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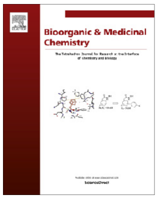

Structure-based optimization of cyclopropyl urea derivatives as potent soluble epoxide hydrolase inhibitors for potential decrease of renal injury without hypotensive action

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ABSTRACT

Epoxyeicosatrienoic acids (EETs) are known to have beneficial pharmacological effects on various cardiovascular events. However, EETs are biologically metabolized by soluble epoxide hydrolase (sEH) to less active metabolites. In our search for potent sEH inhibitors, we optimized a series of cyclopropyl urea derivatives and identified compound **38** as a potent sEH inhibitor with minimal CYP inhibition and good oral absorption in rats. Administration of **38** to DOCA-salt rats suppressed urinary albumin and MCP-1 excretion without affecting systolic blood pressure.

1. Introduction

Epoxyeicosatrienoic acids (EETs) are produced through oxidation of arachidonic acid by CYP 2C and CYP 2J in four distinct regioisomers (5,6-, 8,9-, 11,12-, and 14,15-) each of which is known to play important roles[1] in BK channel, PPAR α and PPAR γ activation[2–4] as well as NF-κB inhibition,[5] which can provide anti-hypertensive and cardiovascular protective effects.[6,7] In addition, it has recently been reported that EETs are involved in pain modulation[8] and psychosis.[9] However, EETs are biologically metabolized via several pathways by soluble epoxide hydrolase (sEH)[10] into dihydroepoxyeicosatrienoic acids (DHETs), which are considered to be less active due to their high solubility and rapid clearance.[11] Maintaining high blood EETs levels by inhibition of sEH has therefore attracted considerable attention as a promising strategy for the treatment of certain cardiovascular events. In fact, DCU[12] and ADU,[13] two sEH inhibitors, have been shown to have anti-hyportensive[14] and vasoprotection[15] effects in rat. Accordingly, various sEH inhibitors have been explored, and several potent agents have been discovered (Fig. 1).[16] For instance, AR9281, which is now under clinical investigation, has been shown to have anti-hypertensive effect in animal models of hypertension and anti-diabetic effect in DIO mouse model.[17]

X-ray co-crystal structure analysis sEH inhibitors binding to the catalytic domain of sEH[18] revealed two critical interactions; one between the hydrogen binding network of Tyr388, Tyr465 and oxygen at the carbonyl group of the ligand and the other between the carboxylic acid of Asp333 and NH group of the ligand.[19] These findings indicate that amide or urea moieties are key functional groups to a tight interaction with sEH catalytic domain (Fig. 2).

Using a combination of virtual screening and fragment-based drug discovery, we have previously reported compound **1** as a promising hit sEH inhibitor (Fig. 3).[20] After parallel synthesis to modify the left hand part of **1**, the obtained substituted piperidine **2**, containing an oxadiazole ring, turned out to have superior sEH inhibitory activity (human sEH IC_{50}: 6.1 nM, rat sEH IC_{50}: 8.3 nM) with preferable lead-like characteristics in terms of molecular size and lipophilicity.[21] However, further optimization of this chemotype was halted due to its inhibition of CYP 2C8 and 2C19. Meanwhile, the phenoxy piperidine **3**, which showed moderate sEH inhibitory activity, had significantly reduced ligand efficiency due to lipophilicity of the Ph group and lower sEH inhibitory activity than **2**. Brief structure–activity relationships of the substituents on the piperidine in hand led to the assumption that lead optimization would be successfully achieved by introducing a carboxylic acid into the inhibitor. In fact, a polar carboxylic acid

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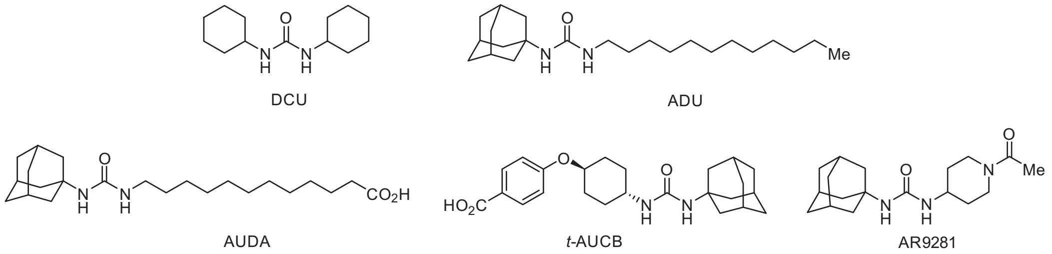

Figure 1. Previously reported representative sEH inhibitors.

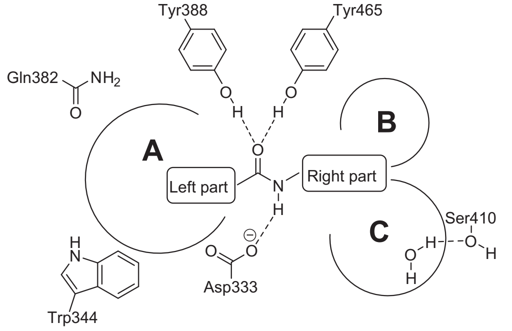

Figure 2. Pharmacophore model of sEH catalytic domain with a proposed interaction network. A, B and C parts are the spacious regions of ligand binding.

functionality would help improve lead-like properties by decreasing lipophilicity of the molecular. Herein we report our investigation of the structure–activity relationship of the left hand part of compound **2** aimed at improving lead compounds ADMET properties using a polar carboxylic acid and cyclopropyl urea moiety.

2. Results and discussion

The desired compounds were synthesized as shown in Scheme 1. Reaction of the commercially available substituted piperidine **4** with *trans*-2-phenylcyclopropyl isocyanate (racemic) under basic conditions provided the urea compounds **3**–**32**. The carboxylic acid compounds **33**–**38** were obtained by standard hydrolysis of the esters **27**–**32**. The tetrazole compound **39** was synthesized by tetrazolylation of the 4-cyanophenoxy compound **26** (Scheme 2).

To understand compound **2** structure–activity relationship around the left hand part, which includes a limited chemical space around the oxadiazole ring, we replaced the 3-(5-isopropyl)oxadiazole with several aryloxy groups (Table 1). Introduction of a phenoxy group (compound **3**) resulted in moderate human and rat sEH inhibitory activity (human sEH IC$_{50}$: 18.0 nM, rat sEH IC$_{50}$: 18.3 nM), while a pyridyloxy group (compounds **5**–**7**) weakened sEH inhibitory activity regardless of nitric positions. Increasing the number of nitrogen atoms by use of a pyrimidyloxy or a pyrazyloxy (compounds **8** and **9**) reduced even more sEH inhibitory activity. On the other hand, introduction of the bulky diphenylmethyloxy group afforded compound **10**, which showed species-dependent sEH inhibitory activity (human sEH IC$_{50}$: 27.1 nM, rat sEH IC$_{50}$: 5.2 nM).

Next, we introduced various substituents at the phenoxy group of compound **3** (Table 2). A Cl, Me, or CF$_3$ group at the 2-, 3-, or 4-position resulted in increased sEH inhibitory activity in the order of 2- > 3- > 4- of the oxy linker (compounds **11**–**19**). Especially, substitution with any of the three groups at the 2-position produced compounds with stronger sEH inhibition than the lead compound **2**. On the other hand, use of OMe or Ph groups at the 2-, 3-, or 4-position afforded compounds with increased sEH inhibitory activity in the order of 3- > 4- > 2-(compounds **20**–**25**). As some of the synthesized compounds had disappointingly bad water solubility (e.g. compound **14** solubility was <0.0002 mg/ml at pH 7.4), we introduced a polar carboxylic acid group or a bioisostere into the phenoxy group.

As expected, introduction of a carboxylic acid improved compounds aqueous solubility to a more than 0.15 mg/ml at pH 7.4 (Table 3A). However, substitution at the 4-position of the phenoxy unit provided compounds with relatively weak human and rat sEH inhibitory activity (compounds **33**–**36**). Although conversion into a tetrazole remarkably improved both human and rat sEH inhibitory activity (compound **39**, human sEH IC$_{50}$: 3.3 nM, rat sEH IC$_{50}$: 6.6 nM), the obtained compound strongly inhibited CYP 2C8 (IC50: 0.8 μM). Next, using SAR information in Table 2, a 2-Chlorine atom was introduced to the prepared 4-carboxylic acid com-

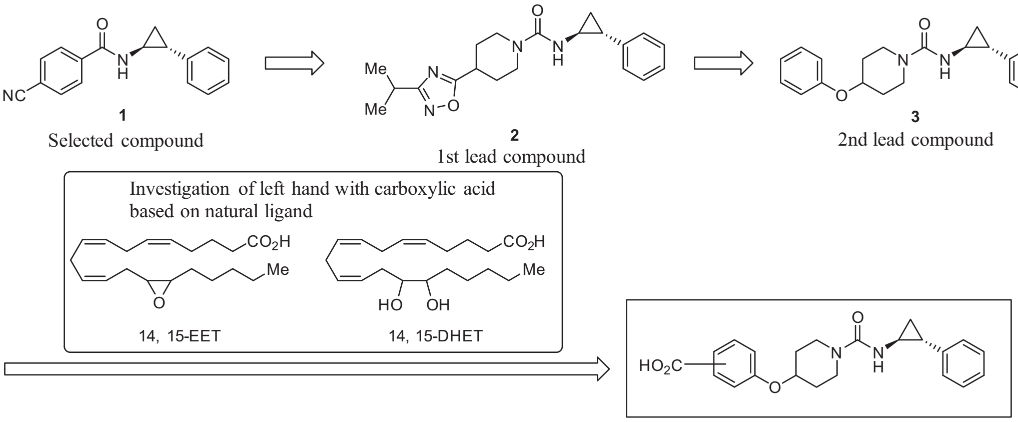

Figure 3. Summary of our strategy to optimize compound **2**.