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The 5-substituted piperazine as a novel secondary pharmacophore greatly improving the physical properties of urea-based inhibitors of soluble epoxide hydrolase

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Abstract

The inhibition of the mammalian soluble epoxide hydrolase (sEH) is a promising new therapy in the treatment of hyper-tension and inflammation. The problems of limited water solubility and high melting points commonly displayed by the active 1,3-disubstituted ureas prevent the further development of potent urea-based sEH inhibitors. Therefore, a new class of potent inhibitors of sEH were designed and synthesized by the introduction of a polar constrained piperazino group in the right side of adasmantyl urea to increase the water solubility. A facile and general synthesis was established to prepare a series of 1-adamantan-1-yl-3-(2-piperazin-2-yl-ethyl)-ureas (**1a–d**) with various 5-substitutions on the 2-piperazino ring, which will advance the SAR study by the efficient making of structurally diverse analogs. The effect of the 5-substitution on the activity and the water solubility was examined. The best potency was exhibited by the 5-benzyl-substituted-piperazine-containing urea with an IC₅₀ value of 1.37 μM against human sEH and good water solubility (*S* = 7.46 mg/mL) and low melting point, in which the 5-substituted piperazine serves as a favorable secondary pharmacophore and a water-solubility enhancing group. Our present work provides a promising new template for the design of orally available therapeutic agents for the disorders that can be addressed by changing the in vivo concentration of the chemical mediators that contain an epoxide.

Keywords

Soluble epoxide hydrolase; 1-adamantan-1-yl-3-(2-piperazin-2-yl-ethyl)-ureas; Piperazine; Chiral pool synthesis; Secondary pharmacophore; Water solubility; Hypertension; Vascular inflammation

1. Introduction

The epoxide hydrolases (EHs) are enzymes present in most living organisms, which transform epoxide-containing compounds to their corresponding diols by the addition of water. The soluble epoxide hydrolase appears selective for epoxides of lipids. In plants and animals, many of these lipid substrates have potent biological activities, such as host defenses, control of development, and regulation of inflammation and blood pressure.¹ There are two well-studied EHs in mammals, the microsomal epoxide hydrolase (mEH) and the soluble epoxide hydrolase

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Supplementary data

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(sEH).² In humans, the sEH plays an important role in the metabolism of endogenous chemical mediators such as arachidonic acid, linoleic acid, and other lipid epoxides. Epoxides of arachidonic acid (epoxyeicosatrienoic acids or EETs) are known effectors of blood pressure³ and modulators of vascular permeability.^{4,5} Hydrolysis of the epoxides by sEH diminishes this activity.³ In both cellular and animal models, continued efficacy of EET-mediated hypertension and cardioprotective effects including inflammation control is dependent upon epoxide hydrolysis by sEH,^{6,7} suggesting that inhibition of the mammalian sEH is a promising new therapy in the treatment of disorders resulting from hypertension and vascular inflammation.^{8–10}

Based on the transition state of the epoxide hydrolysis reaction catalyzed by sEH,^{11,12} 1,3-disubstituted ureas were designed and evaluated as potent sEH inhibitors, which efficiently reduced epoxide hydrolysis in several in vitro and in vivo models.^{7,13,14} The ureas mimicked the structural features of the epoxide ring opening by sEH, with similar charge distribution to the epoxide.¹¹ However, the dialkyl ureas have limited solubility in water and high melting points, leading to poor in vivo efficacy and difficulty in formulation.^{15,16} During the efficient efforts to improve the physical properties, a new concept of a secondary pharmacophore was proposed which consists of a polar functional group located on the fifth/sixth atom (7.5 Å) from the carbonyl group of the urea primary pharmacophore to help improve the binding and the water solubility (Fig. 1).^{17,18} Previous efforts were focused on ester, ketone, alcohol, and ether functionality to determine a favorable secondary pharmacophore. However, the positive effect on the solubility is still quite limited (<2 mg/mL) and the resulting compounds still have relatively high melting points (>100 °C) when the secondary pharmacophore is a polar carbonyl group. How to tackle the problem of the poor physical properties which prevents the potent urea-based sEH inhibitors from entering clinical treatment? We are interested in incorporating a constrained heterocycle as the secondary pharmacophore into the 1,3-dialkyl urea platform. Piperazine is an interesting heterocyclic structure present in many biologically active molecules.^{19,20} Constraining the polar nitrogen atoms into the piperazino ring can confer more drug-like properties to molecules and enhance favorable interactions with macromolecules. In this article, we designed a new class of sEH inhibitors by introducing a substituted piperazino group into the right side of 1-adamantyl-urea to improve the activity and water solubility. A chiral pool approach was established to synthesize the target compounds with various substitutions on the 5-position of the 2-piperazino ring (Fig. 1, compounds **1a–d**). The effect of the 5-substitution on the activity and the water solubility was examined. The best potency was exhibited by the 5-benzyl-substituted-piperazine-containing urea with an IC₅₀ value of 1.37 μM against human sEH, and an exciting water solubility (*S* = 7.46 mg/mL) and low melting point (77–78 °C), providing a promising new scaffold for the further development of orally available sEH inhibitors.

2. Chemistry

An efficient and convenient synthetic approach was developed to prepare the 1-adamantan-1-yl-3-(2-(5-substituted piperazin-2-yl)-ethyl)-ureas. As depicted in Scheme 1, the substituted piperazino moiety was constructed via a chiral pool approach employing the amino acid as the starting material. The free asparagine was easily converted into the *N*^α-Boc-protected form, then the coupling with another amino acid methyl ester afforded the dipeptide **3a–d** allowing the introduction of various substituents of R as the side chain of the amino acid. The Boc removal of **3a–d** followed by treatment with the methanolic NH₃ provided the 2,5-diketopiperazine intermediate **4a–d** in good yield via an intramolecular cyclization. Complete reduction of all amide bonds with BH₃·THF²¹ produced the piperazine-containing amine **5a–d**, in which the amide side chain of the asparagine of the starting material was judiciously converted into the primary amine while the piperazino ring was constructed. The free amine of the side chain became the joining point which was reacted with 1-adamantyl isocyanate to

furnish the final product **1a–d**. In this study, we just synthesized four representative structures, but the methodology is efficient and applicable to the general synthesis of various 5-substituted piperazine-containing analogs for future SAR study. Furthermore, the choice of the starting amino acid can adjust the distance of the urea functionality and the secondary pharmacophore of piperazine. So, from the view point of chemistry, we have established an efficient methodology to synthesize a focused library of piperazine-containing 1,3-dialkyl ureas. Now, the next work is to investigate whether the 5-substituted piperazine is an effective secondary pharmacophore which can enhance the physical properties of this class of sEH inhibitors.

3. Results and discussion

Four novel piperazine-containing dialkyl ureas were designed and synthesized, in which the effect of the substituent on the 5-position of the 2-piperazino moiety on the activity and the water solubility was examined. Four representative structures were chosen as the 5-substituent: the aromatic ring (compound **1a**), the conformational constrained heterocycle (compound **1b**), the alkyl chain (compound **1c**), and the polar hydroxyl group (compound **1d**). We want to determine which type of structure is favored for the binding with the sEH protein when attached on the piperazino ring. A recently developed sensitive fluorescence-based assay²² was used to evaluate the inhibitory activity of these new compounds against human sEH enzyme.

Since the goal of this work is to increase the water solubility of the sEH inhibitors without loss of activity by introducing a polar piperazine group as a secondary pharmacophore, the water solubility (*S*, mg/mL) was determined experimentally at 25 ± 1.0 °C in PBS buffer (0.1 M, pH 7.4).²³ The octanol/water partition coefficient (*P*) is an important indication of the lipophilicity of an organic molecule. The log *P* values were estimated by Crippen's method using CS ChemDraw Ultra version 6.0. On the basis of this log *P* value, log *S* value (solubility in water) was also calculated with the equation suggested by Banerjee et al.²⁴ as reference. All the activity and physical property data are reported in Table 1.

In general, the high melting point was the indication of high stability of crystal structure, which led to lack of solubility in oil and in water. These properties make it difficult to formulate the compounds in either an aqueous or oil base.¹⁷ As shown in Table 1, the piperazine-containing 1-adamantyl-3-alkyl ureas (compounds **1a–d**) pleasantly display improved physical properties: lower melting point (mp < 80 °C) and lipophilicity (log *P* = 2.09–0.54), and better water solubility (*S* = 4.44–8.52 mg/mL) compared to the previously reported best urea inhibitor of sEH (compound **1e**: mp = 114 °C, log *P* = 2.77, *S* = 1.69 mg/mL), which possess an ester functional group as the secondary pharmacophore. It is evident that the conformationally constrained polar piperazine is really an excellent pharmacophore on this position to enhance the drug-like properties in the context of 1,3-dialkyl ureas as sEH inhibitors.

With respect to the inhibitory potency, the 5-substitution on the 2-piperazino ring was found to play an important role. When the substituent was a hydrophobic group, for example, benzyl group, or isopropyl group, the inhibition against human sEH by the resulting piperazine-containing adamantyl ureas was retained potent, with an IC₅₀ value of 1.37 μM for **1a** and 12.6 μM for **1c**, respectively. However, an additional polar substituent such as propanol group on this position remarkably reduced the inhibitory activity (**1d**, IC₅₀ = 100 μM), while the high hydrophilicity confers the best water solubility to compound **1d** (*S* = 8.52 mg/mL). The fused pyrrolo ring with the piperazine endures more rigid constraint and resulted in the lowest melting point in this series (mp = 56–58 °C) at the loss of the potency (**1c**, IC₅₀ = 93.8 μM). So, we anticipate that the optimal combination of the hydrophilic piperazine ring and the hydrophobic substitution on the piperazino ring would confer good physical properties with high potency.

Even though the best compound in our series (compound **1a**) is much less potent than the best one (compound **1e**) of previously reported classes which bear an ester as the secondary pharmacophore, the physical properties are significantly improved by the introduction of the novel piperazine group. Since the poor water solubility and high melting point are the remaining drawbacks of the urea-based inhibitors of sEH, the incorporation of the piperazino group provides a promising new strategy to circumvent the major problem. Furthermore, the initial SAR study has disclosed that the hydrophobic substitution on the 5-position of the piperazino ring is favored for the binding with the sEH, further structural optimization would definitely improve the activity of this new series which inherit good physical properties, and our well-established synthesis in this work will benefit the making of various analogs. In summary, our present work paved a new way for the design of specific and potent sEH inhibitors with enhanced drug-like properties, and will be useful for the design of orally available therapeutic agents for hypertension, vascular and renal inflammation, and other disorders that can be addressed by changing the in vivo concentration of chemical mediators that contain an epoxide.

4. Conclusions

Based on the mode of action of sEH and the structure of the urea inhibitor complexed with the enzyme,^{11,12} we designed and synthesized a new class of sEH inhibitors with remarkably improved physical properties by the introduction of substituted piperazino ring in the urea platform. A practical and efficient synthesis is established to prepare a range of piperazine-containing dialkyl ureas with various 5-substitutions on the 2-piperazino ring, which will benefit further structural elaboration and extensive SAR study. The best potency was exhibited by the 5-benzyl-substituted-piperazine-containing urea with an IC₅₀ value of 1.37 μ M against human sEH and an improved water solubility ($S = 7.46$ mg/mL) and low melting point, in which the 5-substituted piperazine serves as a favorable secondary pharmacophore and a water-solubility enhancing group, providing a promising new template for further development of sEH inhibitors as anti-hypertension and anti-inflammation drugs.

5. Experimental

5.1. Synthesis

The ¹H NMR spectra were recorded on a Varian Mercury-400 MHz spectrometer. The data are reported in parts per million relative to TMS and referenced to the solvent in which they were run. Elemental analyses were obtained using Vario EL spectrometer. Melting points (uncorrected) were determined on a Buchi-510 capillary apparatus. EI-MS spectra were obtained on a Finnigan MAT 95 mass spectrometer. ESI-MS spectra were obtained on a Finnigan LCQ Deca mass spectrometer. Specific rotations (uncorrected) were determined in a Perkin-Elmer 341 polarimeter.

5.2. S-2-tert-Butoxycarbonylamino-succinamic acid (**2**)²⁶

To a suspension of L-Asn·H₂O (1.5 g, 10 mmol) in the solution of dioxane and H₂O (1:1), (BOC)₂O (6.5 g, 30 mmol) was added, with DMF to help increase solubility. Then NaHCO₃ (2.5 g, 30 mmol) was added. The mixture was stirred at room temperature for 24 h, then the solvent was removed at reduced pressure. The residue was acidified with 6 N HCl to pH 2 and filtered. The solid was recrystallized from CH₃OH to afford product **2** as white powder (1.5 g, 65%).

5.3. S,S-2-(2-tert-Butoxycarbonylamino-3-carbamoyl-propionylamino)-3-phenyl-propionic acid methyl ester (**3a**)²⁷

Under ice-salt bath, to the solution of the amino acid methyl ester L-Phe-OMe·HCl (1.3 g, 6 mmol), compound **2** (1.4 g, 6 mmol), and HOBt (1.35 g, 10 mmol) in 20 mL of dry CH₂Cl₂

was added DIPEA (3 mL). The reaction mixture was stirred at 0 °C, added the solution of EDCI (1.91 g, 10 mmol) in 20 mL of dry CH₂Cl₂ dropwise. Then the solution was stirred at room temperature for 24 h. A lot of solid appeared, filtered, and the solid was washed with saturated NH₄Cl aq, 1 N HCl aq, and saturated NaHCO₃ aq, respectively. The crude product was recrystallized from CH₃OH to provide white powder **3a** (1.6 g, 68%). [α]_D²² -9 (*c* = 0.19, MeOH). ¹H NMR (CDCl₃, 400 MHz): δ 7.32–7.14 (5H, m), 6.02–5.99 (1H, m), 5.79–5.78 (1H, m), 5.35–5.34 (1H, m), 4.80–4.78 (1H, m), 4.47–4.45 (1H, m), 3.69 (3H, s), 3.15–3.04 (2H, m), 2.93–2.89 (1H, d, *J* = 16 Hz), 2.57–2.52 (1H, dd, *J*₁ = 16 Hz, *J*₂ = 6.4 Hz), 1.38 (9H, s). Mp: 194–195 °C.

5.4. S,S-1-(2-*tert*-Butoxycarbonylamino-3-carbamoyl-propionyl)-pyrrolidine-2-carboxylic acid methyl ester (**3b**)

Prepared from compound **2** (3.4 g, 15 mmol) and L-Pro-OMe·HCl (2.5 g, 15 mmol) according to the similar procedure to **3a**. The residue was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 10:1) to afford **3b** as white foam (2.2 g, 44%). [α]_D²² -69.7 (*c* = 1.3, CH₂Cl₂). ¹H NMR (CDCl₃, 400 MHz): δ 6.59 (1H, blunt), 5.85–5.83, 5.49–5.47 (2H, blunt), 4.80–4.79 (1H, m), 4.53–4.50 (1H, m), 3.78–3.73 (2H, m), 3.73 (3H, s), 2.71–2.55 (2H, m), 2.25–2.18, 2.05–1.96 (4H, m), 1.45 (9H, s). Mp: 56–58 °C. EI-MS: *m/z* 343 (M⁺).

5.5. S,S-2-(2-*tert*-Butoxycarbonylamino-3-carbamoyl-propionylamino)-3-methyl-butyric acid methyl ester (**3c**)

Prepared from compound **2** (2.9 g, 12.5 mmol) and L-Val-OMe·HCl (2.1 g, 12.5 mmol) according to the similar procedure to **3a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 20:1) to afford **3c** as white foam (3.0 g, 70%). [α]_D²² +0.2 (*c* 0.775, CH₂Cl₂). ¹H NMR (CDCl₃, 400 MHz): δ 7.50 (1H, blunt), 6.20–6.16 (2H, blunt), 5.64 (1H, blunt), 4.52–4.51, 4.47–4.44 (2H, m), 3.73 (3H, s), 2.93–2.89 (1H, dd, *J*₁ = 4 Hz, *J*₂ = 15.6 Hz), 2.65–2.59 (1H, dd, *J*₁ = 6.4 Hz, *J*₂ = 16 Hz), 2.22–2.15 (1H, m), 1.50–1.43 (9H, s), 0.96–0.92 (6H, dd). Mp: 139–141 °C. (Mp₂₈: 141–150 °C). EI-MS: *m/z* 345 (M⁺).

5.6. S,S-2-(2-*tert*-Butoxycarbonylamino-3-carbamoyl-propionylamino)-pentanedioic acid dimethyl ester (**3d**)

Prepared from compound **2** (5.6 g, 24 mmol) and L-Glu(OMe)-OMe·HCl (5.1 g, 24 mmol) according to the similar procedure to **3a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 10:1) to afford **3d** as white foam (8.5 g, 91%). [α]_D²² +17.2 (*c* 1.1, CH₂Cl₂). ¹H NMR (CDCl₃, 400 MHz): δ 7.48–7.47 (1H, blunt), 6.07 (2H, blunt), 5.70 (1H, blunt), 4.58–4.56, 4.49–4.48 (2H, m), 3.73 (3H, s), 3.67 (3H, s), 2.95–2.91 (1H, dd, *J*₁ = 3.2 Hz, *J*₂ = 15.6 Hz), 2.61–2.56 (1H, dd, *J*₁ = 6.4 Hz, *J*₂ = 15.6 Hz), 2.42–2.37 (2H, m), 2.23–2.19 (1H, m), 2.00–1.95 (1H, m), 1.44 (9H, s). Mp: 122–123 °C. EI-MS: *m/z* 389 (M⁺). Anal. Calcd for C₁₆H₂₇N₃O₈: C, 49.35; H, 6.99; N, 10.79. Found: C, 49.17; H, 7.04; N, 10.5.

5.7. S,S-2-(5-Benzyl-3,6-dioxo-piperazin-2-yl)-acetamide (**4a**)

Compound **3a** (1.6 g, 4.2 mmol) was added into the solution of CF₃COOH in CH₂Cl₂ at 0 °C and then stirred for 4 h. The solvent was removed at reduced pressure. The residue was dissolved in NH₃-saturated methanol at 0 °C and stirred for 4 h at room temperature. White solid appeared. The solvent was removed at reduced pressure. The crude solid was recrystallized from H₂O. White powder was obtained (1 g, 92%). [α]_D²² -14 (*c* 0.2, H₂O). ¹H NMR (CD₃OD, 400 MHz): δ 7.34–7.21 (5H, m), 4.31–4.29 (1H, m), 4.13–4.11 (1H, m), 3.32–3.22 (1H, m), 3.03–2.99 (1H, m), 2.62–2.57 (1H, dq, *J*₁ = 4 Hz, *J*₂ = 6.4 Hz), 2.36–2.31 (1H, m). Mp: >200 °C. (Mp₂₉: 268–270 °C) EI-MS: *m/z* 261 (M⁺).

5.8. S,S-2-(1,4-Dioxo-octahydro-pyrrolo[1,2-a]pyrazin-3-yl)-acetamide (4b)

Prepared from compound **3b** (4.9 g, 14 mmol) according to the similar procedure to **4a**. The crude solid was recrystallized from methanol, and white crystal was obtained (1.6 g, 63.8%). $[\alpha]_D^{22}$ -119.9 (*c* 1.0, H₂O). ¹H NMR (DMSO-*d*₆, 400 MHz): δ 7.96 (1H, s), 7.412, 6.91 (2H, s, s), 4.38–4.36 (1H, t, *J*₁ = *J*₂ = 6 Hz), 4.23–4.19 (1H, t, *J*₁ = 6.4 Hz, *J*₂ = 8 Hz), 3.40–3.31 (2H, m), 2.76–2.70, 2.34–2.28 (2H, dq, *J*₁ = 6.0 Hz, *J*₂ = 6.4 Hz, *J*₃ = 16 Hz), 2.12–2.10, 1.89–1.79 (4H, m). Mp: >200 °C. EI-MS: *m/z* 211 (M⁺).

5.9. S,S-2-(5-Isopropyl-3,6-dioxo-piperazin-2-yl)-acetamide (4c)

Prepared from compound **3c** (960 mg, 2.8 mmol) according to the similar procedure to **4a**. The crude solid was recrystallized from H₂O, and white crystal was obtained (520 mg, 88%). $[\alpha]_D^{22}$ -79 (*c* 0.23, H₂O). ¹H NMR (D₂O, 400 MHz): δ 4.58–4.55 (1H, t, *J*₁ = 5.2 Hz, *J*₂ = 8 Hz), 4.09–4.08 (1H, d, *J* = 6.4 Hz), 3.00–2.94, 2.85–2.79 (2H, dq, *J*₁ = 4.8 Hz, *J*₂ = 8 Hz), 2.36–2.32 (1H, m), 1.12–0.98 (6H, dd, *J* = 6.8 Hz). Mp: >200 °C. EI-MS: *m/z* 213 (M⁺). Anal. Calcd for C₉H₁₅N₃O₃: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.86; H, 7.02; N, 19.68.

5.10. 3-(5-Carbamoylmethyl-3,6-dioxo-piperazin-2-yl)-propionic acid methyl ester (4d)

Prepared from compound **3d** (4 g, 10.3 mmol) according to the similar procedure to **4a**. The crude solid was recrystallized from H₂O, and white crystal was obtained (1.4 mg, 52.1%). $[\alpha]_D^{22}$ -34.2 (*c* 0.49, H₂O). ¹H NMR (D₂O, 400 MHz): δ 4.49–4.44 (1H, m), 4.27–4.23 (1H, m), 3.72 (3H, s), 2.89–2.87 (2H, d, *J* = 7.6 Hz), 2.58–2.52 (2H, m), 2.23–2.16 (2H, m). Mp: >200 °C. EI-MS: *m/z* 257 (M⁺). Anal. Calcd for C₁₀H₁₅N₃O₅: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.81; H, 5.82; N, 16.28.

5.11. S,S-2-(5-Benzyl-piperazin-2-yl)-ethylamine (5a)

To the suspension of compound **4a** (260 mg, 1 mmol) in 20 mL THF was added 10 mL of BH₃–THF complex (10 mmol) dropwise with stirring under N₂ over 1 h. Gas emitted and the mixture became nearly solution at the end of the addition. The mixture was re-fluxed for 40 h. It was cooled to room temperature and 20 mL of methanol was added over 1 h. The reaction mixture was concentrated to dryness. The residue was dissolved in methanol (10 mL) and excess methanolic HCl solution was added and the solution was re-fluxed for 2 h. It was cooled and stirred at room temperature overnight. The solvent was removed at reduced pressure, and then a solution of 10% NaOH in water was added into the residue to pH 11. Then H₂O was removed at reduced pressure and dissolved in methanol, and dried with anhydrous Na₂SO₄. Filtered and removed the solvent, purified by silica gel chromatography (CH₂Cl₂/CH₃OH 8:1) to afford **5a** as light yellow oil (177 mg, 80.9%). The methanol saturated with HCl was added to the oil, white solid was obtained. $[\alpha]_D^{22}$ +2.6 (*c* 0.57, MeOH). ¹H NMR (D₂O, 400 MHz): δ 7.46–7.33 (5H, m), 3.13–2.73 (10H, m), 1.85–1.73 (2H, m). Mp: >200 °C. ESI-MS: *m/z* 220.2 [M+H]⁺.

5.12. S,S-2-(Octahydro-pyrrolo[1,2-a]pyrazin-3-yl)-ethylamine (5b)

Prepared from compound **4b** (422 mg, 2 mmol) according to the similar procedure to **5a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 2:1) to afford **5b** as light yellow oil (145 mg, 45%). The methanol solution saturated with HCl was added to the oil, white solid was obtained. $[\alpha]_D^{22}$ -5.5 (*c* 0.83, MeOH). ¹H NMR (DMSO-*d*₆, 400 MHz): δ 3.04–2.47 (10H, m), 2.11–1.94 (2H, m), 1.78–1.25 (6H, m). ESI-MS: *m/z* 170 [M+H]⁺.

5.13. S,S-2-(5-Isopropyl-piperazin-2-yl)-ethylamine (5c)

Prepared from compound **4c** (426 mg, 2 mmol) according to the similar procedure to **5a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 2:1) to afford **5c** as light yellow oil (150 mg, 43.9%). The methanol solution with saturated hydrochloric acid was added to the oil, white solid was obtained. $[\alpha]_D^{22} -13$ (c 0.1, H₂O). ¹H NMR (D₂O, 400 MHz): δ 4.05–3.76–3.49 (6H, m), 3.30–3.26 (2H, t), 2.37–2.31 (2H, m), 2.21–2.19 (1H, m), 1.18–1.12 (6H, dd). Mp: >200 °C. ESI-MS: *m/z* 172 [M+H]⁺.

5.14. S,S-3-[5-(2-Amino-ethyl)-piperazin-2-yl]-propan-1-ol (5d)

Prepared from compound **4d** (1.3 g, 5.1 mmol) according to the similar procedure to **5a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 2:1) to afford **5d** as light yellow oil (640 mg, 68%). The methanol solution saturated with HCl was added to the oil, white solid was obtained. $[\alpha]_D^{22} -3.5$ (c 0.42, H₂O). ¹H NMR (D₂O, 400 MHz): δ 3.962–3.955 (1H, m), 3.871–3.865 (1H, m), 3.73–3.70 (2H, m), 3.67–3.59 (4H, m), 3.29–3.25 (2H, m), 2.35–2.29 (2H, m), 2.00–1.96 (2H, m), 1.77–1.73 (2H, m). Mp: >200 °C. ESI-MS: *m/z* 188.2 [M+H]⁺.

5.15. S,S-1-Adamantan-1-yl-3-[2-(5-benzyl-piperazin-2-yl)-ethyl]-urea (1a)

The solution of 1-isocyanato-adamantane (81 mg, 0.45 mmol) in dry CH₂Cl₂ was added dropwise **5a** (109 mg, 0.5 mmol) in 10 mL of dry CH₂Cl₂ at 0 °C. Then 1.74 μ L DIPEA was added. The mixture was stirred at 0 °C for 3 h and then stirred at room temperature overnight. The solvent was removed at reduced pressure and then purified by silica gel chromatography (CH₂Cl₂/CH₃OH 8:1) to afford **1a** as light yellow solid (44 mg, 24%). $[\alpha]_D^{22} -11$ (c 0.56, CH₂Cl₂). ¹H NMR (CDCl₃, 400 MHz): δ 7.32–7.18 (5H, m), 5.1 (1H, blunt), 4.67 (1H, blunt), 3.35 (1H, m), 3.15–3.11 (1H, m), 2.97–2.75 (8H, m), 2.95–2.71 (7H, m), 2.32–2.28 (2H, m), 2.04–1.58 (17H, m). Mp: 77–78 °C. HRESI-MS *m/z*: 397.2982 [M+H]⁺, calcd 397.2967.

5.16. S,S-1-Adamantan-1-yl-3-[2-(octahydro-pyrrolo[1,2-a]piperazin-3-yl)-ethyl]-urea (1b)

Prepared from compound **5b** (78 mg, 0.46 mmol) according to the similar procedure to **1a**. The procedure was similar as above. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 10:1) to afford **1b** as light yellow solid (60 mg, 44%). $[\alpha]_D^{22} +3.1$ (c 0.4, CH₂Cl₂). ¹H NMR (CD₃OD, 400 MHz): δ 3.34–3.00 (10H, m), 2.7–2.6 (2H, m), 2.04–1.70 (21H, m). Mp: 56–58 °C. HRESI-MS *m/z*: 347.2806 [M+H]⁺, calcd 347.2811.

5.17. S,S-1-Adamantan-1-yl-3-[2-(5-isopropyl-piperazin-2-yl)-ethyl]-urea (1c)

Prepared from compound **5c** (120 mg, 0.7 mmol) according to the similar procedure to **1a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 10:1) to afford **1c** as light yellow solid (30 mg, 14%). $[\alpha]_D^{22} -95$ (c 0.43, CH₂Cl₂). ¹H NMR (CD₃OD, 400 MHz): δ 3.34–3.00 (8H, m), 2.7–2.6 (1H, m), 2.04–1.70 (18H, m), 1.206–0.969 (6H, dd). Mp: 69–70 °C. HRESI-MS *m/z*: 349.2951 [M+H]⁺, calcd 349.2967.

5.18. S,S-1-Adamantan-1-yl-3-[2-[5-(3-hydroxy-propyl)-piperazin-2-yl]-ethyl]-urea (1d)

Prepared from compound **5d** (320 mg, 1.73 mmol) according to the similar procedure to **1a**. The crude product was purified by silica gel chromatography (CH₂Cl₂/CH₃OH 2:1) to afford **1d** as light yellow solid (150 mg, 27%). $[\alpha]_D^{22} 7.8$ (c 0.43, CH₂Cl₂). ¹H NMR (CDCl₃, 400 MHz): δ 4.77 (1H, blunt), 4.45 (1H, blunt), 3.65–3.55 (2H, m), 3.33 (1H, m), 3.14–3.10 (1H,

m), 2.86–2.69 (6H, m), 2.32–2.10 (2H, blunt), 2.10–1.52 (21H, m). Mp: 66–68 °C. HRESI-MS: m/z 365.2921 $[M+H]^+$, calcd 365.2917.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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23. The water solubility (S , mg/mL) was determined experimentally at 25 ± 1.0 °C. For compound **1a** with a UV chromophore, an excess of the test compound was added to a vial containing sodium phosphate buffer, 0.1 M, pH 7.4 (200 μ L), and a suspension of the mixture was equilibrated during

1 h of sonication and 1 h of shaking, followed by centrifugation (5 min, 8000 rpm). The water supernatant was scanned on UV spectrophotometer (HITACHI U-2010) to get its UV absorbance at the wavelength of 254 nm. A regression curve for compound **1a** was obtained from six standard stock solutions ($r = 0.99$) by using UV spectrometry at the same wavelength. Then, the absolute amount of compound **1a** was calculated. For compounds **1b–c** without a UV chromophore, to an exactly weighed amount of test compound was added PBS by an increment of 5 μ L with pipet until the solid was completely dissolved accompanied with frequent shaking at 25 ± 1.0 °C.

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Figure 1.

A representative sEH inhibitor with primary and secondary pharmacophores indicated, and the structures of the designed new sEH inhibitors with substituted piperazine as the secondary pharmacophore.

Scheme 1.

Reagents and conditions: (a) (BOC)₂O, dioxane/H₂O; (b) HOBt, EDCl, DIPEA, L-amino acid methyl ester, CH₂Cl₂; (c) 1—CF₃COOH 50% in CH₂Cl₂; 2—NH₃/CH₃OH; (d) BH₃·THF; (e) 1-adamantyl isocyanate, CH₂Cl₂.

Table 1
Inhibition of human sEH by piperazine-containing 1-adamantyl ureas and the related physical properties

Compound	Structure	Human sEH ^a IC ₅₀ (μM)	Log <i>P</i> ^b	Mp (°C) ^c	Log <i>S</i> ^d	<i>S</i> ^e (mg/mL)
1a		1.37	2.09	77–78	3.65	7.46 ± 0.16
1b		93.8	0.77	56–58	5.47	4.44 ± 0.15
1c		12.6	1.30	69–70	4.67	8.11 ± 0.64
1d		100	0.54	66–68	5.56	8.52 ± 0.04
1e ^f		0.17 ± 0.01	2.77	114	2.27	1.69 ± 0.44

^aThe IC₅₀ values were determined by a recently developed sensitive fluorescence-based assay on human sEH. ²²

^blog *P* (octanol/water partition coefficients) calculated by Crippen's method by using CS ChemDraw 6.0 version.

^cMp, melting point.

^dSolubility in water. It was calculated according to the following equation suggested by Banerjee et al.²⁴: log *P* = 6.5–0.89 (log *S*)-0.015 mp, where mp is melting point.

^eExperimentally obtained solubility in sodium phosphate buffer (0.1 M, pH 7.4) at 25 ± 1.0 °C.²³ Results are means ± SD of two independent experiments.

^fThe compound was reported previously¹⁷ and used here as a reference. Its activity was measured by an NEPC (4-nitrophenyl-*trans*-2,3-epoxy-3-phenylpropyl carbonate) yellow water assay,²⁵ which provides a relatively higher IC₅₀ values compared to the fluorescence-based assay.