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

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Abstract

In the search for bioactive constituents from Vietnam medicinal plants, the leaves and stems of *Murraya paniculata* collected in HoaBinh Province, Vietnam were selected for chemical investigation. From the *n*-hexane fraction, two sterols, including *β*-sitosterol (**6**) and stigmasterol (**7**), and from the chloroform fraction, five coumarins, including mexoticin (**1**), omphalocarpin (**2**), murrangatin (**3**), kimcuongin (**4**), and murracarpin (**5**), were obtained. The structures of the isolated compounds were determined from ESI-MS, HR-ESI-MS, and NMR (1D and 2D) spectroscopic data. Coumarins (**1**–**5**) were elucidated for inhibitory effects against soluble epoxide hydrolase. Among them, coumarins (**2**–**4**) showed soluble epoxide hydrolase inhibitory activity with IC_{50} values 2.2 ± 4.7, 13.9 ± 6.5, and 3.2 ± 4.5 µM, respectively. A kinetic study of the five coumarins revealed the noncompetitive enzymatic mode for **3** and **4**, and a mixture of competitive/noncompetitive enzymatic modes for coumarin **2**. Using molecular modelling, the coumarin kimcuongin (**4**) showed the best binding outline into active sites of human soluble epoxide hydrolase.

Key words

Murraya paniculata · Rutaceae · coumarin · kimcuongin · murracarpin · AutoDock/Vina · soluble epoxide hydrolase (sEH)

Abbreviations

AUDA:	12-(3-adamantan-1-yl-ureido) dodecanoic acid
DHETs:	dihydroxyeicosatrienoicdiols
EETs:	epoxyeicosatrienoic acids
K_i:	inhibitor constant
K_m:	Michaelis-Menten constant
PHOME:	3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxiraneacetic acid
PLIP:	protein-ligand interaction profiler
sEH:	soluble epoxide hydrolase

Supporting information available online at http://www.thieme-connect.de/products

The sEH is the main enzyme that catalyzes the metabolism of EETs into the more polar and usually less potent metabolites DHETs [1]. Early studies have indicated that chemical compounds that can inhibit the sEHs' activity and stabilize the endogenous EET levels may represent potential therapeutic agents for cardiovascular disease [2,3] and the onset of several other diseases [4,5].

Murraya paniculata (L.) Jack (Rutaceae), local name "Nguyet que", is mostly grown as ornaments for its glossy green foliage and white fragrant flowers. It is used as a medicinal plant in Vietnamese traditional medicine. There were several publications of phytochemical studies of *Murraya* (see Supporting Information). In this study, we describe the inhibitory activity against the sEH of coumarins isolated from this plant and explain this effect by molecular modelling.

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, *β*-sitosterol (**6**) and *β*-stigmasterol (**7**), were isolated. From the chloroform fraction of the leaves of *M. paniculata*, five coumarins (**1**–**5**) were subsequently isolated through column chromatography including mexoticin (**1**) [6], omphalocarpin (**2**) [7], murrangatin (**3**) [8], kimcuongin (**4**), and (−)-murracarpin (**5**; **Fig. 1**). The latter two coumarins are reported to possess vasorelaxing activity [9].

The *in vitro* the inhibitory activity of the isolated coumarins (**1**–**5**) from *M. paniculata* leaves was investigated based on the hydrolysis of the sEH on an artificial fluorescent substrate, PHOME, with AUDA as a positive control (IC_{50} 15.7 ± 2.7 nM; **Fig. 2**). With the exception of compounds **1** and **5**, coumarins **2, 3**, and **4** exhibited potential sEH inhibitory activity (assessed at the sEH concentration of 25 µM; **Table 1**). Their IC_{50} values were found to be 3.2 ± 4.5, 13.9 ± 6.5, and 2.2 ± 4.7 µM, respectively (**Fig. 2** and **Table 1**).

Kinetic parameters including maximum velocity (v_{max}), K_m, K_i, and the mode of inhibition of the potent sEH inhibitors **2, 3**, and **4** were obtained using the sEH enzyme as shown in **Table 1**. According to the $K_{i,exp}$ value, omphalocarpin (**2**) showed a higher possibility to interact with the sEH enzyme. In the presence of the two inhibitors **3** and **4**, the K_m values of the sEH enzyme were found to be similar, while the v_{max} and K_m values were gradually decreased and even lower than those of the reaction without the presence of the inhibitor (0.0 µM; **Fig. 3**). This suggested that the inhibitory activity of **3** and **4** on the sEH followed the noncompetitive binding mode and the coumarins might affect the enzyme-substrate complex. While in the case of the reaction catalyzed by coumarin **2**, the v_{max} value decreased, but the K_m value increased compared to that of the reaction without inhibitor. These facts suggest that the inhibitory activity of coumarin **2** on sEH was different from those in the presence of coumarins **3** and **4**. It might follow a mixture (competitive/noncompetitive) binding mode.

In order to investigate the binding mechanisms of the active coumarins to the sEH enzyme, molecular docking using AutoDock Vina software was carried out, where each coumarin was manually docked at the active site of the human sEH (complexed with ligands; **Fig. 4** and **Tables 1 S** and **2 S**, Supporting Information). All three coumarins have hydrophobic interactions to acid amines Asp335A, Leu408A, Leu499A, His524A, and Trp525A (**Fig. 5**). The smallest hydrophobic bond belonged to omphalocarpin (**2**) binding to Leu499A with 2.66 Å. Kimcuongin (**4**) prefered to binding to phenylalanine [Phe267A (3.89 Å) and Phe381A (3.55 and 3.97 Å]. The estimated hydrogen bond formed between the carbonyl groups of the coumarins and the residues

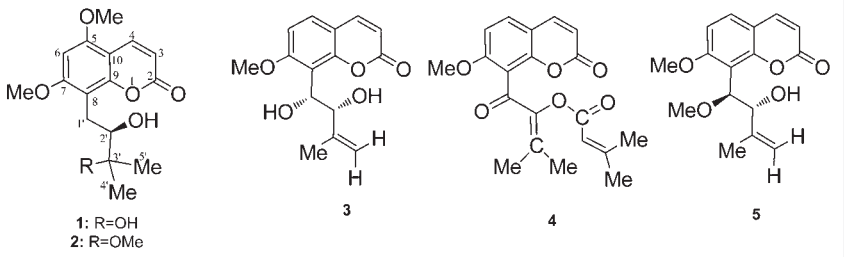


Fig. 1 Structures of the isolated compounds (**1**–**5**).

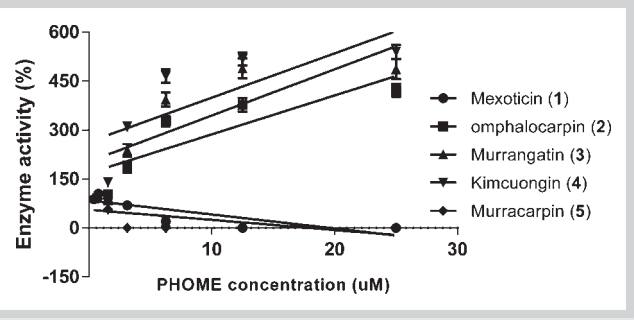


Fig. 2 Inhibitory effect of the isolated coumarins (**1**–**5**) on sEH activity determined using the fluorometric method. The sEH activity is expressed as the percentage of control activity (n = 3). AUDA (IC_{50} 15.7 ± 2.7 nM) was used as a positive control.

showed that the best docked conformation was determined for kimcuongin (**4**) with Tyr383A and Tyr466A of the sEH active site from 2.13–2.77 Å (**Table 2 S**, Supporting Information).

During the docking process, the protein was considered to be rigid while the ligands and those amino acids inside the pocket were flexible. The AutoDock output results represented the docking scores as Gibbs free energy of binding (*Δ*G) values, further converted to the predicted inhibition constants ($pK_{i,pred}$). The designed compounds (**2, 3**, and **4**) were found to have excellent binding affinity to the enzyme, showing binding energies as *Δ*G values of − 6.4, − 6.6 and − 7.8 kcal/mol, respectively. The negative values of *Δ*G indicated that the coumarins bind to sEH spontanously and these values also proved that the compounds possess potential sEH enzyme inhibitory binding activities. Three compounds occupy the same cavity (**Fig. 1 S**, Supporting Information) with only some differences in amino acid residue involvement because of their dihedral rotation and conformational mismatch. The small variance in *Δ*G values and binding posed inside the pocket may be attributed to the differences in the position of the functional groups in the selected compounds. The *Δ*G values were further converted to $pK_{i,pred}$, where K_i was calculated by the formula $K_i = IC_{50}/1 + [S]/K_m = \exp(\Delta G/R \times T)$, so $IC_{50} = \exp(\Delta G/R \times T) \times 1 + [S]/K_m$ (**Table 1**).

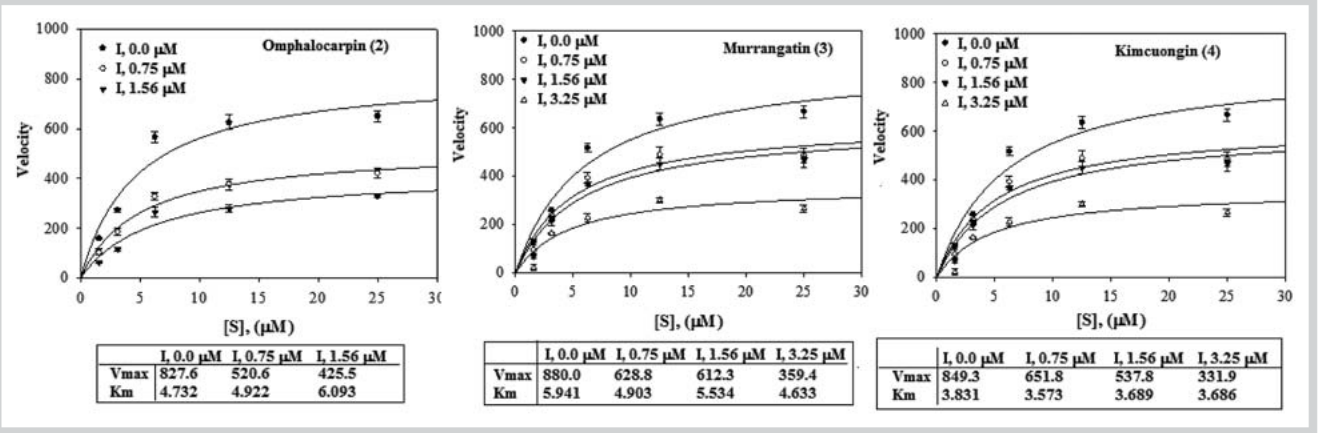
The docking results of the three compounds were analyzed by a bioinformatic tool, giving an interaction diagram and a table of interaction data for each binding site. For all compounds, the binding was dominated by hydrophobic interactions and hydrogen bonds. The three diagrams, in agreement with the *Δ*G calculation results, showed that compounds **2** and **3** had a similar set of binding profiles (seven hydrogen interactions and one hydrogen bond), while **4** had the best binding outline (nine hydrogen bonds and two hydrogen bonds; **Fig. 4**).

The amino acids presented at the binding site are computationally mutated to alanine in an ABS-Scan tool to perform an *in silico* alanine scanning mutagenesis for binding site residues in the protein-ligand complex (**Fig. 5**). The binding energy is computed for each mutant and the corresponding energy differencies (*ΔΔ*G) values between the wild-type protein and the mutated one are also calculated. The *ΔΔ*G profile shared by murrangatine (**3**) and kimcuongin (**4**) was not totally repeated by omphalocarpin (**2**), which showed the smallest binding energy. The docked poses of coumarin derivatives clearly demonstrated the binding positions of the ligand with the enzyme. The main binding force is due to the interactions of Tyr466 and Tyr383 with the coumarin derivatives, while other important interactions were found with amino acid residues Trp 525, Trp 336, and Phe 267. The coumarin

Table 1 **A** *In vitro* sEH inhibitory activity with IC_{50} values, kinetic study of coumarins (**1**–**5**) and v_{max}, K_m, and $K_{i,exp}$ values [Substrate concentration of 0.75 µM. AUDA was used as a positive control 15.7 ± 2.7 (nM)]. **B** Predicted and experimental binding parameters of coumarins **2**–**4** to sEH enzyme.

No.	Compounds	A. Kinetic parameters					B. Predicted and experimental binding parameters				
		IC_{50} (µM)	Binding mode	v_{max}	K_m	$K_{i,exp} = 1/K_m$	*Δ*G (kcal/mol)	$K_{i,pred}$	$pK_{i,pred}$	$IC_{50,pred}$ (µM)	$pK_{i,exp}$
1	Mexoticin	> 25	ND								
2	Omphalocarpin	2.2 ± 4.7	Mixture (competitive/ noncompetitive)	520.6	4.922	0.203 169	− 6.4	2.02E-05 M	4.69	2.5	5.66
3	Murrangatin	13.9 ± 6.5	noncompetitive	628.8	4.903	0.203 956	− 6.8	1.03E-05 M	4.99	(ND)	4.87
4	Kimcuongin	3.2 ± 4.5	noncompetitive	651.8	3.573	0.279 876	− 7.8	1.89E-06 M	5.72	(ND)	5.49
5	Murracarpin	> 25	ND								

ND = not determined



	I, 0.0 µM	I, 0.75 µM	I, 1.56 µM
Vmax	827.6	520.6	425.5
Km	4.732	4.922	6.093

	I, 0.0 µM	I, 0.75 µM	I, 1.56 µM	I, 3.25 µM
Vmax	880.0	628.8	612.3	359.4
Km	5.941	4.903	5.534	4.633

	I, 0.0 µM	I, 0.75 µM	I, 1.56 µM	I, 3.25 µM
Vmax	849.3	651.8	537.8	331.9
Km	3.831	3.573	3.689	3.686

Fig. 3 Effects of coumarins **2, 3**, and **4** on the activity of enzyme sEH for hydrolysis of the PHOME substrate.

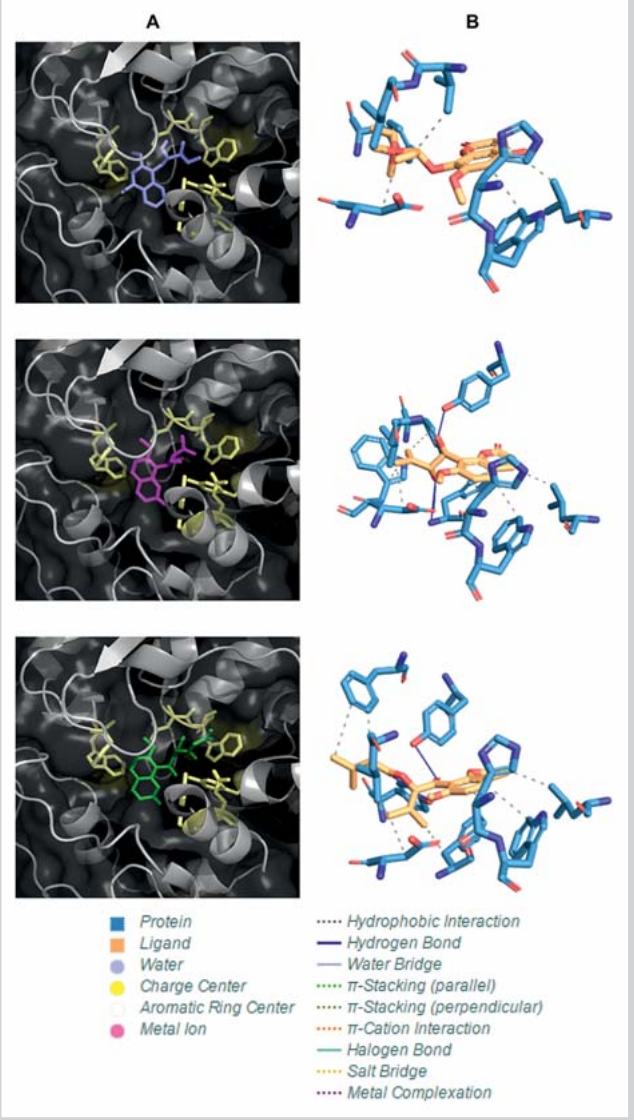


Fig. 4 **A** Docked complexes of omphalocarpin (**2**, blue), murrangatin (**3**, magenta), and kimcuongin (**4**, green) into human sEH shown in cartoon representation. **B** PLIP 3D-interaction map of the ligands with sEH residues playing an essential role for binding.

kimcuongin (**4**), furthermore, showed good affinity towards Tyr 383, Gln 384, and Phe 387, which were responsible for better sEH binding energy and its potential inhibitory activity.

In summary, among five coumarins isolated from the chloroform fraction of the leaves and stems of *M. paniculata*, three coumarins, omphalocarpin (**2**), murrangatin (**3**), and kimcuongin (**4**), showed sEH inhibitory activity with IC_{50} values 2.2 ± 4.7, 13.9 ± 6.5, and 3.2 ± 4.5 µM, respectively, in noncompetitive (**3, 4**) and mixture-kinetic mode (**2**). Using a computational approach, kimcuongin (**4**) showed the best binding outline, characterized by the smallest binding energy (ΔG = − 7.8 kcal/mol), a good affinity towards Tyr 383, Gln 384, and Phe 387, and its potential sEH inhibitory activity. These three coumarins might be worthy to further investigate in order to develop a new scaffold of therapeutic agents for cardiovascular diseases.

Materials and Methods

The leaves and twigs of *M. paniculata* were collected in Cuc Phuong National Park, Hoa Binh Province, North Vietnam. The plant was identified by botanist Dr. Tran The Bach, Institute of Ecology and Biological Resources (VAST). A voucher specimen (C-425) is deposited in the herbarium of the Institute of Natural Products Chemistry (VAST), Hanoi, Vietnam.

Dried powdered leaves and twigs of *M. paniculata* (3.2 kg) were extracted with MeOH 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), ethyl acetate (EtOAc), and water to give the corresponding solvent-soluble fractions *n*-hexane (6.7 g), chloroform (7.2 g), EtOAc (16.7 g), and water (60 mL). Repeated chromatography of *n*-hexane and chloroform fractions on silica gel columns with different eluting solvents resulted in the separation of compounds **1**–**5** and **6, 7**, respectively.

Supporting information

General experimental procedures, extraction and isolation, sEH bioassays, and molecular modelling are described in detail in Supporting Information.

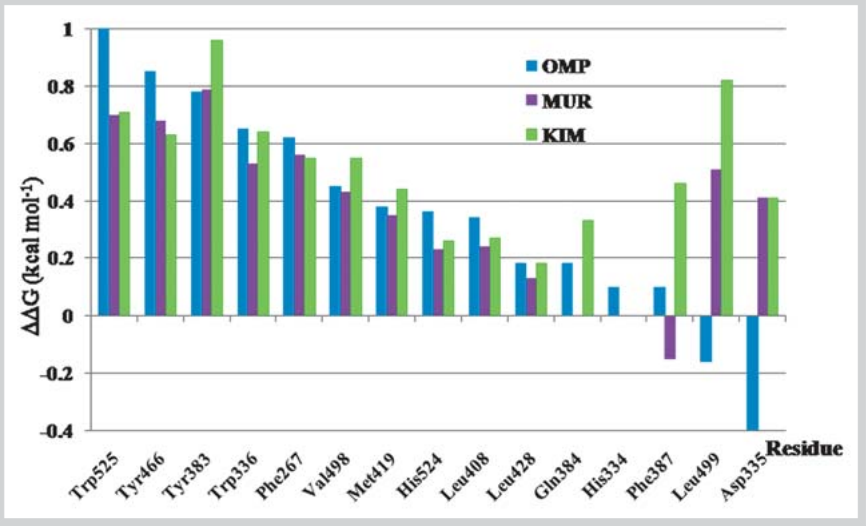


Fig. 5 ABS-Scan energy plot. ΔΔG values recorded after alanine scanning mutation of the single residues involved in the binding of omphalocarpin (blue), murrangatin (magenta), and kimcuongin (green).

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Conflict of Interest

The authors declare no conflict of interest.

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