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Three-dimensional rational approach to the discovery of potent substituted cyclopropyl urea soluble epoxide hydrolase inhibitors

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ABSTRACT

We have previously reported a series of cyclopropyl urea derivatives as potent orally available soluble epoxide hydrolase (sEH) inhibitors. Here, we designed and synthesized three substituted cyclopropane derivatives that occupy all available pockets of sEH catalytic domain. Compound **14** with a diphenyl substituted cyclopropyl moiety showed good sEH inhibitory activity. Co-crystal structure of this compound and human sEH hydrolase catalytic domain revealed enzyme pockets occupied by the phenoxypiperidine part and the diphenyl cyclopropyl moiety. Furthermore, investigation of the phenoxypiperidine part of compound **14** resulted in the discovery of compound **19**, which showed potent sEH inhibitory activity (sub-nM sEH IC_{50} values).

Soluble epoxide hydrolase (sEH) is a cytosolic enzyme expressed ubiquitously in several organs, particularly in the kidney and the liver.[1] The principal role of sEH involves endogenous metabolism of epoxyeicosatrienoic acids (EETs) to dihydroeicosatrienoic acids (DHETs).[2] EETs, which consist of four distinct regioisomers (5,6-, 8,9- , 11,12- and 14,15-), are known to play vital roles[3] in BK channel, PPAR α and PPAR γ activation[4–6] as well as NF-κB inhibition,[7] which is believed to be inherently associated with anti-hypertensive and cardiovascular protective effects.[8,9] Therefore, regulation of blood EETs levels by inhibition of sEH has attracted considerable attention as a promising strategy for the treatment of certain cardiovascular events. Based on these findings, a number of drug discovery research programs have, over the past decade, focused on the development of sEH inhibitors.[10] Although Loch et al. have reported that ADU, an sEH inhibitor, produces notable anti-hypertensive effect in DOCA-salt rat,[11] non-significant decrease in blood pressure in several animal models treated with sEH inhibitors has also been reported.[12] On the other hand, it has been also suggested that EETs are associated with pain modulation[13] and psychosis.[14] Hammock co-workers have reported EETs have an effect on suppress the tonic component of seizure related excitability through modulating GABA activity.[15] In addition, the sEH inhibitor, 4-phenylchalcone oxide (4-PCO) treatment showed significantly reduced neuronal death and improve memory function after cardiac arrest/cardiopulmonary resuscitation.[16] Thus, the effectiveness of EETs and sEH inhibitor for the treatment of central nervous system disorders are expected recently.

We have recently reported that carboxylic acid containing cyclopropyl urea based sEH inhibitors not only have potent inhibitory activity toward sEH, but can also reduce renal injury without hypotensive action in DOCA-salt rats.[17] In this Letter, we attempted structure based optimization of the cyclopropane moiety using X-ray crystallography of sEH hydrolase domain and compound **1**, a similar with our reported compounds (Fig. 1).

After intense investigation, we successfully obtained the co-crystal structure of compound **1** with human sEH catalytic domain (Fig. 2). As reported with various sEH inhibitors,[20,21] compound **1** bound to sEH through two critical hydrogen binding networks; (1) the hydroxyl group of Tyr 383, Tyr 466 and oxygen at the carbonyl and (2) the carboxylic acid of Asp 335 NH group of compound **1**. Meanwhile in our molecule, the 4-phenoxypiperidine part and the cyclopropylphenyl part were placed in the enzyme pocket, that is, in A and B parts, respectively, leaving the C part of the pocket around the geminal hydrogen position of the cyclopropyl part vacant. Compound **2**[18] and **3**[19] reported by Boehringer Ingelheim research group (Fig. 1) suggested to fill all regions of the catalytic site of sEH (A, B and C). Compound **2** occupied the diphenyl group of the right part, while Compound **3** occupied the *ortho*- and *para*- dichloro substituted benzene of the right part. We believe that these compounds demonstrate excellent

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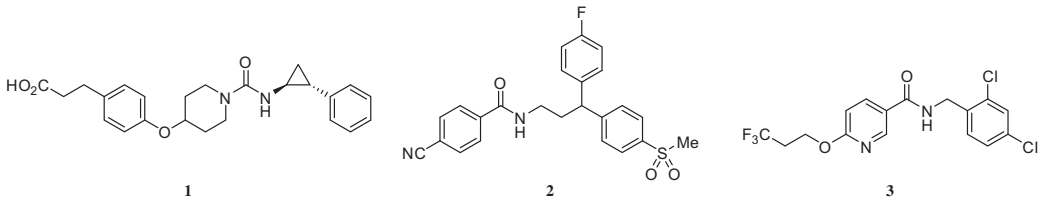


1 2 3

Figure 1. Structures of the carboxylic acid containing cyclopropyl urea based sEH inhibitor **1** and two benzamide based sEH inhibitors reported by Boehringer Ingelheim research group (compounds **2**[18] and **3**[19]).

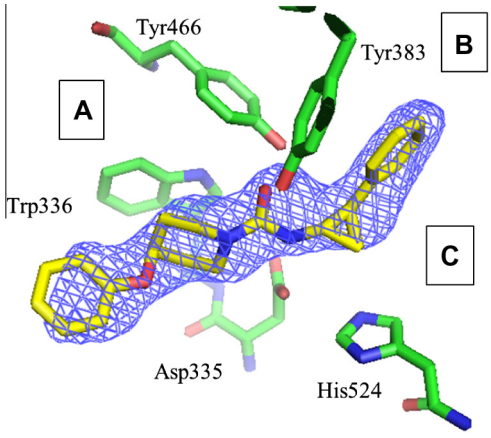


Figure 2. X-ray co-crystal structure of compound **1** with human sEH hydrolase domain. 2Fo-Fc map contoured at 1 sigma. The electron density of propionic acid moiety could not be identified. Absolute configuration of **1** in the crystal structure could not be determined by electron density. Here it is represented by one enantiomer (PDB code 4X6Y).

human sEH inhibitory activity because they effectively take advantage of all available binding regions. We therefore hypothesized that introduction of a substituent at the geminal hydrogen position of compound **1**, that is, in *trans* configuration at the urea part and *cis* configuration at the phenyl group of the cyclopropyl ring would increase hydrophobic interaction with the C part.

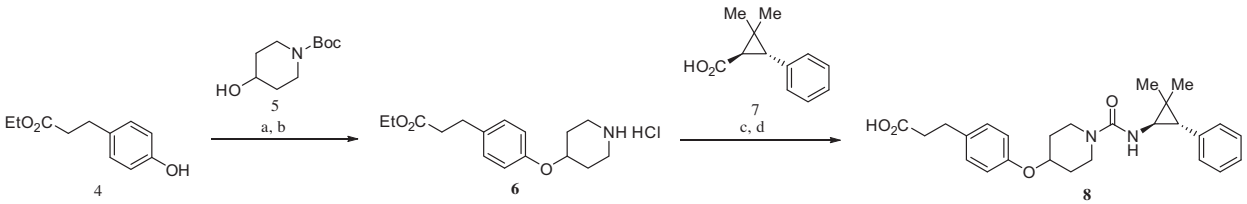
To date, a number of cyclopropane-based conformations,[22] such as amino acids,[23] peptides[24] and nucleosides[25] have been appended to molecular mimetics. In addition, increase of bioactivity based on conformational restriction by cyclopropane have been reported.[26] However, the use of three substituted cyclopropane as a base for three-dimensional conformational regulation has not been explored. Based on these findings, we considered introducing substituents at the cyclopropyl moiety of compound **1** to increase sEH inhibitory activity.

The general synthetic procedures for urea formation of the substituted cyclopropyl carboxylic acids and phenoxypiperidines are shown in Scheme 1. The key intermediate **6** was prepared by Mitsunobu reaction of the commercially available **4** and **5**, followed by deprotection of the Boc group under usual conditions. On the other hand, the substituted cyclopropyl carboxylic acid **7** was synthesized as previously reported.[27] Compound **7** was converted to an isocyanate by Curtius rearrangement with DPPA and the HCl salt of compound **6** added to the reaction mixture to form the urea linkage. Finally, hydrolysis of the ester group under standard basic conditions provided the target compound **8**.

Other substituted cyclopropyl carboxylic acids were prepared as shown in Scheme 2. Stereoselective synthesis of the desired cyclopropane configuration was achieved by coupling reaction of the ethyl diazoacetate and $ClCuP(OiPr)_3$ using *trans* or *cis*-β-methylstyrene, or *cis*-stylbene as starting substrate.[28] The substituted cyclopropyl ester could not be hydrolyzed under basic conditions probably due to the steric hindrance of the substituents on the cyclopropyl ring. To overcome low nucleophilicity, TMSOK, which is used without H_2O, was applied to the reaction to give key carboxylic acids. The prepared carboxylic acids were used for Curtius rearrangement according to the procedure described in Scheme 1. Meanwhile, the non-carboxylic acids **16**–**19** were obtained by Curtius rearrangement of intermediate **11c** followed by corresponding amines.

Structure–activity relationship (SAR) study of the substituted cyclopropyl moiety is summarized in Table 1. Introduction of the geminal Me group **8** markedly decreased sEH inhibitory activity (h-sEH IC_{50} >100 nM, r-sEH IC_{50} >100 nM).[29] On the other hand, a mono Me group placed in the *cis* configuration together with a phenyl group (compound **12**) resulted in good sEH inhibitory activity (h-sEH IC_{50} 12.0 nM, r-sEH IC_{50} 11.7 nM) without species (human and rat) difference. Meanwhile, a mono Me group placed in the *trans* configuration provided weak inhibitory activity (h-sEH IC_{50} 75.7 nM, r-sEH IC_{50} >100 nM) (**13**). Put together, these findings show that a *cis* configuration together with a phenyl group on the cyclopropyl ring lead to increased sEH inhibitory activity as we speculated in the X-ray analysis. Considering the C part in the catalytic domain, a bulky substituent seemed to be appropriate. In fact, introduction of a phenyl group further enhanced sEH inhibitory activity more than 8-fold as compared to **1** (compound **14**, h-sEH IC_{50} 2.3 nM, r-sEH IC_{50} 3.9 nM). To achieve additional interaction with Ser 412 and Phe 497 through a combined water, a 4-fluoro-phenyl group was used instead of a phenyl group. However, this modification led to no beneficial effect on the inhibitory activity (compound **15**, h-sEH IC_{50} 2.1 nM, r-sEH IC_{50} 3.2 nM).

We succeed in analyzing the X-ray co-crystal structure of compound **14** and the hydrolase domain of human sEH (Fig. 3).



Scheme 1. Synthesis of compound **8**. Reagents and conditions: (a) DIAD, PPh_3, **5**, THF, room temperature; (b) 4 M HCl/dioxane, CH_2Cl_2, room temperature; (c) **7**, DPPA, TEA, toluene, 100 °C, then **6**, room temperature; (d) 2 M NaOH aq/THF/MeOH = 1:1:1, room temperature.