Published in final edited form as:

Medchemcomm. 2012 March 1; (3): 379–384. doi:10.1039/C2MD00288D.

Structure-based optimization of the piperazino-containing 1,3-disubstituted ureas affording sub-nanomolar inhibitors of soluble epoxide hydrolase

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Abstract

The inhibition of the soluble epoxide hydrolase (sEH) is a promising new therapy in the treatment of hypertension, inflammation and other cardiovascular disorders. Piperazino functionality as the tertiary pharmacophore remarkably improved the drug-like profile of the 1,3-disubstituted urea sEH inhibitors. However, the potency was more dependent on the overall best balance of the hydrophilicity and lipophilicity. Based on the sEH-inhibitor complex structure, further structural optimization on the piperazino-containing 1,3-disubstituted urea scaffold was conducted for an improved potency. The 1-adamantylacetamide and *para*-phenylcarbonyl group were identified to be an optimal primary pharmacophore and secondary pharmacophore motif, respectively, generating sub-nanomolar sEH inhibitors with favorable water solubility.

Keywords

soluble epoxide hydrolase inhibitor; piperazine; EETs; 1,3-disubstituted urea; 1-adamantylacetamide

In recent years, soluble epoxide hydrolase (sEH) has drawn particular interest in the medicinal community for its potential application in the treatment of cardiovascular diseases.¹ The sEH belongs to the α/β hydrolase fold family of enzymes and are involved in the metabolism of endogenously derived fatty acid epoxides and other lipid epoxides.² The epoxyeicosatrienoic acids (EETs), as the primary metabolites of cytochrome P450 epoxygenases of arachidonic acid,³ are known to regulate blood pressure and inflammation.^{4,5} In addition, the EETs have vascular protective effects such as suppression of reactive oxygen species following hypoxia-reoxygenation,⁶ attenuation of vascular

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smooth muscle migration,⁷ and enhancement of a fibrinolytic pathway.⁸ However, the hydrolysis of EETs to the dihydroxy epoxyeicosatrienoic acids (DHETs) by sEH often leads to reductions in these biological activities.⁹ Therefore, stabilizing the *in vivo* concentration of EETs through pharmacological intervention by sEH inhibitors is a novel and potentially therapeutic avenue to treat hypertension, inflammation, and other cardiovascular disorders.¹⁰

So far an impressive number of sEH inhibitors have been reported,¹¹ among which the 1,3-disubstituted ureas displayed significant anti-hypertension and anti-inflammatory effects through inhibition of EET hydrolysis in several cellular and animal models (representative examples seen in Fig. 1).^{1,12,13} However, the urea-based inhibitors often suffer from high melting point, poor solubility and stability, which hinders their pharmacological use *in vivo*. The incorporation of a substituted piperazino group as the secondary or tertiary pharmacophore into the 1-adamantyl-urea scaffold was proved to be an efficient solution (exemplified by compounds **1** and **2** in Fig. 1).^{14,15} However, the potency was observed to be sacrificed more or less when the polarity of the molecule was improved for the gain of solubility.^{14,15} Therefore, an overall best balance of the hydrophilicity and lipophilicity of the molecule was required. This observation initiated our further exploration on the primary and secondary pharmacophores on the piperazino-containing 1,3-disubstituted urea scaffold for an optimal matching. For this purpose, the newly discovered potent sEH inhibitor **2** was chosen as the lead compound (Fig. 1).¹⁵

To guide a rational lead optimization, a docking model of **2** in human sEH was first established on the basis of the X-ray crystallographic structure of the previously reported protein-inhibitor complex (Fig. 2).¹⁶ Besides the anticipated hydrogen bond interactions between the urea N-H and Asp333 and between the urea carbonyl and Tyr381/Tyr465, an interesting π -stacking interaction was identified between the secondary pharmacophore phenyloxy and the phenyl ring of Trp381. According to the binding mode, the location of the 1-(methylsulfonyl)piperazino moiety and the adamantyl group seemed tunable for the two functionalities to stretch out into the catalytic domain and gain additional interactions with the key residues. Therefore, the structural optimization on the piperazino-containing sEH inhibitors was conducted around the urea nucleus, including the exploration on the right-hand linker between the primary urea pharmacophore and the tertiary piperazino pharmacophore as well as the left-hand linker between the primary urea pharmacophore and the adamantyl group, as described in Figure 3. The phenyl butyl ether functionality, i.e. the secondary pharmacophore and its linker with the tertiary piperazine pharmacophore, was replaced with *m/p*-phenylcarbonyl, 8-propyloxy-5-quinoliny and 4-(4-methylpiperidin-1-yl)-4-oxobutanacyl, respectively, to investigate the distance, orientation, rigidity and aromatic features (Fig. 3, series **3–6**). Furthermore, the piperazino group was examined as the secondary pharmacophore by merging into the urea functionality (Fig. 3, series **7**). On the other hand, the 1-adamantyl urea was changed into 1-adamantylacetamide (Fig. 3, series **8**) to increase the flexibility and solubility of the molecule.

Schemes 1 and 2 outline the syntheses of the 1-adamantan-1-yl-3-substituted ureas having different secondary pharmacophore and linker between the urea group and the piperazino group (compounds **3–6a–d**). The piperazino group was *N*⁴-substituted with various acyl groups, located on the right-hand side terminus. These 1-adamantan-1-yl-3-(piperazin-1-

yl)aryl/alkyl ureas were readily synthesized by coupling 1-adamantyl isocyanate with various amines in CH₂Cl₂.

As shown in Scheme 1, the preparation of various amines was commenced with commercially available *m*- or *p*-nitrobenzoic acid and *tert*-butyl piperazine-1-carboxylate. The amidation product *tert*-butyl 4-(3/4-nitrobenzoyl)piperazine-1-carboxylate (**9m**, **p**) was reduced by hydrogen followed by condensation with 1-adamantyl isocyanate furnishing the *N*, *N'*-disubstituted ureas (**3–4c**). Further deprotection and acetylation/sulfonylation on the *N*⁴ atom of the piperazino ring yielded the corresponding *N*⁴-unsubstituted piperazine-containing compounds (**3–4a**) and the *N*⁴-acetyl/sulfonyl protected derivatives (**3–4b–d**), respectively. Other urea compounds (**5–6a–c**) were synthesized analogously, but through a different sequence. For example, the quinolin-8-yl-bridged ureas **5b–c** were synthesized by employing *tert*-butyl 4-(3-((5-aminoquinolin-8-yl)oxy)propyl)piperazine-1-carboxylate as the amine component to react with 1-adamantyl isocyanate, then varying the *N*⁴-substituent on the piperazine ring of the resulting 1-adamantyl-urea. However, the 4-methylpiperidin-1-yl-bridged ureas **6a–c** were synthesized via a different strategy. The 1-adamantyl urea was first formed to serve as the starting material, then the treatment with succinic anhydride yielded 4-(4-((3-adamantan-1-yl)ureido)methyl)piperidin-1-yl)-4-oxobutanoic acid **12**. Further amidation by substituted piperazines afforded the desired products **6a–c**.

1-Adamantanylacetamide compounds **8b–d** were synthesized from 1-adamantaneacetic acid and the correspond amines. As depicted in Scheme 2, the coupling in the presence of HOBt and EDCI followed by sequential deprotection and acylation afforded the target compounds **8b–d** in good yields.

The effect of the secondary pharmacophore and the linker on enzyme inhibition and physical properties was investigated. The biological activities of these ureas were summarized in Table 1. Obviously, the distance and functionality between the primary and the tertiary pharmacophores played an important role in the inhibitory potency against human sEH enzyme. When the flexible phenyloxy alkyl chain was replaced with the rigid benzoyl moiety, the orientation of the urea primary pharmacophore and the tertiary piperazine pharmacophore bridged by the benzoyl linker became vital to the interaction with the enzyme, i.e. the *para*-substituted pattern (**4a–d**) generally showed superior activity and solubility over the corresponding *meta*-substituted analogs (**3a–d**). More significantly, the *N*⁴-*tert*-butyloxy carbonyl substituted piperazine containing *para*-substituted urea **4c** was more potent than the lead compound **2** by a factor of 2-fold with acceptable water solubility. As indicated previously,¹⁵ the *N*⁴-piperazine substituent can adjust the overall hydrophobicity and polarity of the molecule to achieve an optimal balance between the potency and the physical properties of the sEH inhibitor. For the rigid benzoyl bridged urea series, the *tert*-butyloxy carbonyl substituent was more favorable binding than the methylsulfonyl substitution (**3c** vs **3d**, **4c** vs **4d**), probably due to its more hydrophobic nature resulting in an appropriate overall CLogP value (around 5). Undoubtedly, the unsubstituted piperazine containing analogs displayed weak activity but the best water solubility owing to its high polarity (**3a**, **4a**, **6a**).

On the other hand, the incorporation of the pyridine into the phenyl core ring exerted a different effect on the binding potency (**5b–c** compared to **15b–c**), which was dependent on the *N*⁴-piperazine substituent. When the *N*⁴-piperazine was substituted by acetyl group, the 8-propoxyquinolin-5-yl bridged urea (**5b**, IC₅₀ = 142 nM) was almost 43-fold less potent than the corresponding 4-propyloxyphen-1-yl bridged analog (**15b**, IC₅₀ = 3.3 nM), while the *N*⁴-*tert*butyloxycarbonyl substituted piperazine containing 8-propoxyquinolin-5-yl bridged urea was 2-fold more potent than the 4-propyloxyphen-1-yl bridged counterpart (**5c**, IC₅₀ = 6.8 nM vs **15c**, IC₅₀ = 14.6 nM). The reason might be related with the overall balance of the lipophilicity and the polarity of the molecule. Accordingly, the aliphatic piperidinylcarbonyl or piperazinyl replacement for the phenyloxy secondary pharmacophore (**6a–c**, **7a–c**) caused a substantial drop in the inhibitory activity regardless of the *N*⁴-piperazine substituent, indicating the importance of the aromatic moiety and the appropriate lipophilic nature of the core region. The resulting molecules (**5–6**)**a–c** possessed high hydrophilicity (indicated by the low ClogP value) which was disfavored for binding to the hydrophobic pocket of the sEH enzyme.

Interestingly, the left-hand side alteration with more flexibility turned out beneficial for the binding. The replacement of the 1-adamantyl urea with 1-adamantanylacetamide resulted in a general enhancement in the inhibitory activity (**8b–c**, IC₅₀ = 0.4~0.6 nM) relative to the lead compound (**2**, IC₅₀ = 1.0 nM). As expected, the more flexible 1-adamantanylacetamide gained more interaction with the protein. Although these amide-based sEH inhibitors possessed desired melting point, the water solubility was reduced. However, the 1-adamantanylacetamide as primary pharmacophore provided a new scaffold for the potent sEH inhibitor design.

To better understand the molecular basis for the improved potency of the *para*-benzoyl urea and 1-adamantanylacetamide analogues, two representative compounds (**4d** and **8d**)¹⁸ were docked into the structure of human sEH previously reported to be cocrystallized with compound 4-(3-cyclohexyluriedo)butyric acid (PDB code: 1ZD3)¹⁶ (Figure 4a, b for docking studies). Compounds **4d** and **8d** were docked into the catalytic site using the program Autodock^{19,20} assuming the same binding mode and exhibiting the same hydrogen bonding interaction pattern with the enzyme as observed for the 4-(3-cyclohexyluriedo)butyric acid. The lowest energy conformation was adopted as the final binding conformation.

As shown previously, the active site in human sEH is formed by a channel in the protein between two large, solvent accessible pockets, with the active site tyrosine and aspartic residues encircling the ligand.¹⁶ The costructure of **4d** (Figure 4a) reveals the hydrophobic adamantyl moiety was buried in the middle of the active site of sEH, with the carbonyl oxygen of the urea directed toward the two tyrosines and the urea N-H directed toward the aspartic acid in the active site. Interestingly, the structure shows the phenyl ring formed a π -stacking interaction with the imidazole ring of the His523 residue. Furthermore, the piperazine ring resides well to position the sulfonyl oxygens in hydrogen bonding distance to Trp524 and Arg408 residues, which may further contribute to the inhibitor's good binding affinity.

For the potent 1-adamantylacetamide inhibitor **8d**, besides the common hydrogen bond interactions between the amide N-H and Asp333 and between the amide carbonyl and Tyr381/Tyr465, and the similar π -stacking interaction between the phenyloxy and the phenyl ring of Tyr381, the adamantyl group resides deep into the active site to form an additional π - σ interaction with the indole ring of Trp334. Moreover, the piperazino nitrogen is ionized to form electronic interactions with the carboxyl oxygens of Asp528 and Asp495, which may further stabilize the binding conformation.

In conclusion, based on the docking model of the potent piperazino-containing urea inhibitor in human sEH, we successfully conducted the structural optimization around the urea nucleus by stretching the primary pharmacophore and restricting the tertiary pharmacophore. The 1-adamantylacetamide and *para*-phenylcarbonyl group were identified to be an optimal primary pharmacophore and secondary pharmacophore motif, respectively, generating sub-nanomolar sEH inhibitors with favorable water solubility. The improved binding potency was nicely rationalized by the molecular modeling established on the X-ray crystal structure of the sEH enzyme, providing superior scaffold for the potent sEH inhibitor design and development.

Acknowledgments

National Natural Science Foundation of China (81021062, 20872154 and 81072527) is greatly appreciated for the financial supports.

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18. **4d**: pale yellow powder, Mp 112–114°C; ^1H NMR (300 MHz, CDCl_3) δ 1.68 (br s, 6H), 2.00 (br s, 6H), 2.08 (br s, 3H), 2.81 (s, 3H), 3.22 (br s, 4H), 3.70 (br s, 4H), 5.12 (s, 1H), 7.17–7.24 (m, 4H); EI-MS m/z : 460 (M^+). **8d**: pale yellow powder, Mp 122–124°C; ^1H NMR (300 MHz, CDCl_3) δ 1.68–1.82 (m, 16H), 1.99 (br s, 3H), 2.08 (s, 2H), 2.46 (m, 2H), 2.54–2.58 (m, 4H), 2.77 (s, 3H), 3.23–3.26 (m, 4H), 3.99 (t, 2H, $J = 5.7$ Hz), 6.63 (dd, 1H, $J_1 = 6.3$ Hz, $J_2 = 1.8$ Hz), 6.84 (dd, 1H, $J_1 = 5.4$ Hz, $J_2 = 2.1$ Hz), 7.00 (s, 1H), 7.18 (t, 1H, $J = 8.4$ Hz), 7.41 (t, 1H, $J = 2.4$ Hz); EI-MS m/z : 503 (M^+).
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Figure 1.

The representative urea-based sEH inhibitors

t-AUCB, *trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid; **APAU**, *N*-(1-acetylpiperidin-4-yl)-*N'*-(adamant-1-yl)urea; **AMCU**, 1-adamantan-1-yl-3-(4-(3-morpholinopropoxy)cyclohexyl)urea; **CPTU**, 1-(1-(cyclopropanecarbonyl)piperidin-4-yl)-3-(4-(trifluoromethoxy)phenyl)urea

Figure 2. Minimized docking model of lead compound **2** (purple) in human sEH (the key residues within the binding pocket were indicated in green). The dashed lines illustrate atom pairs within hydrogen bond distance. The 3-D picture was prepared by Accelrys Discovery Studio. The model is based on the X-ray crystallographic structure of 1ZD3 (PDB code): human sEH complexed with 4-(3-cyclohexyluriedo)butyric acid.¹⁶

Figure 3.

The structural variations around the urea nucleus of the 1,3-disubstituted urea sEH inhibitors functionalized with piperazine as the tertiary pharmacophore

a. sEH Inhibitor 4d

b. sEH Inhibitor 8d

Figure 4.

Putative binding modes of the optimized analogs **4d** (a) and **8d** (b) in human sEH, predicted by the molecular modeling. The 3-D picture was prepared by Accelrys Discovery Studio, while the 2-D picture was drawn by Molecular Operating Environment.

Scheme 1. Reagents and conditions

a) *tert*-butyl piperazine-1-carboxylate, HOBt, EDCI, DIPEA, CH₂Cl₂, 86%–94%; b) H₂, 10% Pd/C, MeOH, rt, 99%; c) adamantyl isocyanate, CH₂Cl₂, 0 °C to rt, 66%–75%; d) TFA, CH₂Cl₂, 0 °C to rt, 66%–80%; e) (CH₃CO)₂O, CH₂Cl₂ (for the preparation of **3–6b**), or methanesulfonyl chloride, Et₃N, CH₂Cl₂ (for the preparation of **3–4d**), or succinic anhydride, Et₃N, CH₂Cl₂ (for the preparation of **12**), 54%–98%; f) *tert*-butyl piperazine-1-carboxylate, Et₃N, THF, reflux, 28%.

Scheme 2. Reagents and conditions

a) 1-adamantaneacetic acid, HOBT, EDCI, Et₃N, CH₂Cl₂, rt, 14%; b) TFA, CH₂Cl₂, 0°C to rt, 83%; c) (CH₃CO)₂O, CH₂Cl₂ (for the preparation of **8b**), 68%; or methanesulfonyl chloride, Et₃N, CH₂Cl₂ (for the preparation of **8d**), 90%.

Table 1

Inhibition of human sEH and physical properties of piperazine-containing sEH inhibitors with variation on the secondary or primary pharmacophore domain

Compd.	Structure	R	Mp ^a (°C)	S ^b (mg/mL)	CLogP ^c	IC ₅₀ ^d (nM)
3a		H	138–140	1.15	2.93	133.4
3b			140–144	0.89	2.52	46.3
3c			114–117	0.21	4.91	7.3
3d			112–114	0.13	2.94	41.8
4a		H	143–145	1.96	2.93	67.1
4b			118–121	0.53	2.52	9.0
4c			130–132	0.50	4.91	0.5
4d			112–114	0.86	2.94	1.0

Compd.	Structure	R	Mp ^a (°C)	S ^b (mg/mL)	CLogP ^c	IC ₅₀ ^d (nM)
5b			130–132	0.13	4.62	142
5c			105–107	0.15	7.01	6.8
6a		H	Oil	8.00	1.74	77.3
6b			ND	ND ^e	1.33	44.7
6c			100–102	0.47	3.73	18.8
7a		H	> 200	ND	2.39	100000
7b			182–185	ND	1.99	321
7c			190–192	ND	4.38	490
7e		CH ₃	175–178	ND	2.97	63.7
8b			Oil	0.16	5.14	0.6

Compd.	Structure	R	Mp ^a (°C)	S ^b (mg/mL)	CLogP ^c	IC ₅₀ ^d (nM)
8c			54–57	0.18	7.53	0.4
8d			122–124	0.17	5.56	0.5
2f			63–65	2.97	4.28	1.0
15b ^f			170–172	0.32	3.86	3.3
15e ^f			92–94	ND	6.25	14.6

^aMp, melting point;

^bExperimentally obtained solubility in sodium phosphate buffer (0.1 M, pH 7.4) at 25±1.0°C.¹⁴ Results are means of two independent experiments.

^cThe IC₅₀ values were averages of three replicates, determined by a sensitive fluorescent assay on human sEH.¹⁷ The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.¹⁷

^dCLogP, calculated LogP (octanol/water partition coefficients) predicted by Crippen's method by using CS ChemDraw 6.0 version.

^eND: not determined.

^fThe reference compounds **2** and **15b**, **c** were cited from our previous work.¹⁵