation of India. The algal samples were thoroughly washed with tap water to remove extraneous matter, shade-dried, and finally pulverised. Powdered samples (~1 kg) were initially de-pigmented with n-hexane ($600 \times 2$) followed by extraction with MeOH/EtOAc solvent system (1:1 v/v,
500 mL \times 3) at room temperature for 12 h, until the removal of pigment materials, and filtered through Whatman No. 1 filter paper over anhydrous sodium sulfate (Na$_2$SO$_4$). The pooled extract was concentrated with a rotary vacuum evaporator (at about 45 °C) (Heidolf, Germany) (Dhara and Chakraborty 2020) to obtain a viscous material, which was labelled as the crude concentrate of *U. lactuca* (45 g, yield on dry basis 4.5%).

### 3.3. Bioactivity-directed chromatographic purification and spectral analyses

Crude extract (20 g) of *U. lactuca* was mixed with silica gel (60–120 mesh, 2 g), before being charged into a glass column (120 cm \times 4.5 cm), which was previously filled with silica gel (60–120 mesh, 60 g) (Dhara and Chakraborty 2020). Elution was commenced with an increasing order of polarity (n-hexane/EtOAc, EtOAc/MeOH, 9:1 to 6:4 v/v) to produce 13 fractions. The obtained fractions were pooled to 6 fractions (UF$_1$-UF$_6$) based upon RP-C$_{18}$ HPLC (acetonitrile ACN-MeOH, 50:50 v/v) and TLC (n-hexane/EtOAc 80:20, v/v) experiments. Semi-purified fractions were subjected to autophotography with DPPH reagent on a silica gel pre-coated TLC plate (eluted at 2:8 v/v, EtOAc: n-hexane) to locate the most active fraction. The UF$_2$ fraction (4.1 g), which was found to possess greater radical decolourising potential, was loaded to a silica gel column (230–400 mesh) with step wise gradient of EtOAc/n-hexane (0–100% EtOAc) to yield 6 fractions (UF$_{2,1}$-UF$_{2,6}$) (Table S1). Among these fractions, based on comparatively greater antioxidant activity (IC$_{50}$ 0.6–0.7 mg/mL) as well as ability to inhibit 5-LO (IC$_{50}$ 1.16 mg/mL), UF$_{2,1}$ was subjected to preparative HPLC (using MeOH-ACN solvent system, 3:2 v/v, 4 ml min$^{-1}$ flow rate) to yield ulvapyrone (60 mg), whereas the purity of isolated compound was analysed by TLC (silica/GF 254, EtOAc/n-hexane 1:4, v/v) and RP-C$_{18}$ HPLC (MeOH-ACN, 3:2 v/v).

### 3.4. Physicochemical and spectroscopic data

White amorphous solid; UV (MeOH) $\lambda_{\text{max}}$ (log $\epsilon$): 217 nm (4.21); TLC (Si gel GF$_{254}$ 15 mm; n-hexane/EtOAc 80:20, v/v) $R_\text{f}$: 0.45; melting point (decom.) 168-171°C; HPLC-$R_\text{f}$ (MeOH-ACN 3:2 v/v): 3.4 min; $^1$H NMR (CDCl$_3$): $\delta$H 5.95 (1H, d, J = 5.88 Hz), 5.82 (1H, d, J = 6.24 Hz), 5.69 (3H, s), 5.23 (1H, d, J = 7.48), 4.25 (1H, m), 4.21 (1H, dd, J = 7.27, 7.48 Hz), 4.08 (2H, q, J = 8.88 Hz), 3.97 (1H, m), 2.70 (1H, d, J = 5.88 Hz), 2.65 (1H, d, J = 7.27 Hz), 2.11 (1H, m), 1.89 (3H, s), 1.60 (1H, t, J = 7.04 Hz), 1.41 (1H, t, J = 8.05 Hz), 1.24 (3H, t, J = 6.73 Hz), 1.15 (3H, d, J = 6.01 Hz), 0.88 (3H, d, J = 6.06 Hz); $^{13}$C NMR (CDCl$_3$): $\delta$C 15.8, 17.9, 20.4, 24.7, 24.9, 38.2, 41.9, 45.5, 65.1, 69.1, 71.6, 74.9, 98.6, 114.8, 116.4, 128.1, 129.0, 130.9, 133.2, 136.6, 139.9, 156.3, 168.5, 173.3; IR (KBr, cm$^{-1}$) (stretching = $\nu$, bending = $\mu$): 3438 (br, O-H$_\nu$), 2828 (C-H$_\nu$), 1748 (C = O$_\nu$), 1632 (C = C$_\nu$), 992 (C-H$_\mu$), etc (Figure S10); HRESIMS, found *m/z* 429.1923 [M + H]$^+$, cal. for C$_{24}$H$_{29}$O$_7$ 429.1913 (Δ = 0.52 ppm) (Figure S9).

### 3.5. Bioactivity evaluation

Anti-inflammatory property was deduced by the inhibitory properties against 5-LO enzyme (Maneesh and Chakraborty 2017b). Antioxidant activities were assessed using
the stable DPPH and ABTS scavenging activities (Maneesh and Chakraborty 2018). The results of the radical scavenging activities and enzyme inhibition activities were displayed by IC\textsubscript{50} (concentration of compounds at that they inhibit 50% of radical/ enzyme activities), which were expressed as mg mL\textsuperscript{-1} values. Steric bulkiness, hydrophobicity, and electronic parameters of the purified compound and standards were determined by ACD ChemSketch (version 12.0; Advanced Chemistry Development Inc., Toronto, CA) and ChemDraw Ultra (Cambridge Soft Corp., MA; version 12.0) softwares.

### 3.6. Pharmacokinetic parameters and molecular modeling

SwissADME web tools were used to calculate pharmacokinetic parameters and oral bioavailability (Daina et al. 2017). AutoDock 4 (AutoDock, version 1.5.6) software was used for \textit{in silico} molecular docking of ulvapyrone (built in ACD/ChemSketch and converted in to MDL Molfiles) with 5-LO (Gilbert et al. 2012) (PDB: 3V99; resolution 2.4 Å, crystal structure downloaded from \texttt{www.pdb.org}) and energetically reduced (Swiss-PdbViewer, version 4.1.0). The grid box values were selected as \(x = 44.775, y = 37.875, z = -19.495\) (52 Å \times 58 Å \times 110 Å) for 5-LO by Auto Grid algorithm. Molecular docking analysis was carried out by Genetic Algorithm and Lamarckian genetic algorithm methods, whereas Cygwin (I and II) terminals were used to run the docking algorithm. Molecular docking analyses were visualised by USCF Chimera (University of California, San Francisco, version 1.11.2). After the autodocking, root-mean-square deviations of atomic positions were assessed, and the docked conformations were built on their docking scores and binding energies.

### 3.7. 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-directed chromatographic separation of organic extract of \textit{U. lactuca} afforded an unreported pyrone-linked benzochromene derivative, ulvapyrone, characterised as 2-\{(6a'-hydroxyethyl-4'-methyltetrahydro-2H-pyran-2'-one)-6'-yl\}-4-methyl-7-ethylacetate-8-hydroxy-7, 8-dihydrobenzo [de]chromene with potential attenuation properties against arachidonate 5-LO. Bioactivity of the titled compound was compared with standards used in various commercial preparations, besides to that physicochemical properties, lipophilicity, water solubility, drug-likeness and pharmacokinetic prediction study also suggested the oral bio-availability of ulvapyrone. Structure-bioactivity correlation analysis recognised that higher electronic properties along with optimum hydrophobicity of ulvapyrone favored the interaction with active site of 5-LO enzyme, and also corroborated by lesser docking parameters. The results illustrated that ulvapyrone could be developed as a promising marine-originated lead for use against inflammation.
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Conflict of interest

The authors declare no competing financial interest.

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Data availability

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

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