Supporting Information

Coumarins Isolated from *Murraya paniculata* in Vietnam and their Inhibitory Effects Against Enzyme Soluble Epoxide Hydrolase (sEH)

Pham Ngoc Khanh¹, Ottavia Spiga², Alfonso Trezza², Young Ho Kim³, Nguyen Manh Cuong¹

Affiliations

¹Department of Bioactive products, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam

²Department of Biotechnology chemistry and Pharmacology, University of Siena, Italia

³Department of Natural Products, College of Pharmacy, Chungnam National University, Daejeon, Korea

Correspondence

*Assoc. Prof. Nguyen Manh Cuong*

Institute of Natural Products Chemistry

Vietnam Academy of Science and Technology (VAST)

18 Hoang Quoc Viet Street

122100 CauGiay

Hanoi
In traditional medicine, *Murraya paniculata* was used as an analgesic, digestion stimulant, anti-swelling, anti-ecchymotic, expectorant, tonic, and toothache remedy [1, 2]. Phytochemical studies of *Murraya* lead to the isolation of indol akaloid [3], polymethoxylated flavonoid [4], essential oils [5], and a number of coumarins [6-8].

The methanol extract of the dry leaves of *M. paniculata* was suspended with water and subsequently fractioned with *n*-hexane, chloroform, and ethyl acetate. From the *n*-hexane fraction, two sterols, \( \beta \)-sitosterol (6) and \( \beta \)-stigmasterol (7), were isolated. From the chloroform
fraction of the leaves of *M. paniculata*, five coumarins (1-5) were subsequently isolated through column chromatography.

Compound 1 was obtained as a white solid, soluble in acetone, chloroform. Its molecular formula was determined as C\textsubscript{16}H\textsubscript{20}O\textsubscript{6} from the peak at *m/z* 331.1158 ([M + Na]\textsuperscript{+}: 331.1157) in the HR-ESI-MS. The \textsuperscript{1}H-NMR spectrum of 1 showed the signals of 2 ortho-protons at \(\delta_\text{H} 6.12\) (d, H-3) and 8.11 (d, H-4) (with \(J = 9.5\) Hz) and one proton at \(\delta_\text{H} 6.60\) (s, H-6) characteristic for a 5,7,8-trisubstituted coumarin. The \textsuperscript{13}C-NMR spectrum of 1 displayed 16 carbon signals (1 × C=O, 5 × C\textsubscript{4}, 4 × CH, 2 × Me and 2 × OMe). In the HMBC spectrum, HMBC correlations of methoxy protons at \(\delta_\text{H} 3.98\) (s, H-5)/3.96 (s, H-7) and corresponding carbons C-5 (\(\delta_\text{C} 157.3)/C-7 (163.5) indicated the position of these methoxys at the carbons C-5 and C-7 of the coumarin ring. The side chain (C\textsubscript{6}H\textsubscript{13}O\textsubscript{2}), including an exo-methylene at \(\delta_\text{H} [2.96 (1H, dd, \(J = 9.5; 14.0\) Hz, H-1’a); 2.90 (1H, dd, \(J = 3.5; 14.0\) Hz, H-1’b)], an oxygenated methine at \(\delta_\text{H} 3.76\) (1H, dd, \(J = 3.5; 9.5\) Hz, H-2’), and two methyls at \(\delta_\text{H} 1.26/1.25\) (3H each), was confirmed to attach at carbon C-8 of the coumarin ring by the HMBC cross-peaks of protons H\textsubscript{2}-1’ (\(\delta_\text{H} 2.96/2.90\) and carbons C-7 (\(\delta_\text{C} 163.5), C-8 (109.2), C-9 (155.4), and C-2’ (76.8). Based on these spectroscopic evidences and compared to published papers, compound 1 was identified as mexoticin (2H-8-[2,3-dihydroxy-3-methylbutyl]-5,7-dimethoxy-1-benzopyran-2-one), which was isolated by Chakraborty et al. from *Murraya exotica* leaves (Fig. 1) [9].

Compound 2 was obtained as a white solid with a molecular formula of C\textsubscript{17}H\textsubscript{22}O\textsubscript{6} based on the HR-MS peak at *m/z* 345.1348 ([M + Na]\textsuperscript{+}: 345.1314). The \textsuperscript{1}H-, \textsuperscript{13}C-, and DEPT-NMR spectra of 2 were closely similar to those of 1, characterized for the presence of the 5,7-dimethoxy-8-substituted coumarin system. Different from 1, the side chain of 2 was one methoxy less (C\textsubscript{5}H\textsubscript{10}O), including a methylene group at \(\delta_\text{H} 2.96\) (2H, \(J = 9.0\) Hz, H-1’), a methine at \(\delta_\text{H} 3.73\),
(dd, 1H, J = 3.5, 9.0, H-2’), an aliphatic methoxy at $\delta_H$ 3.29 (s, 3’-OCH$_3$), and two methyls at $\delta_H$ 1.28 (s, 6H, 4’-CH$_3$). The downfield shift of the methine C-2’ ($\delta_C$ 76.3) suggested that this carbon attached to one hydroxyl group. The side chain was thus identified as 2’-hydroxy-3’-methoxy-3’-methylbutyl attached to C-8 through the methylene group C-1’. This reveals the structure of compound 4 as omphalocarpin [10].

Compound 3 was obtained from chloroform fractions as a white needle crystal, m.p. (183-185°C). The $^1$H-NMR spectrum of compound 3 showed the presence of the 7-methoxy-8-substituted coumarin nucleus by the characteristic signals of aromatic protons at $\delta_H$ 6.27 (H-3)/7.97 (H-4, $J_{3,4} = 9.5$ Hz) and $\delta_H$ 7.59 (H-5)/7.06 (H-6, $J_{5,6} = 8.5$ Hz) and a methoxy proton at $\delta_H$ 3.88 (7-OMe). The remaining signals were due to the side chain C$_2$H$_9$O$_2$ at the C-8 position. Two broad signals at $\delta_H$ 4.95 and 4.85 belonged to two hydroxyl groups (l’-OH and 2’-OH). The methyl protons, which can theoretically couple with two vinylic protons at $\delta_H$ 4.61 and 4.57 (H$_2$-4’), appeared as a sharp singlet at $\delta_H$ 1.80. A proton signal at the $\delta_H$ 5.10 (H-l’) doublets due to the interaction with a proton at $\delta_H$ 4.70 (H-2’) with coupling constant $J_{l’-2’} = 7.5$ Hz. The side chain was thus identified as 3’-methyl-but-3’-ene-1’,2’-diol, attached to C-8 through C-1’, leading to the expression of compound 3 for murrangatin [10-12]. This murrangatin and its erythrodiastereoisomer minumicrolin (murpanidin) had been obtained from the leaves and root bark of M. paniculata var. omphalocarpa (Hayata) Tanaka, which is indigenous in Taiwan [10, 11, 13].

Compounds 4 and 5 were obtained as a white powder and identified as kimcuongin (4; C$_{20}$H$_{20}$O$_6$, M = 356) and (-)-murracarpin (5; C$_{16}$H$_{18}$O$_5$, M = 290). These two coumarins possessed vasorelaxing activity with IC$_{50}$ values 37.7 μM and 139.3 μM, respectively, on the rat aorta ring precontracted with 60 mM K$^+$ [2, 14].
Materials and Methods

General experimental procedures

$^1$H-NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were measured on a Bruker AVANCE 500 spectrometer. The ESI-MS spectra were obtained with an ESI-MicroQ-TOF III (Bruker Daltonics, Inc.) and an FT-ESI-MS (Varian, Inc.) mass spectrometer. UV and IR spectra were obtained on JASCO V-630 and Impact 410 Nicolet FT-IR spectrometers, respectively. Column chromatography (CC) was carried out on silica gel (Si 60 $F_{254}$, 230-400 mesh, Merck). All solvents were distilled before use. Precoated plates of silica gel 60 $F_{254}$ were used for analytical purposes. Compounds were visualized under UV radiation (254, 365 nm) and by spraying plates with 10% H$_2$SO$_4$ followed by heating with a heat gun.

Extraction and isolation

Dried powdered leaves and twigs of *M. paniculata* (3.2 kg) were extracted with MeOH over the period of 5 days at room temperature and concentrated under reduced pressure to yield a black crude MeOH extract (120 g). The crude MeOH extract was suspended in hot MeOH-water (1:1, v/v) and successively partitioned with $n$-hexane, dichloromethane (DCM), and ethyl acetate (EtOAc). The resulting fractions were concentrated under reduced pressure to give the corresponding solvent-soluble fractions $n$-hexane (6.7 g), chloroform (7.2 g), EtOAc (16.7 g), and water (60 mL).

The chloroform fraction (7.0 g) was subjected to CC on a flash silica gel column (400-630 mesh) with gradient solvents of $n$-hexane/EtOAc (1:0, 40:1, 20:1, 10:1, 5:1, and 0:1, v/v, 1.0 L /each).
and EtOAc/MeOH (1:0, 3:1; 1:1, 1:3, and 0:1, v/v, 1.0 L / each) to afford 9 fractions (F-2A to F-2I). Fraction F-2E was subjected to CC on a silica gel column (230-400 mesh) using n-hexane-acetone (5/1 - 1/1, v/v) as eluting solvents to obtain 4 subfractions (F-2E-1–2E-4). Subfraction F-2E-2 was slowly crystallized at 4°C overnight to obtain compound 1 (20 mg) as a white powder. Fraction F-2E-3 was further chromatographed on a silica gel column eluting with n-hexane-acetone (3/1, v/v) to yield compound 2 (17 mg). Fraction F-2C was further purified on a silica gel column (230-400 mesh) eluting with n-hexane-EtOAc (2:1) to produce 5 fractions. From fraction F-2C-2, a white compound was crystallized and cleaned-up 3 times with cold MeOH (1 mL) to obtain compound 3 (23 mg). Subfraction F-2B (3.1 g) was further chromatographed on a silica gel column (230-400 mesh) and eluted with a gradient of n-hexane in EtOAc (100% n-hexane to 100% EtOAc) to obtain 29 fractions (F-2B-1 to F-2B-29). Fraction F-2B-8 (55 mg) was subjected to CC on a silica gel column eluting with n-hexane/EtOAc (2/1, v/v) to obtain compound 4 (42 mg). A white crystal precipitated from subfraction F-2B-11 was filtered and washed with cold MeOH (1 mL) to yield compound 5 (20 mg). The n-hexane fraction (red brown pasta, 6.7 g) was chromatographed on a silica gel column using solvent gradients of n-hexane-acetone (40:1, 20:1, 10:1, 5:1 and 1:1, v/v, 1.0 L each) to afford 15 subfractions (H1 to H15). The white participate from subfraction 13 (n-hexane-acetone, 10:1, v/v) was filtered out, washed with cold methanol (2 × 1 mL), and crystallized in n-hexane to obtain compound 6 (20 mg). The filtrate was rechromatographed on a silica gel column with n-hexane-acetone (10:1, v/v) to obtain compound 7 (10 mg). These two compounds were identified only by comparison of their \(^1\)H-NMR spectra and TLC pattern to those of authentic compounds.
Spectral and physical data

**Mexoticin (1):** 2H-8-(2,3-dihydroxy-3-methylbutyl)-5,7-dimethoxy-1-benzopyran-2-one, white powder, C_{16}H_{20}O_{6} (M = 308), m.p. 188-189°C. \(^1\)H-NMR \(\delta\) (ppm): 6.12 (1H, d, \(J = 9.5\) Hz, H-3), 8.11 (1H, d, \(J = 9.5\) Hz, H-4), 3.98 (3H, s, 5-OMe), 6.60 (1H, s, H-6), 3.96 (3H, s, 7-OMe), 2.96 (1H, dd, \(J = 9.5\); 14.0 Hz, H-1’a); 2.90 (1H, dd, \(J = 3.5\); 14.0 Hz, H-1’b), 3.76 (1H, dd, \(J = 3.5\); 9.5 Hz, H-2’), 1.26 (3H, s, H-4’), 1.25 (3H, s, H-5’); \(^13\)C-NMR \(\delta\) (ppm): 163.9 (s, C-2), 110.7 (d, C-3), 141.0 (d, C-4), 157.3 (s, C-5), 56.7 (s, 5-OMe), 92.2 (d, C-6), 163.5 (s, C-7), 56.7 (s, 7-OMe), 109.2 (s, C-8), 155.4 (s, C-9), 104.8 (s, C-10), 25.7 (t, C-1’), 76.8 (d, C-2’), 78.8 (s, C-3’), 21.7 (q, C-4’), 21.2 (q, C-5’). LC-ESI-MS: 346.2788, 331.1158 (calcd. for C_{16}H_{20}O_{6}Na: 331.1157), 291.1220 (calcd. for [M - H_2O + H] \+ C_{16}H_{19}O_{5}: 291.1232); [\(\alpha\)]_D^{25} -36.6° (CHCl_3).

**Omphalocarpin (2):** 2H-8-(2-hydroxy-3-methoxy-3-methylbutyl)-5,7-dimethoxy-1-benzopyran-2-one, white powder, C_{17}H_{22}O_{6} (M = 322), m.p. 148-149°C. \(^1\)H-NMR (CDCl_3) \(\delta\) (ppm): 6.14 (1H, d, \(J = 9.5\) Hz, H-3), 7.98 (1H, d, \(J = 9.5\) Hz, H-4), 6.34 (1H, s, H-6), 3.98 (6H, s, 5- and 7-OMe), 2.95 (2H, m, H-1’), 3.73 (1H, dd, \(J = 3.5\) & 9.0 Hz, H-2’), 3.29 (3H, s, 2’-OMe), 1.28 (6H, s, -Me). \(^13\)C-NMR \(\delta\) (ppm): 161.4 (s, C-2), 110.9 (d, C-3), 138.8 (d, C-4), 155.6 (s, C-5), 90.5 (d, C-6), 154.2 (s, C-9), 108.3 (s, C-8), 103.9 (s, C-10), 76.6 (d, C-2’), 56.2 (s, 5-OMe), 55.9 (s, 7-OMe), 49.3 (s, 2’-OMe), 24.6 (t, C-1’), 21.0 (q, C-4’), 20.3 (q, C-5’). HR-ESI-MS: 645.2438, 597.1941, 511.1517, 345.1161, 291.1114 (calcd. for [M - CH_3OH] \+ C_{16}H_{19}O_{5}: 291.1232).

**Murrangatin (3):** 2H-8-[(1S,2S)-1,2-dihydroxy-3-methyl-3-buten-1-yl]-7-methoxy-1-benzopyran-2-one, white needle crystal, soluble in MeOH and CHCl_3, C_{15}H_{16}O_{5} (M = 276), m.p. 133-135°C. \(^1\)H-NMR (DMSO- \textit{d}_6, 500 MHz) \(\delta\) (ppm): 7.97 (1H, d, \(J = 9.5\) Hz, H-4); 6.27 (1H, d, \(J = 9.5\) Hz, H-3); 7.59 (1H, d, \(J = 9.0\) Hz, H-5); 7.06 (1H, d, \(J = 8.5\) Hz, H-6); 5.10 (1H, dd, \(J_1 = 7.5Hz, J_2 = 8.5Hz, H-2’); 4.95 [1H, s (br), -OH]; 4.85 [1H, s (br.), -OH]; 4.70 (1H, d, \(J =
7.5Hz, H-1'); 4.60 (1H, d, J = 4.5Hz, H-4'a); 4.57 (1H, dd, J1 = 5.0 Hz, J2 = 8.0 Hz, H-4'b); 3.88 (3H, s, 7-OMe); 1.80 (3H, s, 5'-Me). 13C-NMR δ (ppm): 160.9 (s, C=O, C-2), 112.2 (d, C-3), 144.8 (d, C-4), 128.3 (d, C-5), 108.5 (d, C-6), 160.2 (s, C-7), 56.3 (q, 7-OMe), 118.4 (s, C-8), 112.6 (s, C-9), 153.1 (s, C-10), 66.1 (d, C-1'), 75.5 (d, C-2'), 143.8 (s, C-3'), 112.4 (dd, =CH2, C-4'), 17.6 (q, C-5'). [α]D25-10.3º (c = 0.29, CHCl3).

Kimcuongin (4): 2H-8-(1-oxo-2-scenecioyl-3-methyl-butene-2)-7-methoxy-1-benzopyran-2-one; white crystal, soluble in CHCl3, orange spot on TLC plate with Dragendorff reagent, C20H20O6 (M = 356), m.p. 149-151°C. 1H-NMR (CDCl3) δ: 6.23 (1H, d, J = 1.5 and 9.5 Hz, H-3), 7.61 (1H, d, J = 9.5 Hz, H-4), 7.42 (1H, d, J = 8.5 Hz, H-5), 6.82 (1H, d, J = 8.5 Hz, H-6), 3.85 (3H, s, H-11), 1.83 (3H, s, H-4'), 2.30 (3H, s, H-5'), 5.44 (1H, s, H-2''), 1.78 (3H, s, H-4'') and 1.99 (3H, s, H-5''). 13C-NMR (CDCl3) δ: 159.9 (s), 113.7 (d), 142.9 (d), 129.4 (d), 107.7 (d), 159.5 (s), 117.9 (s), 151.7 (s), 112.5 (s), 56.4 (s), 186.9 (s), 142.6 (s), 140.4 (s), 21.6 (q), 20.3 (q), 163.9 (s), 114.2 (d), 159.1 (s), 27.3 (q) and 20.1 (q). IR (KBr) cm⁻¹: 2979 (aromatic C-H), 1729 (coumarin C=O), 1611 (aromatic ether CO). UV λmax (CHCl3) nm (log ε): 324 (2.45), 270 (2.04). HR-ESI-MS m/z: 357.10408 (100%) [M + H]+ (calcd. for C20H21O6: 357.13327), 273.06050 (4%) [calcd. for C15H13O5 ([M - C5H7O]+): 273.07629], 203.02536 (3%) [calcd. for C11H7O4 ([M-side chain (C10H13O3)+CO]+): 203.03443], 177.04809 (3.5%) (calcd. for C10H9O3: 177.05517). ESI-MS m/z: 357.1 [M + H]+.

Murracarpin (5): 2H -8-[(1S,2S)-2-hydroxy-1-methoxy-3-methyl-3-buten-1-yl]-7-methoxy-1-benzopyran-2-one, white compound, soluble in MeOH and CHCl3, C16H18O5 (M = 290), m.p. 148-149°C. 1H-NMR (DMSO-d6) δ: 6.27 (1H, d, J = 9.5 Hz, H-3), 7.96 (1H, d, J = 9.5 Hz, H-4), 7.62 (1H, d, J = 8.5 Hz, H-5), 7.07 (1H, d, J = 8.5 Hz, H-6), 3.88 (3H, s, H-11), 4.81 (d, J = 8.5 Hz, H-1'), 4.86 (dd, J = 3.5, 8.5 Hz, H-2'), 5.17 (d, 1H, J = 3.5 Hz, -OH), 4.50 (brs, H-4'a), 4.39
(brs, H-4'b), 1.48 (3H, s, H-5'), 3.15 (3H, s, H-6'). $^{13}$C-NMR $\delta$: 159.9 (s), 112.2 (d), 144.8 (d), 129.4 (d), 108.4 (d), 160.8 (s), 113.7 (s), 153.1 (s), 112.6 (s), 56.3 (s), 78.1 (d), 75.9 (d), 145.2 (s), 112.0 (dd), 16.7 (q), 57.1 (s). $\alpha_{D}^{25}$ -15.6° ($c = 0.063$, CHCl$_3$).

**sEH inhibitory assay**

*Chemical reagents:* 3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxiraneacetic acid (PHOME), 12-[[tricyclo[3.3.1.13,7] dec-1-ylamino)carbonyl] amino]-dodecanoic acid (AUDA), purified recombinant sEH, and 6-methoxy-2-naphtaldehyde (internal standard for fluorometric assays) were obtained from Santa Cruz Biotechnology.

*Fluorometric method for evaluating sEH inhibitory activity:* All preparations for this study were carried out in a dry nitrogen atmosphere unless otherwise specified. The enzyme was frozen in multiple small aliquots and thawed once immediately before use.

The sEH inhibitory activity was determined using a hydrolysis reaction of PHOME in the presence of the sEH enzyme as described previously. The final reaction volume was 200 μL, and contained 25 mM Bis-Tris buffer (including 0.1% bovine serum albumin, pH 7.0), 1 μM PHOME, 3 nM sEH enzyme, and various concentrations of samples or the positive control AUDA (dissolved in DMSO). Reaction systems were incubated at 30°C for 1 h, and fluorescence intensity was then monitored every 3 min (during 1 h) using a Geniosmicroplate reader (Tecan) at excitation and emission wavelengths of 330 and 465 nm, respectively. The sEH inhibitory activity for each sample was calculated as follows:

$$\text{Enzyme activity (\%) = } \left[ \frac{(S - S_0)}{(C - C_0)} \right] \times 100$$

where C is the fluorescence of the control (enzyme, buffer, MeOH, and substrate) after 60 min of incubation, $C_0$ is the fluorescence of the control at zero time, S is the fluorescence of the tested
samples (enzyme, buffer, sample solution, and substrate) after incubation, and $S_0$ is the fluorescence of the tested samples at zero time. The IC$_{50}$ values were determined based on regression of four to five data points with a minimum of two points in the linear region of the curve on either side of the IC$_{50}$ [15].

**Determination of enzyme kinetic parameters:** $v_{\text{max}}$, maximum velocity, and $K_m$, Michaelis-Menten constant, for the enzymatic reaction were assessed from nonlinear regression plots of velocity versus substrate concentrations. $K_m$ is half of the $v_{\text{max}}$. For the inhibition constant, $K_i$ and the mode of inhibition determination, a series of experiments were conducted. Experiments were carried out with 3 to 5 concentrations of a specific substrate with a range of inhibitor concentrations (0-100 μM).

**Statistical analysis:** All data are presented as means ± standard deviation (SD; n = 3). In all comparisons, $p < 0.05$ was considered significant. The sEH-mediated activities in the presence of inhibitors are expressed as percentages of the corresponding control values. The pharmacological response to each substance is given as IC$_{50}$. A sigmoid-shaped curve was fitted to the data, and the enzyme inhibition parameter IC$_{50}$ was calculated by fitting the Hill equation to the data using nonlinear regression (least squares best fit modeling) of the plot of percentage of control activity versus concentration of the test inhibitor using GraphPad Prism 5.0 (GraphPad Software, Inc.). Significant differences among groups were determined using analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test ($p < 0.05$). The values for $v_{\text{max}}$ and $K_m$ were determined using nonlinear regression plots using GraphPad Prism 5.0 (GraphPad Software, Inc.). Kinetic parameters, $K_i$, were determined by linear regression using Lineweaver-Burk plots using Microsoft Excel software.
Molecular modelling

The docking simulation study of omphalocarpin (2), murrangatin (3), and kimcuongin (4) was performed by using a flexible side chains protocol based on Iterated Local Search Global Optimizer Algorithm of AutoDock/Vina v.1.1.2 [16]. The crystal structures of human sEH complexed with N-cyclohexyl-N’-(4-iodophenyl)urea, 4-[(cyclohexylamino)carbonyl amino]butanoic acid and N-(5-chloro-1,3-benzoxazol-2-yl)-2-cyclopentylacetamide were downloaded from Brookhaeven Protein Data Bank (entry 1VJ5, 1ZD3 or 3PDC) (www.rcsb.org/pdb). Ligand structures were prepared using ChemBioDraw Ultra 12.0 (HyperChem Professional 7.51, Hypercube, Inc.) and the energy minimizations of the prepared ligands were carried out with Chem3D Ultra, while the pdbqt format essential for the docking simulation was generated using script of Molecular Graphics Laboratory (MGL) tools [17]. The computational software was downloaded from the website http://scripps.edu, operated under Microsoft Windows 8, installed on an Intel i7 PC with a 3.2 GHz processor and 8 GB RAM. The ligand molecules were docked into a refined sEH enzyme using AutoDock/Vina v.1.1.2 (Trott and Olson, 2010). The generation and affinity grid maps, viewing of docking poses, and analysis of virtual screening results were obtained using AutoDock plug-in of PyMOL (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC). The AutoDock output results represent the docking scores as Gibbs free energy of binding ($\Delta G$) values, further converted to the predicted inhibition constants ($K_{i,pred}$). In silico alanine scanning mutagenesis was performed by using the ABS-Scan tool 2 [18]. Each amino acid residue present at the binding site was computationally mutated to alanine and the ligand interaction energy was recalculated for each mutant. The corresponding $\Delta G$ values were computed by comparing them to the wild-type protein, thus allowing the evaluation
of individual residue contribution towards ligand interaction. The interactive three-dimensional maps were constructed using the PLIP bioinformatics tool [19].
**Table 1S** Hydrophobic interactions and binding modes of compounds 2-4 with protein residues.

<table>
<thead>
<tr>
<th>Index</th>
<th>Residue</th>
<th>AA</th>
<th>Distance</th>
<th>Ligand Atom</th>
<th>Protein Atom</th>
<th>Distance</th>
<th>Ligand Atom</th>
<th>Protein Atom</th>
<th>Distance</th>
<th>Ligand Atom</th>
<th>Protein Atom</th>
</tr>
</thead>
<tbody>
<tr>
<td>42A</td>
<td>PHE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ASP</td>
<td></td>
<td>3.26</td>
<td>3339</td>
<td>1076</td>
<td>3.47</td>
<td>3337</td>
<td>1076</td>
<td>3.19</td>
<td>3337</td>
<td>1076</td>
</tr>
<tr>
<td>111A</td>
<td>TRP</td>
<td></td>
<td>3.94</td>
<td>3336</td>
<td>1086</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>156A</td>
<td>PHE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.97</td>
<td>3344</td>
<td>1542</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.55</td>
<td>3342</td>
<td>1540</td>
</tr>
<tr>
<td>183A</td>
<td>LEU</td>
<td></td>
<td>3.87</td>
<td>3329</td>
<td>1836</td>
<td>3.56</td>
<td>3326</td>
<td>1836</td>
<td>3.34</td>
<td>3327</td>
<td>1836</td>
</tr>
<tr>
<td>241A</td>
<td>TYR</td>
<td></td>
<td>3.47</td>
<td>3337</td>
<td>2438</td>
<td>3.72</td>
<td>3336</td>
<td>2438</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>273A</td>
<td>VAL</td>
<td></td>
<td>3.57</td>
<td>3338</td>
<td>2792</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>274A</td>
<td>LEU</td>
<td></td>
<td><strong>2.66</strong></td>
<td>3339</td>
<td>2801</td>
<td>3.25</td>
<td>3337</td>
<td>2801</td>
<td>3.19</td>
<td>3342</td>
<td>2802</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.77</td>
<td>3337</td>
<td>2801</td>
</tr>
<tr>
<td>299A</td>
<td>HIS</td>
<td></td>
<td>3.54</td>
<td>3320</td>
<td>3050</td>
<td>3.21</td>
<td>3321</td>
<td>3050</td>
<td>3.32</td>
<td>3321</td>
<td>3050</td>
</tr>
<tr>
<td>300A</td>
<td>TRP</td>
<td></td>
<td>3.44</td>
<td>3320</td>
<td>3069</td>
<td>3.52</td>
<td>3322</td>
<td>3069</td>
<td>3.62</td>
<td>3322</td>
<td>3069</td>
</tr>
</tbody>
</table>
**Table 2S** Hydrogen bonds and binding modes of compounds 2-4 with protein residues.

<table>
<thead>
<tr>
<th>Index</th>
<th>Residue</th>
<th>AA</th>
<th>Distance H-A</th>
<th>Distance D-A</th>
<th>Donor Angle</th>
<th>Protein donor?</th>
<th>sidechain donor?</th>
<th>Donor Atom</th>
<th>Acceptor Atom</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Omphalocarpin (2)</strong></td>
<td>241A</td>
<td>TYR</td>
<td>3.07</td>
<td>3.66</td>
<td>118.77</td>
<td>x</td>
<td>x</td>
<td>2441[O3]</td>
<td>3333[O3]</td>
</tr>
<tr>
<td><strong>Murrangatin (3)</strong></td>
<td>42A</td>
<td>PHE</td>
<td>3.08</td>
<td>3.85</td>
<td>135.43</td>
<td>x</td>
<td>0</td>
<td>377[Nam]</td>
<td>3338[O3]</td>
</tr>
<tr>
<td>110A</td>
<td>ASP</td>
<td>3.23</td>
<td>3.71</td>
<td>113.73</td>
<td>x</td>
<td>x</td>
<td>1078[O3]</td>
<td>3338[O3]</td>
<td></td>
</tr>
<tr>
<td>158A</td>
<td>TYR</td>
<td>2.15</td>
<td>2.65</td>
<td>109.82</td>
<td>0</td>
<td>x</td>
<td>3332[O3]</td>
<td>1569[O3]</td>
<td></td>
</tr>
<tr>
<td><strong>Kimcuongin (4)</strong></td>
<td>158A</td>
<td>TYR</td>
<td>2.13</td>
<td>2.92</td>
<td>135.13</td>
<td>x</td>
<td>x</td>
<td>1569[O3]</td>
<td>3332[O2]</td>
</tr>
<tr>
<td>241A</td>
<td>TYR</td>
<td>2.77</td>
<td>3.70</td>
<td>154.00</td>
<td>x</td>
<td>x</td>
<td>2441[O3]</td>
<td>3341[O2]</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1S A The active site of the hydrolase domain of human sEH complexed with omphalocarpin (2) in blue, murrangatin (3) in magenta, and kimcuongin (4) in green as obtained from docking simulation. The amino acid residues represented in pale yellow sticks are those principally involved in the binding interactions. B The hydrophobic interactions and distances (Å) from the three coumarins to the amino acid residues.
References


5. Rout PK, Rao YR, Naik S. Liquid CO₂ extraction of Murraya paniculata Linn. flowers. Ind Crops Prod 2010; 32: 338-342


