STUDIES ON STRUCTURE-ACTIVITY RELATIONSHIPS AND PREPARATION OF PHYSIOLOGICALLY ACTIVE SPIROCYCLIC DIAMINE-BASED UREAS

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Contents

Abbreviation

Boc	*tert*-butoxycarbonyl
Bn	benzyl
Bu	butyl
DIPEA	*N*-*N*-diisopropylethylamine
DMF	*N*-*N*-dimethylformamide
EETs	Epoxyeicosatrienoic acids
Et	ethyl
GBM	glomerular basement membrane
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HETE	hydroxyeicosatetraenoic acid
HPETE	hydroperoxyeicosatetraenoic acid
LDA	lithium diisopropylamide
Me	methyl
Ms	mesyl
PDB	Protein Data Bank
PG	prostaglandin
SAR	structure–activity relationship
SPR	structure–property relationship
sEH	Soluble epoxide hydrolase
SHR	spontaneously hypertensive rat
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TX	thromboxane

Chapter 1

General introduction

1) Arachidonate cascade as a therapeutic target

Arachidnic acid is derived from a phospholipid and converted into a variety of fatty acids through the arachidonate cascade (Figure 1-1). Some of these fatty acids are involved in the control of body functions such as inflammation. Because of the important role of these fatty acids, enzymes and receptors involved in the arachidonate cascade have been identified as therapeutic targets. Cyclooxygenase has a key role in the production of prostaglandins. Some cyclooxygenase inhibitors (e.g., aspirin and diclofenac) are marketed as therapeutic agents for treatment of pain and inflammation. Leukotrienes are derived from metabolism of arachidonic acid by lipoxygenase (LOX). Leukotriene receptor antagonists (e.g., pranlukast) have been used for treatment of asthma and seasonal allergies.

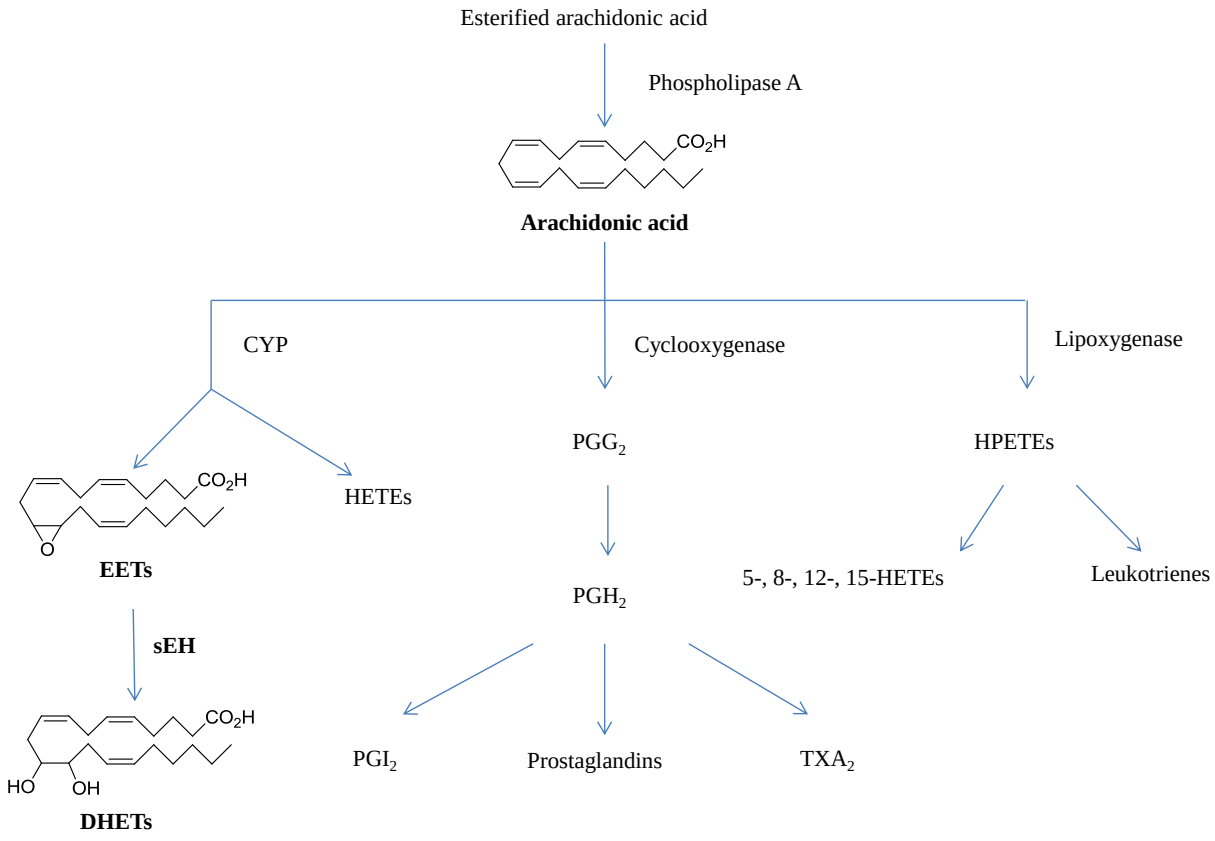

Figure 1-1. Major pathways of the arachidonate cascade.

2) Soluble epoxide hydrolase

Epoxyeicosatrienoic acids (EETs) are produced by epoxidation of arachidonic acid by CYP2J and CYP2C of the cytochrome P450 (CYP) superfamily (Figure 1-1). EETs exhibit physiologically beneficial effects such as vasodilatation, vasoprotection, and anti-inflammation. Soluble epoxide hydrolase (sEH), which is located in mainly liver, kidney, and vascular tissue[1,2], converts EETs to dihydroxyeicosatrienoic acids (DHETs) [3]. sEH inhibition produces effects expected from an increase in EETs level.

3) Treatment of hypertension with sEH inhibitors

Renal sEH expression is upregulated in angiotensin II hypertensive rat[4] and spontaneously hypertensive rat (SHR),[5] but not in normotensive rat. Several preclinical studies have indicated that sEH inhibitors significantly reduce blood pressure in angiotensin II hypertensive rat and SHR, but have no effect in normotensive rat. These findings suggest that sEH inhibitors have potential use for treating hypertension without causing any hypotensive side effects.

4) Treatment of chronic kidney disease with sEH inhibitors

Chronic kidney disease is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health. People with end-stage kidney disease (ESKD) (known as stage 5 chronic kidney disease) need to be treated with dialysis or translant.[6] These treatments lower patients' quality of life, so chronic kidney disease should be treated from an early stage. The standard of care for patients with chronic kidney disease is administration of blood pressure-lowering drugs such as angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists, both of which slow down the progression of chronic kidney disease. However, no drugs can cure or reverse the disease. According to a recent report,[7a] sEH in proximal tubular cells is upregulated in chronic proteinuric kidney diseases. According to that study, 1-(1-methylsulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)urea reduces long-term elevated serum creatinine levels, interstitial inflammation, fibrosis, and α-smooth muscle actin expression in adriamycin-induced

nephropathic mice. These findings suggest that sEH inhibitors have potential use in treating chronic proteinuric kidney diseases. [7]

5) sEH inhibitors

The catalytic pocket of sEH consists of Tyr381, Tyr465, and Asp333, which are responsible for the enzymatic activity. X-ray crystal structures have been reported for sEH inhibitors bound to sEH (e.g., Protein Data Bank (PDB) code: 1VJ5). The structures suggested that amide or urea derivatives may bind to the catalytic pocket via hydrogen bonds between the amide or urea carbonyl oxygen and Tyr381 or Tyr465, and between the urea or amide NH and Asp333 (Figure 1-2).[8]

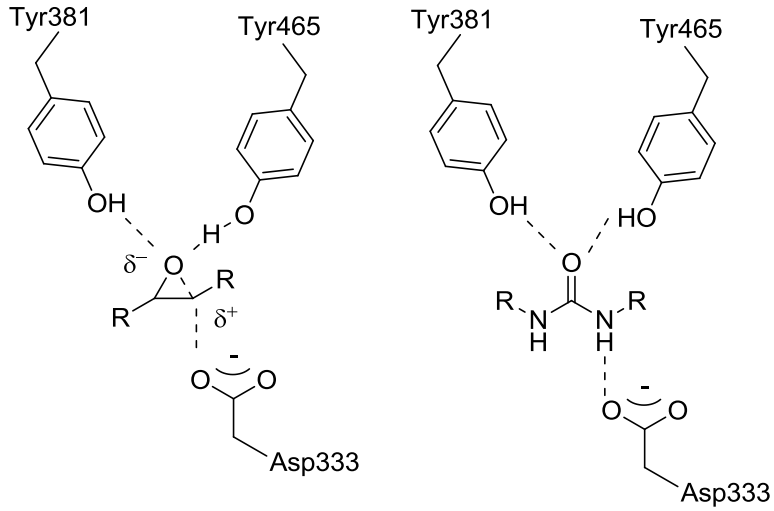

Figure 1-2. Transition state for epoxide opening catalyzed by sEH. (left) and general binding mode of a dialkylurea to sEH (right)

Morisseau et al. reported that 1,3-disubstituted ureas with a adamantan-1-yl group are potent sEH inhibitors (an example is AUDA; Figure 1-3).[9] In subsequent studies, a number of sEH inhibitors containing amide, urea, and isoxazole moieties have been identified.[10,11] The sEH inhibitor AR9281 (Figure 1-3) has good oral bioavailability, antihypertensive effects in SHR, and antidiabetic effects in diet-induced obesity mouse. AR9281 was advanced to human clinical trial involving obese patients with stage 1 hypertension and impaired glucose tolerance. A variety

of cyclic amine-based trisubstituted ureas that are potent sEH inhibitors have also been reported (Figure 1-3).[12] Although a number of sEH inhibitors have potent *in vitro* activity, only a few sEH inhibitors have *in vivo* efficacy. Oral administration of some sEH inhibitors failed to reduce blood pressure in SHR but elevated EET levels in the kidney.[12] Thus, development of sEH inhibitors with *in vivo* potency was thought to be a challenging task.

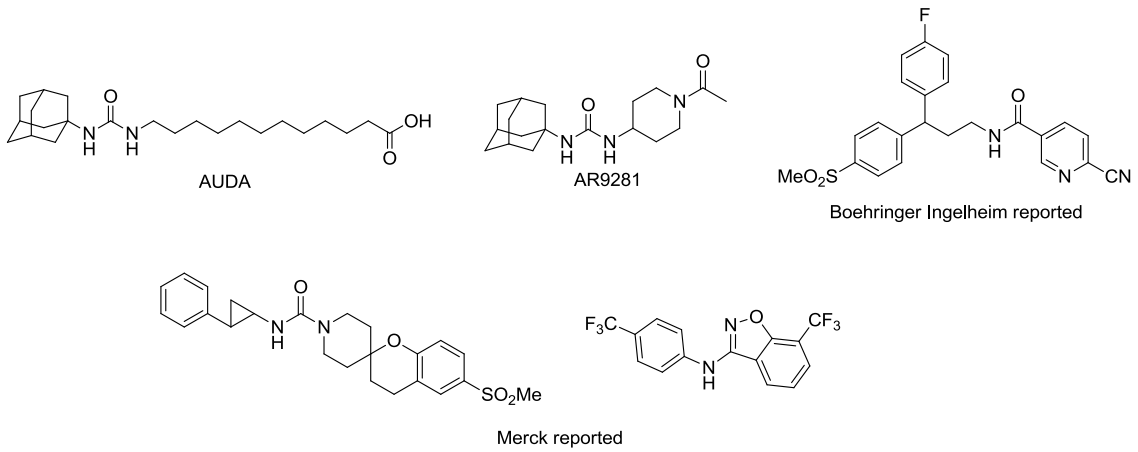

Figure 1-3. Examples of reported sEH inhibitors.

6) Goal of this research

The *in vivo* efficacy of sEH inhibitors remains unclear; nevertheless, considering the *in vivo* potency of some sEH inhibitors (e.g., AR9281, Figure 1-2) and the beneficial effects described above, our laboratory was motivated to embark on a search for novel sEH inhibitors. The goal of this research was to identify orally active sEH inhibitors for the treatment of hypertension and chronic kidney disease.

7) Plan for developing orally active sEH inhibitors

In addition to referring to the reports mentioned above, I planned to utilize docking studies for the design of novel sEH inhibitors. A docking study is a powerful tool in medicinal chemistry, and predicts the binding orientation of a ligand to a target protein. Docking studies help medicinal chemists design new ligands with higher affinity to the target protein. If a docking study shows that around the ligand binding pocket of the target protein there are any amino acid residues that might form hydrogen bonds with a ligand, installing hydrogen bond donors

or acceptors in the parent ligand could improve the binding affinity. X-ray crystal structures have been reported for human sEH (e.g., PDB code: 1VJ5) and mouse sEH (e.g., PDB code: 1EK1) bound to sEH inhibitors. However, there is no X-ray crystal structure of rat sEH. A docking study using these reported X-ray crystal structures could facilitate the discovery of sEH inhibitors.

In the development of orally active small molecule drugs, scientists are often faced with difficult problems. Some problems in drug discovery relate to *in vitro* biological activity and pharmacokinetics. With respect to *in vitro* biological activity, it is often necessary to identify compounds that have activity toward a target protein in both humans and animals, because it is necessary to extrapolate efficacy for human diseases from biological tests using animal models of target diseases. Pharmacokinetic profiles, which relate to efficacy, are described with parameters such as oral bioavailability, clearance (CL), biological half-life ($t_{1/2}$), maximum drug concentration (C_{max}), area under the blood concentration-time curve (AUC), and volume of distribution (V_d). The oral bioavailability of a compound depends on its lipid membrane permeability, stability to CYP-mediated metabolism, solubility in gastric and intestinal fluids and carrier-mediated transport. Lipinski's rule of five describes the likelihood that a drug will be orally active.[13] This rule says that, generally speaking, an orally active drug has no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular mass of less than 500 Da, and an octanol-water partition coefficient log P no greater than 5. Because it would be impractical to evaluate the pharmacokinetic profiles and *in vivo* efficacies of all the compounds that couple possibly be synthesized, I planned to search for compounds that satisfy Lipinski's rule and that have *in vitro* inhibitory activity against human, mouse, and rat sEH; solubility in biologically relevant medium; and stability to CYP-mediated metabolism. The pharmacokinetic profiles and *in vivo* efficacies of the identified compounds were then evaluated.

Following this plan, the search for novel sEH inhibitors was started. Details of particular studies are given in Chapter 2-5.

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Chapter 2

Discovery of 2,8-diazaspiro[4.5]decane-based trisubstituted ureas as highly potent soluble epoxide hydrolase inhibitors and orally active drug candidates for the treatment of hypertension

Abstract: The identification of 2,8-diazaspiro[4.5]decane-based trisubstituted urea derivatives as highly potent soluble epoxide hydrolase (sEH) inhibitors and orally active agents for treating hypertension is described. Docking studies using human and mouse sEH X-ray crystal structures revealed steric hindrance around the side chain of Phe406 of mouse sEH. The trifluoromethyl moiety (**II-21**) was replaced with a trifluoromethoxy moiety (**II-22**) to prevent steric clash, and improved mouse sEH inhibitory activity was observed. The oral administration of **II-22**, **II-30**, and **II-47** at a dose of 30 mg/kg reduced blood pressure in spontaneously hypertensive rat, but had little effect on blood pressure in normotensive rat.

Introduction

Motivated by the expected beneficial effects of sEH inhibitors as described in Chapter 1, I began to search for sEH inhibitors to serve as agents for treating hypertension without causing hypotensive side effects.

Design

The linear structures of epoxyeicosatrienoic acids and the reported X-ray crystal structural analysis of sEH inhibitors bound to sEH suggest an elongated cylindrical hydrophobic pocket around the catalytic site of sEH. Amide and urea derivatives with linear hydrophobic substituent were expected to be favored as ligands for catalytic pocket of sEH. Recently, there has been much interest in spirocyclic diamine scaffolds in medicinal chemistry,[1] leading to studies on the synthesis and structure–activity relationship (SAR) of diazaspirocyclic compounds. The reason why I focused on these scaffolds is that their rigidity may contribute to efficient interaction with target proteins. Because of this feature, the diazaspiro-based ureas for use as sEH inhibitors were designed (Figure 2-1). I aimed to design the sEH inhibitors following Lipinski's rule (see Chapter 1-6) in order to discover orally active drugs.

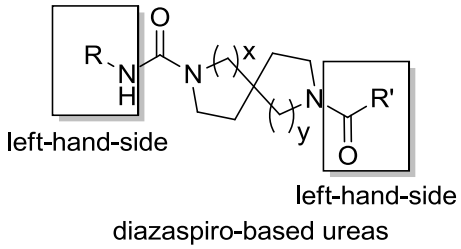
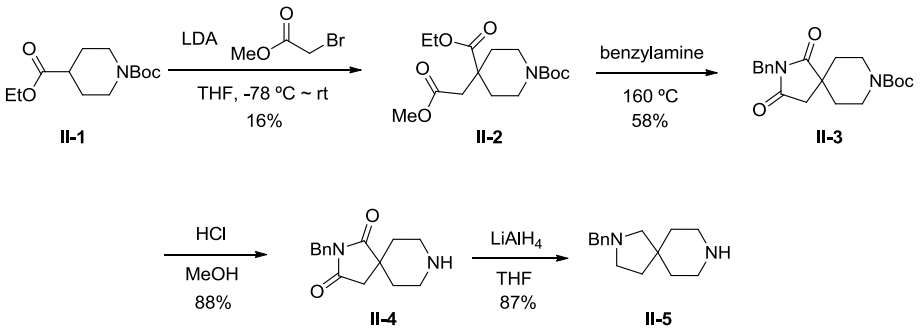


Figure 2-1. Example of spirocyclic diamine scaffold and sEH inhibitor design.

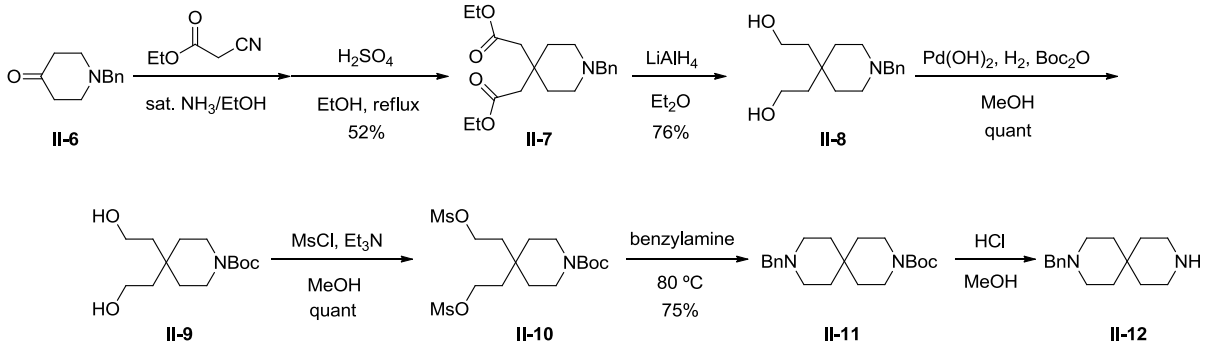
Chemistry

The starting materials **II-5** and **II-12** were synthesized according to the literature.[2] The synthesis of

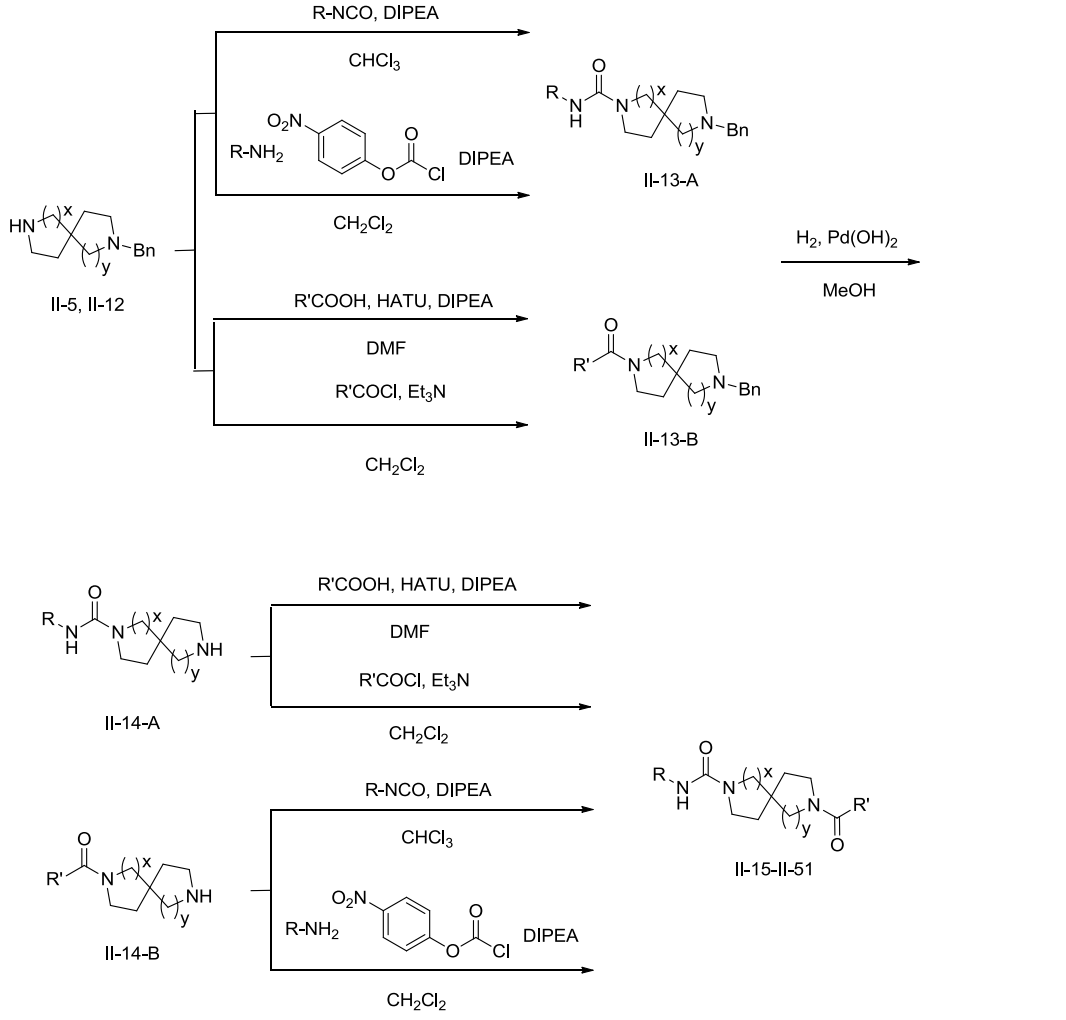
2,8-diazaspiro[4.5]decane scaffold **II-5** is shown in Scheme 2-1. Ester **II-1** was deprotonated with LDA and alkylated. **II-2** was treated with benzylamine affording **II-3**. Deprotection of the Boc group using HCl led to **II-4**. Reduction of imide **II-4** was carried out with $LiAlH_4$ providing **II-5**. The synthesis of 3,9-diazaspiro[5.5]undecane scaffold **II-12** is shown in Scheme 2-2. **II-6** was treated with ethyl 2-cyanoacetate in basic media, and then the product was treated with H_2SO_4 to obtain diester **II-7**. **II-8** was obtained by reducing diester **II-7** with $LiAlH_4$. Removing the benzyl group and protecting an amine with a Boc group through $Pd(OH)_2$-catalyzed hydrogenation in the presence of $(Boc)_2O$ afforded **II-9**. Dimesylation of **II-9** was carried out, and **II-10** was obtained. **II-10** was treated with benzylamine to obtain **II-11**. Removal of the Boc group in **II-11** with HCl gave **II-12**. The general procedure for synthesizing the series of target compounds is shown in Scheme 2-3. **II-13-A** was formed by treating **II-5** or **II-12** with isocyanate or with carbamate prepared from *p*-nitrophenyl chloroformate and amine. Removal of the benzyl protecting groups by $Pd(OH)_2$-catalyzed hydrogenation provided **II-14-A**. Then, condensation with carboxylic acid afforded the target compounds. Otherwise, **II-5** or **II-12** was condensed with carboxylic acid to produce **II-13-B**. Removal of the benzyl protecting groups by $Pd(OH)_2$-catalyzed hydrogenation provided **II-14-B**. Then, the target compounds were formed by treating **II-14-B** with isocyanate or with carbamate prepared from *p*-nitrophenyl chloroformate and amine.



Scheme 2-1. Synthesis of 2,8-diazaspiro[4.5]decane scaffold **II-5**.



Scheme 2-2. Synthesis of 3,9-diazaspiro[5.5]undecane scaffold **II-12**.



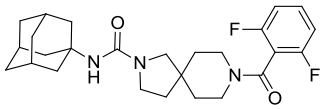
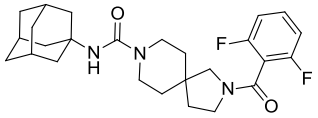
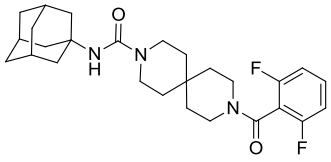
Scheme 2-3. Synthesis of diazaspiro-based ureas (x = 1, 2; y = 1, 2).

Results and discussion

The diazaspiro-based urea derivatives listed in Tables 2-1 to 2-6 had 1 or 2 hydrogen bond donors, 4-9 hydrogen bond acceptors, molecular mass of 424-501 Da, and an octanol-water partition coefficient log P of 0.75-4.15. These derivatives almost satisfied Lipinski's rule.

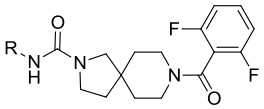
SAR studies of various diazaspiro scaffolds were performed (see Table 2-1). The adamantan-1-yl group was selected as the left-hand substituent while 2,6-difluorobenzoyl was as the right-hand-side substituent. The highest inhibitory activity against human sEH was observed for the 2,8-diazaspiro[4.5]decane framework (**II-15**), which was therefore utilized in subsequent SAR and structure–property relationship (SPR) studies.

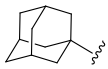
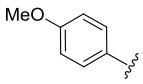
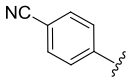
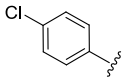
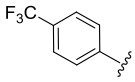
Table 2-1. SARs of diazaspiro scaffolds.

Structure	Compound	Human sEH IC_{50} (nM)
	**II-15**	175.6
	**II-16**	>200
	**II-17**	466.3

SAR and SPR studies of the left-hand side were performed (Table 2-2). Replacing the adamantan-1-yl group of **II-15** with 4-methoxyphenyl (**II-18**) slightly improved inhibitory activity against human sEH. The 4-cyano derivative **II-19** was a more potent human sEH inhibitor than **II-18**, but the inhibitory activity of **II-19** against mouse sEH was lower. 4-Chloro derivative **II-20** showed high inhibitory activity against human sEH, but only modest inhibitory activity against mouse sEH. Introduction of a trifluoromethyl moiety at the 4-position led to excellent human sEH inhibitory activity (**II-21**).

Table 2-2. SARs and SPRs of the left-hand side with 4-substituted phenyl.



R	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1[b] (μg/mL)	Solubility JP2[c] (μg/mL)	Microsomal stability[a]
	**II-15**	175.6	N.D.	N.D.	80	80	0.485
	**II-18**	151.6	731.0	N.D.	N.D.	N.D.	N.D.
	**II-19**	51.7	1445.0	N.D.	77	77	0.022
	**II-20**	6.7	489.0	N.D.	71	64	N.D.
	**II-21**	0.3	228.0	6.1	23	21	0.043

[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia

[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia

N.D.: Not determined.

However, a major challenge that arose was dealing with the approximately 800-fold difference between the mouse and human sEH inhibitory activities. For this reason, I attempted to design derivatives with sufficient mouse sEH inhibitory activity for evaluating efficacy in a mouse disease model. Also, to elucidate the difference in inhibitory activity between human and mouse sEHs for **II-21**, docking studies of human and mouse sEHs with **II-21** were performed using X-ray crystal structures (Figure 2-2). The results revealed that the trifluoromethyl

moiety of **II-21** sterically clashes with the side chain of Phe406 in mouse sEH, which is replaced with Leu406 in human sEH, and the oxygen atom of the urea moiety of **II-21** is bound to Tyr381, Tyr465, and Asp333 in the catalytic pocket of both sEHs. This steric hindrance caused the lower binding affinity of **II-21** to mouse sEH than to human sEH. To resolve this steric hindrance, I envisioned inserting an oxygen atom between the aromatic ring and the trifluoromethyl functional group to keep the trifluoromethyl moiety of **II-21** apart from the side chain of Phe406 in mouse sEH and I designed **II-22**. A docking study of 4-trifluoromethoxy derivative **II-22** with mouse sEH suggested that **II-22** should bind to the catalytic pocket of mouse sEH without steric hindrance (Figure 2-3). In line with my expectation, **II-22** was found to have sufficient inhibitory activity in human, rat, and mouse sEHs (Table 2-3). On the other hand, the insertion of the single methylene linkage resulted in decreased inhibitory activities of both human and mouse sEHs (**II-23**).

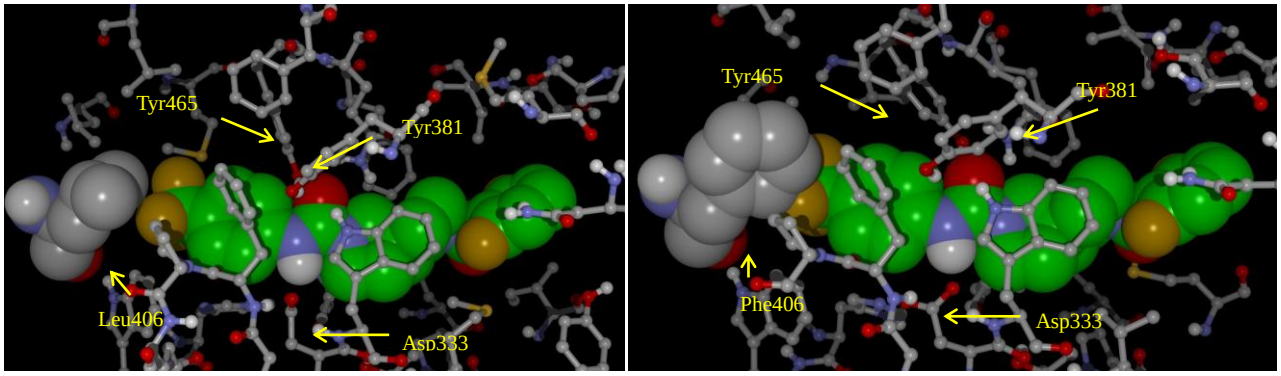
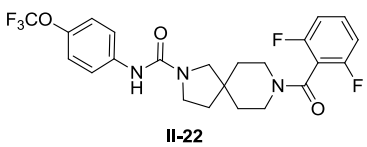


Figure 2-2. Docking studies of human sEH (left; PDB code: 1VJ5) and mouse sEH (right; PDB code: 1EK1) with **II-21** (depicted by space filling model). The residues Leu406 in human sEH and Phe406 in mouse sEH are shown as gray space filling models.

a)



II-22

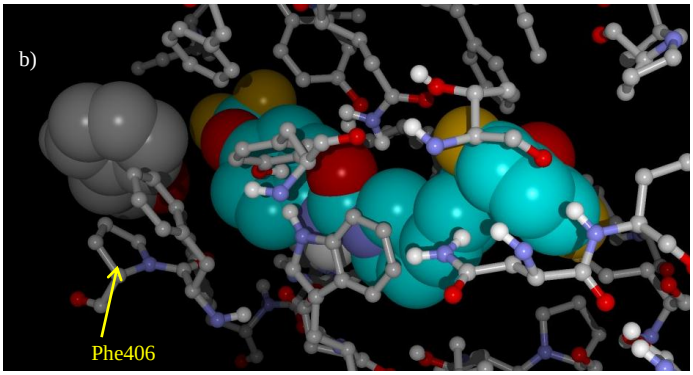
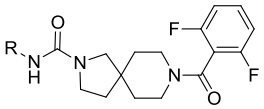
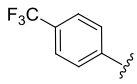
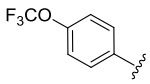
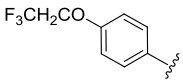


Figure 2-3. a) Structure of **II-22**. b) Docking studies of murine sEH (PDB code: 1EK1) with **II-22** (depicted by space filling model). The residue Phe406 was highlighted by space filling model in gray.

Table 2-3. SARs and SPRs of the left-hand side with 4-substituted phenyl.



R	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1[b] (μg/mL)	Solubility JP2[c] (μg/mL)	Microsomal stability[a]
	**II-21**	0.3	228.0	6.1	23	21	0.043
	**II-22**	0.4	25.7	4.5	24	21	0.000
	**II-23**	5.7	97.5	N.D.	60	54	0.003

[a] Units: mL/min/mg protein.

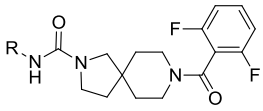
[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia

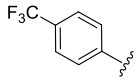
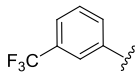
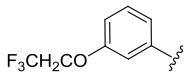
[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia

N.D.: Not determined.

I speculated that 3-substituted phenyl derivatives would not have the steric hindrance described above and designed **II-24** and **II-25** (see Table 2-4). The 3-substituted phenyl derivatives **II-24** and **II-25** were also examined. Compared with **II-21**, the 3-trifluoromethyl derivative **II-24** showed improved inhibitory activity against mouse sEH, suggesting that substitution at the 3-position alleviated steric hindrance, leading to the enhanced inhibitory activity. However, substitution with a bulky group (**II-25**) was found to be ineffective in alleviating the steric hindrance.

Table 2-4. SARs and SPRs of the left-hand side with 3-substituted phenyl.



R	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1[b] (μg/mL)	Solubility JP2[c] (μg/mL)	Microsomal stability[a]
	**II-21**	0.3	228.0	6.1	23	21	0.043
	**II-24**	16.1	72.7	N.D.	26	23	0.034
	**II-25**	35.3	439.0	N.D.	N.D.	N.D.	N.D.

[a] Units: mL/min/mg protein.

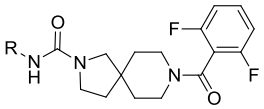
[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia

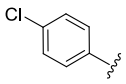
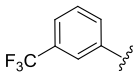
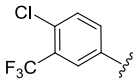
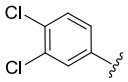
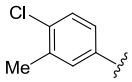
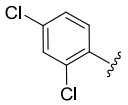
[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia

N.D.: Not determined.

Considering the study results in Table 2-4, I envisioned that substitution at the 3-position of **II-20** would enhance the murine sEH inhibitory activity of **II-20**. 4-Chloro derivatives with additional substituents at the 3-position (**II-26** to **II-28**) were also studied and found to exhibit inhibitory activity against mouse sEH which was approximately 8-fold that of 4-chloro **II-20**. In contrast, compounds with substituents at the 2-position (**II-29**) showed sEH inhibitory activity much lower than that of **II-20** (see Table 2-5). Thus, I speculate that the substituent at the 2-position affects the interaction between the urea moiety and the amino acid residues in the hydrolase catalytic pocket. From the above results, the derivative with a 4-trifluoromethoxyphenyl substituent in the left-hand moiety (**II-22**) was selected for *in vivo* studies. It showed good metabolic stability and tolerable solubility.

Table 2-5. SARs and SPRs of the left-hand side with 3,4-substituted phenyl.



R	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1[b] (μg/mL)	Solubility JP2[c] (μg/mL)	Microsomal stability[a]
	**II-20**	6.7	489.0	N.D.	71	64	N.D.
	**II-24**	16.1	72.7	N.D.	26	23	0.034
	**II-26**	1.1	25.2	N.D.	4	4	N.D.
	**II-27**	3.5	61.0	N.D.	8	7	0.069
	**II-28**	3.4	31.6	N.D.	24	21	0.079
	**II-29**	115.0	1112.0	N.D.	N.D.	N.D.	N.D.

[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia

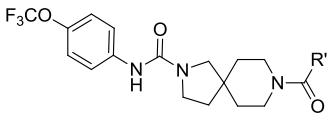
[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia

N.D.: Not determined.

Finally, the SAR and SPR of the right-hand side (see Table 2-6) were studied in order to improve the solubility of **II-22**. The solubility was slightly improved by altering the substituent of the phenyl ring on the right-hand side (**II-30** to **II-32**). The replacement with heteroaromatic rings (**II-33** to **II-39**) and alkyl groups (**II-40** to **II-51**) also

gave good solubility. In terms of sEH inhibitory activity, benzamides (**II-30** to **II-32**), heteroaromatic amides (**II-33** to **II-39**), and alkyl amides (**II-40** to **II-51**) were well tolerated for human and rat sEH, but this was not the case in mouse sEH. The comparison between **II-30** and **II-31** revealed that a hydroxy group introduced onto benzamide enhanced mouse sEH inhibitory activity. **II-35** showed potency for human sEH inhibition 573-fold that for mouse sEH inhibition. Compared with **II-22**, alkyl amides **II-42**, **II-49**, and **II-51** were more potent mouse sEH inhibitors, but were labile to CYP-mediated metabolism.

Table 2-6. SARs and SPRs of the right-hand side.



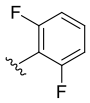
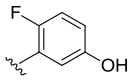
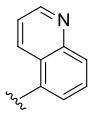
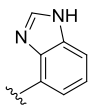
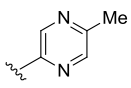
R’	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1 [b] (μg/mL)	Solubility JP2 [c] (μg/mL)	Microsomal stability[a]
	**II-22**	0.4	25.7	4.5	24	21	0.000
	**II-30**	0.7	40.6	5.3	56	47	0.021
	**II-31**	0.2	11.0	4.1	38	35	0.044
	**II-32**	0.3	N.D.	5.2	35	30	0.01
	**II-33**	0.2	64.5	6.6	94	58	0.057
	**II-34**	0.8	91.5	22.2	92	86	0.001
	**II-35**	0.4	229.0	11.9	87	86	0.008

Table 2-6. SARs and SPRs of the right-hand side.

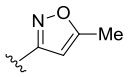
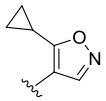
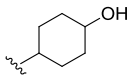
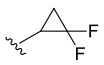
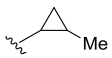
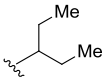
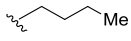
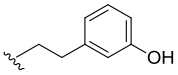
R'	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1 [b] (μg/mL)	Solubility JP2 [c] (μg/mL)	Microsomal stability[a]
	**II-36**	0.4	N.D.	5.3	82	80	0.058
	**II-37**	0.2	67.4	11.7	86	82	0.000
	**II-38**	0.5	40.6	N.D.	79	72	0.128
	**II-39**	0.9	N.D.	9.5	78	75	0.019
	**II-40**	2.8	108.0	N.D.	83	81	N.D.
	**II-41**	3.0	116.0	N.D.	85	85	N.D.
	**II-42**	0.7	21.5	9.0	64	60	0.314
	**II-43**	1.2	N.D.	22.4	87	86	0.020
	**II-44**	0.3	N.D.	4.7	88	88	0.007
	**II-45**	0.5	N.D.	15.8	77	78	0.019
	**II-46**	1.3	83.8	13	77	77	0.054
	**II-47**	0.6	N.D.	6.4	73	74	0.021

Table 2-6. SARs and SPRs of the right-hand side.

R'	Compound	Human sEH IC_{50}(nM)	Murine sEH IC_{50}(nM)	Rat sEH IC_{50}(nM)	Solubility JP1 [b] (μg/mL)	Solubility JP2 [c] (μg/mL)	Microsomal stability[a]
	**II-48**	0.6	65.5	9.4	75	75	0.015
	**II-49**	0.3	13.1	N.D.	79	78	0.089
	**II-50**	0.8	64.3	N.D.	74	75	0.195
	**II-51**	0.4	21.7	N.D.	37	33	0.483

[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia.

[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia.

N.D.: Not determined.

In the next study, I investigated the efficacy of **II-22**, **II-30**, and **II-47** at inducing a hypotensive effect in spontaneously hypertensive rat (SHR) and normotensive rat. These derivatives were selected based on other biological tests The oral administration of these compounds at a dose of 30 mg/kg reduced blood pressure in SHR (Figure 2-4, top), but had little effect on blood pressure in normotensive rat (Figure 2-4, bottom).

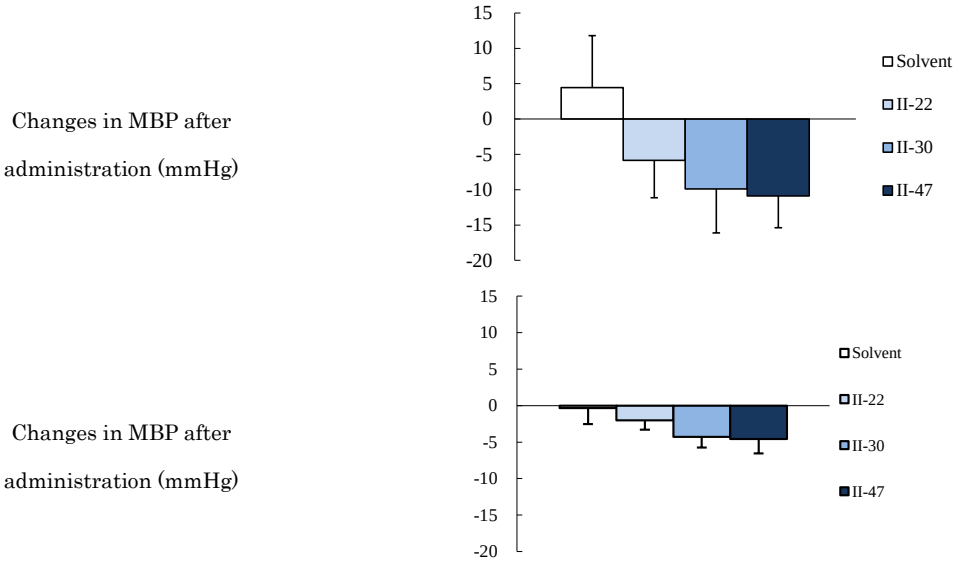


Figure 2-4. Hypotensive effect of **II-22**, **II-30**, and **II-47** in WKY rat. Mean blood pressure (MBP) change from baseline (mmHg) for **II-22**, **II-30**, and **II-47** (30 mg/kg, po) in SHR (top) and normotensive rat (bottom) at 6 h after administration. Solvent: methylcellulose/tween.

Note that no reduction in blood pressure has been observed in SHR after the administration of several sEH inhibitors.[3] The Doris group has reported that several haplotypes of SHR are insensitive to sEH inhibitors,[4] but the reason for the difference between their results and mine is currently unclear.

Summary

In summary, 2,8-diazaspiro[4.5]decane-based trisubstituted ureas were identified as highly potent sEH inhibitors and orally active agents for treating hypertension. In SAR studies on its left-hand side, the potent human sEH inhibitor **II-21** was found to be a weak mouse sEH inhibitor. Docking studies of human and mouse sEHs using X-ray crystal structures revealed steric hindrance around the side chain of Phe406 in mouse sEH with **II-21**. From the results of this study, I adopted a trifluoromethoxy moiety instead of a trifluoromethyl moiety in order to prevent such steric hindrance and, in this way, succeeded at improving the mouse sEH inhibitory activity. In terms of inhibitory activity, various substituents on the right-hand side were well tolerated in human and rat sEHs, and

the solubility of **II-22** was improved by changing the substituent on the right-hand side. Oral administration of **II-22**, **II-30**, and **II-47** at a dose of 30 mg/kg reduced blood pressure in SHR, but had little effect on blood pressure in the normotensive rat.

Experimental Section

General Information

All reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. ^{1}H NMR spectra were recorded on JNM-AL400 at 400 MHz and are referenced to an internal standard of tetramethylsilane (TMS, δ=0). Chemical shifts are given in ppm. Coupling constants (*J*) are given in Hz. Multiplicities are abbreviated as singlet (s), doublet (d), triplet (t), quartet (q), doublet - doublet (dd), multiplet (m), and broad (br). Mass spectra were recorded with electron-spray ionization (ESI) on a Waters ZQ-2000. Thin layer chromatography was performed using Merck Kieselgel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV-light at 254 nm and color reagents. Flash chromatography was performed using Yamazen HI-FLASH COLUMNS (Particle Size：40 μm). Solvents were removed by rotary evaporation.

1-*tert*-Butyl 4-ethyl 4-(2-methoxy-2-oxoethyl)piperidine-1,4-dicarboxylate (II-2).

Under argon atmosphere, *n*-BuLi (10.9 ml, 17.1 mmol, 1.57 M in-*n*-hexane) was added to a solution of diisopropylamine (2.44 ml, 17.1 mmol) in THF (30 ml) cooled at -78 °C. The solution was stirred at -78 °C for 30 min. A solution of 1-*tert*-butyl 4-ethyl piperidine-1,4-dicarboxylate (II-1) (4.0 g, 15.5 mmol) in THF (15 ml) was added, and the solution was stirred at -78 °C for 1 h. Methyl 2-bromoacetate (2.24 ml, 24.3 mmol) was added, and the solution was stirred at -78 °C for 1 h. The solution was warmed up to rt and stirred for 18 h. A saturated aqueous solution of NH_4Cl was added, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-2** in 16% yield.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.28 (3H, t, *J* = 6.8 Hz), 1.46 (9H, s), 1.50-1.56 (2H, m), 2.07-2.13 (2H, m), 2.61 (2H, brs), 3.13-3.22 (2H, m), 3.66 (3H, s), 3.68- 3.72 (2H, m), 4.21 (2H, q, *J* = 6.8 Hz).

MS (ESI) m/z 330 $[M+H]^+$.

***tert*-Butyl 2-benzyl-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylate (II-3).**

A mixture of 1-*tert*-butyl 4-ethyl 4-(2-methoxy-2-oxoethyl)piperidine-1,4-dicarboxylate (**II-2**) (817 mg, 2.48 mmol) and benzylamine (2.7 ml, 24.8 mmol) was stirred at 160 °C for 18 h. 3 M aqueous HCl was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-3** in 58% yield.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.46 (9H, m), 1.92-2.04 (2H, m), 2.58 (2H, m), 2.92-3.01 (2H, m), 4.01 (2H, brs), 4.64 (2H, s), 7.28-7.34 (5H, m).

MS (ESI) m/z 359 $[M+H]^+$.

2-Benzyl-2,8-diazaspiro[4.5]decane-1,3-dione (II-4).

To a solution of *tert*-butyl 2-benzyl-1,3-dioxo-2,8-diazaspiro[4.5]decane-8-carboxylate (**II-3**) (512 mg, 1.43 mmol) in methanol (4 ml) was added HCl in methanol (20%, 6 ml). The solution was stirred at rt for 4 h. The solution was neutralized with 1 M aqueous NaOH, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **II-4** in 88% yield.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.42-1.46 (2H, m), 1.93-2.01 (2H, m), 2.60 (2H, s), 2.65-2.71 (2H, m), 3.10-3.15 (2H, m), 4.64 (2H, s), 7.28-7.34 (5H, s).

MS (ESI) m/z 259 $[M+H]^+$.

2-Benzyl-2,8-diazaspiro[4.5]decane (II-5).

To a solution of 2-benzyl-2,8-diazaspiro[4.5]decane-1,3-dione (**II-4**) (280 mg, 1.084 mmol) in THF (5 ml) was added $LiAlH_4$ (206 mg, 5.42 mmol). The mixture was stirred at rt for 4 h. Water was added, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **II-5** in 87% yield.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.50-1.53 (4H, m), 1.62-1.66 (2H, m), 2.37 (2H, s), 2.58 (2H, t, J = 6.8 Hz), 2.74-2.78 (4H, m), 3.58 (2H, s), 7.30-7.38 (5H, m).

MS (ESI) m/z 231 $[M+H]^+$.

(2-Benzyl-2,8-diazaspiro[4.5]decan-8-yl)(2,6-difluorophenyl)methanone (II-13B1).

To a solution of 2-benzyl-2,8-diazaspiro[4.5]decane (120 mg, 0.52 mmol) and 2,6-difluorobenzoyl chloride (76 μl, 0.6 mmol) in dichloromethane (3 ml) was added DIPEA (145 μl, 1.04 mmol). The solution was stirred at rt for 2 h. Water was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-13B1** in 76% yield.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.55-1.72 (6H, m), 2.38 (1H, d, *J* = 9.2 Hz), 2.43 (1H, d, *J* = 9.2 Hz), 2.56-2.65 (2H, m), 3.20-3.24 (2H, m), 3.55 (1H, d, *J* = 13.6 Hz), 3.62 (1H, d, *J* = 13.6 Hz), 3.65-3.72 (1H, m), 3.77-3.82 (1H, m) , 6.91-6.95 (2H, m) , 7.22-7.36 (6H, m).

MS (ESI) m/z 371 $[M+H]^+$.

(2,6-Difluorophenyl)(2,8-diazaspiro[4.5]decan-8-yl)methanone (II-14B1).

To a solution of (2-benzyl-2,8-diazaspiro[4.5]decan-8-yl)(2,6-difluorophenyl)methanone (**II-13B1**) (147 mg, 0.04 mmol) in methanol (3 ml) was added 10% $Pd(OH)_2$ on carbon (45 mg). Under hydrogen atmosphere, the mixture was stirred at rt for 18 h and filtered through a pad of Celite. The solvent was removed under reduced pressure to afford **II-14B1** in quantitative yield.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.60-1.80 (4H, m), 1.95-2.02 (2H, m), 3.12-3.26 (1H, m), 3.29-3.34 (2H, m), 3.42-3.49 (3H, m), 3.76-3.84 (2H, m), 6.92-7.02 (2H, m), 7.34-7.38 (1H, m).

MS (ESI) m/z 281 $[M+H]^+$.

***N*-(Adamantan-1-yl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (II-15).**

To a solution of (2,6-difluorophenyl)(2,8-diazaspiro[4.5]decan-8-yl)methanone (50 mg, 0.18 mmol) and adamantan-1-yl isocyanate (40 mg, 0.21 mmol) in dichloromethane (1 ml) was added DIPEA (50 μl, 0.29

mmol). The solution was stirred at rt for 16 h. Water was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatograph y (ethyl acetate-*n*-hexane) to afford **II-15** in 81% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50-1.63 (2H, m), 1.64-1.75 (8H, m), 1.80-1.88 (2H, m), 1.98-2.01 (6H, m), 2.05-2.12 (3H, m), 3.20-3.42 (6H, m), 3.62-3.73 (1H, m), 3.90-3.99 (2H, m), 6.91-7.00 (2H, m), 7.26-7.41 (1H, m).

MS (ESI) m/z 458 $[M+H]^+$.

***N*-((adamantan-1-yl)-2-benzyl-2,8-diazaspiro[4.5]decane-8-carboxamide (II-13-A1).**

Starting from 2-benzyl-2,8-diazaspiro[4.5]decane (**II-5**) the title compound was obtained following the pr ocedure described for-*N*-(Adamantan-1-yl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**I I-15**) in 51% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.53-1.56 (4H, m), 1.62-1.68 (9H, m), 1.96-1.98 (6H, m), 2.06 (3H, brs), 2.37 (2H, s), 2.60 (2H, t, *J* = 6.8 Hz), 3.20-3.25 (4H, m), 3.58 (2H, s), 4.18 (1H, brs) , 7.23-7.31 (5H, m).

MS (ESI) m/z 408 $[M+H]^+$.

***N*-(adamantan-1-yl)-2-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-8-carboxamide (II-16).**

To a solution of *N*-((adamantan-1-yl)-2-benzyl-2,8-diazaspiro[4.5]decane-8-carboxamide (**II-13-A1**) (22 mg、0.054 mmol) in methanol (3 ml) was added 10% $Pd(OH)_2$ on carbon (22 mg). Under hydrogen atmosph ere, the solution was stirred at rt for 18 h and filtered through a pad of Celite. The solvent was remove d under reduced pressure. To a solution of the resulting residue and 2,6-difluorobenzoyl chloride (7.4 μl, 0.06 mmol) in dichloromethane (0.5 ml) was added DIPEA (14 μl, 0.08 mmol). The solution was stirre d at rt for 2 h. Water was added, and the aqueous layer was extracted with dichloromethane. The combi ned organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pr essure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to aff

ord **II-16** in 87% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52-1.62 (4H, m), 1.63-1.70 (6H, m), 1.80-1.90 (2H, m), 1.95-2.01 (6H, m), 2.05-2.11 (3H, m), 3.12 (2H, s), 3.15-3.25 (2H, m), 3.28-3.45 (4H, m), 3.58 (1H, s) , 3.75 (1H, t, *J* = 6.8 Hz), 4.08 and 4.12 (1H, brs), 6.92-6.98 (2H, m) , 7.33-7.38 (1H, m).

MS (ESI) m/z 458 $[M+H]^+$.

Diethyl 2,2'-(1-benzylpiperidine-4,4-diyl)diacetate (II-7).

1-Benzylpiperidin-4-one (10 g, 53 mmol) and ethyl 2-cyanoacetate (11.3 ml, 106 mmol) were dissolved to a saturated ethanol solution of ammonia, and the solution was stirred at 0 °C for 16 h. The solution was filtered, and the residue was dissolved to 50% aqueous H_2SO_4 (45 ml). The solution was refluxed for 3 days. The solution was evaporated under reduced pressure, and the residue was azeotroped with ethanol. To the residue ethanol (50 ml) was added, and the mixture was refluxed for 20 h. A saturated aqueous solution of Na_2CO_3 was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **II-7** in 52% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.24 (6H, t, *J* = 6.8 Hz), 1.68 (4H, t, *J* = 5.2 Hz), 2.45-2.49 (4H, m), 2.56 (4H, s), 3.51 (2H, s), 4.10 (4H, q, *J* = 6.8 Hz), 7.23-7.30 (5H, m).

MS (ESI) m/z 348 $[M+H]^+$.

2,2'-(1-benzylpiperidine-4,4-diyl)diethanol (II-8).

To a suspension of $LiAlH_4$ (2.1 g, 55 mmol) in diethyl ether (200 ml) cooled at -30 °C was added a solution of diethyl 2,2'-(1-benzylpiperidine-4,4-diyl)diacetate (**II-7**) (12.2 g, 35 mmol) in diethyl ether (25 ml). The mixture was stirred at rt for 16 h. 1 M aqueous NaOH was added, and the mixture was filtered through a pad of Celite. The aqueous layer was extracted with diethyl ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **II-8** in 76% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52 (4H, t, *J* = 5.6 Hz), 1.68 (4H, t, *J* = 6.8 Hz), 2.40-2.45 (4H, m), 3.51 (2H, s),

3.73 (4H, t *J* = 6.8 Hz), 7.24-7.32 (5H, m).

MS (ESI) m/z 264 $[M+H]^+$.

***tert*-Butyl 4,4-bis(2-hydroxyethyl)piperidine-1-carboxylate (II-9).**

To a solution of 2,2'-(1-benzylpiperidine-4,4-diyl)diethanol (**II-8**) (415 mg, 1.58 mmol) and di-*tert*-butyl dicarbonate in methanol (12 ml) was added 10% $Pd(OH)_2$ on carbon (45 mg). Under hydrogen atmosphere, the mixture was stirred at rt for 16 h and filtered through a pad of Celite. The solvent was removed under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-9** in quantitative yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43-1.46 (13H, m), 1.71 (4H, t, *J* = 6.4 Hz), 3.40 (4H, t, *J* = 5.6 Hz), 3.76 (4H, t, *J* = 6.4 Hz).

***tert*-Butyl 4,4-bis(2-((methylsulfonyl)oxy)ethyl)piperidine-1-carboxylate (II-10).**

To a solution of *tert*-butyl 4,4-bis(2-hydroxyethyl)piperidine-1-carboxylate (**II-9**) (431 mg, 1.58 mmol) and methanesulfonyl chloride (0.27 ml, 3.47 mmol) in dichloromethane (7 ml) was added triethylamine (0.6 ml, 4.34 mmol). The solution was stirred at -20 °C for 2 h. 1 M aqueous HCl was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-10** in quantitative yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46-1.49 (13H, m), 1.90 (4H, t, *J* = 6.8 Hz), 3.04 (6H, s), 3.42 (4H, t, *J* = 6.0 Hz), 4.32 (4H, t, *J* = 6.8 Hz).

***tert*-Butyl 9-benzyl-3,9-diazaspiro[5.5]undecane-3-carboxylate (II-11).**

A mixture of *tert*-butyl 4,4-bis(2-((methylsulfonyl)oxy)ethyl)piperidine-1-carboxylate (**II-10**) (300 mg, 0.70 mmol) and benzylamine (0.38ml, 3.49mmol) was stirred at 80 °C for 16 h. Water was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine,

dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by s ilica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-11** in 75% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38-1.43 (4H, m), 1.44 (9H, s), 1.49-1.52 (4H, m), 2.38-2.42 (4H, m), 3.30-3.38 (4H, s), 3.50 (2H, s), 7.24-7.26 (1H, m), 7.28-7.31 (4H, m).

MS (ESI) m/z 345 $[M+H]^+$.

(9-Benzyl-3,9-diazaspiro[5.5]undecan-3-yl)(2,6-difluorophenyl)methanone (II-13-B2).

To a solution of *tert*-butyl 9-benzyl-3,9-diazaspiro[5.5]undecane-3-carboxylate (**II-11**) (181 mg, 0.53 mmol) in methanol (5 ml) was added HCl in methanol (20%, 5 ml). The solution was stirred at rt for 2 h. The solvent was removed under reduced pressure. To a solution of the resulting residue and 2,6-difluorobenzoyl chloride (66.1 μl, 0.53 mmol) in dichloromethane (1 ml) was added triethylamine (0.11 ml, 0.79 mmol). The solution was stirred at rt for 2 h. Water was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-13-B2** 91% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (2H, t, *J* = 6.0 Hz), 1.51-1.61 (6H, m), 2.40 (4H, t, *J* = 5.6 Hz), 3.25 (2H, t, *J* = 5.6 Hz), 3.50 (2H, s), 3.76 (2H, t, *J* = 6.4 Hz), 6.91-6.94 (2H, m), 7.23-7.36 (6H, m).

MS (ESI) m/z 385 $[M+H]^+$.

***N*-(Adamantan-1-yl)-9-(2,6-difluorobenzoyl)-3,9-diazaspiro[5.5]undecane-3-carboxamide (II-17).**

To a solution of (9-benzyl-3,9-diazaspiro[5.5]undecan-3-yl)(2,6-difluorophenyl)methanone (**II-13-B2**) (153m g、0.39 mmol) in methanol (4 ml) was added 10% $Pd(OH)_2$ on carbon (51 mg). Under hydrogen atmosp here, the solution was stirred at rt for 18 h and filtered through a pad of Celite. The solvent was remov ed under reduced pressure. To a solution of the resulting residue (25 mg) and adamantan-1-yl isocyanate (19 mg, 0.11 mmol) in dichloromethane was added (0.5 ml) DIPEA (23 μl, 0.13 mmol). The solution was stirred at rt for 30 min. Water was added, and the aqueous layer was extracted with dichloromethan e. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated und

er reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-h exane) to afford **II-17** (33 mg).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45-1.63 (8H, m), 1.65-1.69 (6H, m), 1.98-2.00 (6H, m), 2.05-2.12 (3H, m), 3.26-3.33 (6H, m), 3.75-3.82 (2H, m), 4.21 (1H, s), 6.91-7.00 (2H, m), 7.30-7.38 (1H, m).

MS (ESI) m/z 472 $[M+H]^+$.

Starting from corresponding isocyanates compounds in Table 2-4 were obtained following the procedure described for-*N*-(adamantan-1-yl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**II-15**).

Table 2-4. Data of compunds.

Compound	Name	yield (%)	^{1}H NMR ($CDCl_3$, 400MHz)	MS(ESI) $[M+H]^+$
II-18	8-(2,6-difluorobenzoyl)-*N*-(4-methoxyphenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	65	1.55-1.66 (2H, m), 1.71-1.79 (2H, m), 1.89-1.99 (2H, m), 3.28-3.45 (4H, m), 3.50-3.61 (2H, m), 3.64-3.75 (1H, m), 3.78 (3H, s), 3.95-4.05 (1H, m), 6.02 (1H, s), 6.84 (2H, d, *J* = 8.8 Hz), 6,92-6.98 (2H, m), 7.26-7.39 (3H, m).	430
II-19	N-(4-cyanophenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	16	1.55-1.64 (2H, m), 1.72-1.74 (2H, m), 1.89-2.01 (2H, m), 3.28-3.48 (4H, m), 3.52-3.55 (2H, m), 3.65-3.75 (1H, m), 3.93-4.02 (1H, m), 6.52 (1H, s), 6.91-6.98 (2H, m), 7.31-7.41 (1H, m), 7.53-7.55 (4H, m).	425
II-20	N-(4-chlorophenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	71	1.57-1.65 (2H, m), 1.70-1.79 (2H, m), 1.90-1.99 (2H, m), 3.26-3.45 (4H, m), 3.50-3.61 (2H, m), 3.62-3.75 (1H, m), 3.92-4.01 (1H, m), 6.11 (1H, s), 6,93-7.00 (2H, m), 7.21-7.39 (5H, m).	434
II-21	8-(2,6-difluorobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	56	1.50-1.63 (2H, m), 1.70-1.77 (2H, m), 1.90-2.01 (2H, m), 3.28-3.50 (4H, m), 3.53-3.65 (2H, m), 3.62-3.78 (1H, m), 3.95-4.03 (1H, m), 6.29 (1H, s), 6.92-7.00 (2H, m), 7.26-7.41 (1H, m) , 7.52-7.54 (4H, m).	468
II-22	8-(2,6-difluorobenzoyl)-*N*-(4-(trifluorometho	66	1.55-1.64 (2H, m), 1.71-1.76 (2H, m), 1.89-2.01 (2H, m), 3.28-3.47 (4H, m), 3.51-3.62 (2H, m),	484

	xy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide		3.65-3.76 (1H, m), 3.95-4.05 (1H, m), 6.20 (1H, s), 6.92-6.99 (2H, m), 7.25 (2H, d, *J* = 8.0 Hz), 7.31-7.41 (1H, m), 7.42 (2H, d, *J* = 8.0 Hz).	
II-24	8-(2,6-difluorobenzoyl)-*N*-(3-(trifluoromethyl)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	77	1.59-1.66 (2H, m), 1.71-1.78 (2H, m), 1.91-1.99 (2H, m), 3.29-3.66 (6H, m), 3.67-3.79 (1H, m), 3.92-4.02 (1H, m), 6.24 (1H, s), 6,93-7.00 (2H, m), 7.23-7.72 (5H, m).	468
II-27	N-(3,4-dichlorophenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	70	1.60-1.67 (2H, m), 1.71-1.79 (2H, m), 1.91-2.01 (2H, m), 3.28-3.50 (4H, m), 3.51-3.68 (2H, m), 3.68-3.79 (1H, m), 3.91-4.05 (1H, m), 6.76 (1H, s), 6.91-7.00 (2H, m), 7.21-7.39 (3H, m), 8.26 (1H, d, *J* = 8.8 Hz).	469
II-29	N-(2,4-dichlorophenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	71	1.60-1.67 (2H, m), 1.71-1.79 (2H, m), 1.91-2.01 (2H, m), 3.28-3.50 (4H, m), 3.51-3.68 (2H, m), 3.68-3.79 (1H, m), 3.91-4.05 (1H, m), 6.76 (1H, s), 6.91-7.00 (2H, m), 7.21-7.39 (3H, m), 8.26 (1H, d, *J* = 8.8 Hz).	469

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (II-26).**

To a solution of 4-nitrophenyl chloroformate (36 mg, 0.18 mmol) in dichloromethane (0.50 ml) were added 4-chloro-3-(trifluoromethyl)aniline (35 mg, 0.18 mmol) and DIPEA (31 μl, 0.18 mmol) in dichloromethane (1.0 ml). The solution was stirred at rt for 5 min. (2,6-difluorophenyl)(2,8-diazaspiro[4.5]decan-8-yl)methanone (38 mg, 0.13 mmol) in dichloromethane (1.5 ml) and DIPEA (54 μl, 0.31 mmol) was added. The solution was stirred at rt for 2 h. A saturated aqueous solution of $NaHCO_3$ was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-26** in 30% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60-1.65 (2H, m), 1.71-1.79 (2H, m), 1.91-1.99 (2H, m), 3.29-3.50 (4H, m), 3.51-3.65 (2H, m), 3.65-3.79 (1H, m), 3.92-4.05 (1H, m), 6.26 (1H, s), 6.92-7.01 (2H, m), 7.30-7.43 (2H, m), 7.61-7.74 (2H, m).

MS (ESI) m/z 502 $[M+H]^+$.

Starting from corresponding amines compounds in Table 2-5 were obtained following the procedure described for-*N*-(4-chloro-3-(trifluoromethyl)phenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**II-26**).

Table 2-5. Data of compunds.

Compound	Name	yield (%)	^{1}H NMR ($CDCl_3$, 400MHz)	MS(ESI) $[M+H]^+$
II-23	8-(2,6-difluorobenzoyl)-*N*-(4-(2,2,2-trifluoroethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	71	1.55-1.65 (2H, m), 1.70-1.78 (2H, m), 1.89-1.99 (2H, m), 3.25-3.47 (4H, m), 3.50-3.61 (2H, m), 3.61-3.75 (1H, m), 3.92-4.03 (1H, m), 4.31 (2H, dd, *J* = 8.1, 16.3 Hz), 6.06 (1H, s), 6.86-6.99 (4H, m), 7.29-7.38 (3H, m).	498
II-25	8-(2,6-difluorobenzoyl)-*N*-(3-(2,2,2-trifluoroethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	61	1.60-1.67 (2H, m), 1.71-1.79 (2H, m), 1.91-2.01 (2H, m), 3.28-3.50 (4H, m), 3.51-3.68 (2H, m), 3.68-3.79 (1H, m), 3.91-4.05 (1H, m), 6.76 (1H, s), 6.91-7.00 (2H, m), 7.21-7.39 (3H, m), 8.26 (1H, d, *J* = 8.8 Hz).	498
II-28	N-(4-chloro-3-methylphenyl)-8-(2,6-difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	62	1.58-1.65 (2H, m), 1.70-1.77 (2H, m), 1.90-1.99 (2H, m), 2.34 (3H, s), 3.24-3.46 (4H, m), 3.49-3.61 (2H, m), 3.62-3.75 (1H, m), 3.92-4.03 (1H, m), 6.07 (1H, s), 6.89-7.01 (3H, m), 7.11-7.39 (3H, m).	448

***tert*-Butyl 2-benzyl-2,8-diazaspiro[4.5]decane-8-carboxylate (II-52).**

To a solution of 2-benzyl-2,8-diazaspiro[4.5]decane (**II-5**) (5.0 g, 21 mmol) in water (20 ml) were added di-*tert*-butyl dicarbonate (7.2 g, 33 mmol) and NaOH (2.64 g, 66 mmol). The solution was stirred at rt for 3 h. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **II-52** in 84% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (9H, s), 1.47-1.52 (2H, m), 1.59-1.67 (4H, m), 2.37 (2H, s), 2.54-2.64 (2H, m), 3.24-3.40 (4H, m), 3.58 (2H, s), 7.22-7.25 (1H, m), 7.29-7.33 (4H, m).

***tert*-Butyl 2,8-diazaspiro[4.5]decane-8-carboxylate (II-53)**.

Starting from *tert*-butyl 2-benzyl-2,8-diazaspiro[4.5]decane-8-carboxylate (**II-52**) the title compound was o btained following the procedure described for (2,6-difluorophenyl)(2,8-diazaspiro[4.5]decan-8-yl)methanone (**II-14B1**) in 94% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (9H, s), 1.50-1.70 (8H, m), 3.35-3.50 (6H, m).

***tert*-Butyl 2-((4-(trifluoromethoxy)phenyl)carbamoyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (II-54).**

Starting from *tert*-butyl 2,8-diazaspiro[4.5]decane-8-carboxylate (**II-53**) and 4-(trifluoromethoxy)phenyl is ocyanate the title compound was obtained following the procedure described for-*N*-(adamantan-1-yl)-8-(2,6 -difluorobenzoyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**II-15**) in 68% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (9H, s), 1.52-1.58 (4H, m), 1.88 (2H, t, *J* = 6.9 Hz), 3.28-3.35 (4H, m), 3.46-3.56 (4H, m), 6.16 (1H, brs), 7.14 (2H, d, *J* = 8.7 Hz), 7.43 (2H, d, *J* = 8.7 Hz).

***N*-(4-(Trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (II-55).**

To a solution of *tert*-butyl 2-((4-(trifluoromethoxy)phenyl)carbamoyl)-2,8-diazaspiro[4.5]decane-8-carboxylat e (**II-54**) (1.0 g, 2.26 mmol) in dichloromethane (10 ml) was added TFA (1.22 ml, 15.8 mmol). The sol ution was stirred at rt for 2 h. A saturated aqueous solution of $NaHCO_3$ was added, and the aqueous lay er was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **II-55** in 75% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.54-1.58 (4H, m), 1.87 (2H, t, *J* = 7.3 Hz), 2.76-2.95 (4H, m), 3.34 (2H, s), 3.53 (2H, t, *J* = 7.3 Hz), 6.18 (1H, brs). 7.14 (2H, d, *J* = 8.2 Hz), 7.43 (2H, d, *J* = 8.2 Hz).

MS (ESI) m/z 344 $[M+H]^+$.

8-(2-Methylcyclopropanecarbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (II-46).

To a solution of 2-methylcyclopropanecarboxylic acid (26 mg, 0.26 mmol) in DMF (1 ml) were added HATU (80 mg, 0.21 mmol), DIPEA (61 μl, 0.35 mmol) and-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**II-55**) (60 mg, 0.18 mmol). The solution was stirred at rt for 16 h. Water was added, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **II-46** in quantitative yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.55-0.62 (1H, m), 1.12 (3H, d, *J* = 5.9 Hz), 1.13-1.19 (1H, m), 1.26 (1H, t, *J* = 6.8 Hz), 1.41-1.47 (1H, m), 1.52-1.68 (4H, m), 1.92 (2H, t, *J* = 6.8 Hz), 3.40 (2H, s), 3.56 (4H, t, *J* = 6.8 Hz), 3.71-3.80 (2H, m), 6.19 (1H, s), 7.15 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).

MS (ESI) m/z 426 $[M+H]^+$.

Starting from corresponding aminoacids compounds in Table 2-6 were obtained following the procedure described for 8-(2-methylcyclopropanecarbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**II-46**).

Table 2-6. Data of compunds.

Compound	Name	yield (%)	^{1}H NMR ($CDCl_3$, 400MHz)	MS(ESI) $[M+H]^+$
II-30	8-(2-fluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	87	1.50-1.62 (2H, m), 1.68-1.78 (2H, m), 1.88-2.00 (2H, m), 3.23-3.48 (4H, m), 3.48-3.78 (3H, m), 3.90-4.02 (1H, br), 6.16 (1H, s), 7.07-7.17 (3H, m), 7.22 (1H, t, *J* = 3.6 Hz), 7.36-7.45 (4H, m).	466
II-31	8-(2-fluoro-5-hydroxybenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazas	68	1.53-1.78 (4H, m), 1.88-2.00 (2H, m), 3.32-3.50 (4H, m), 3.50-3.62 (2H, m), 3.65-3.75 (1H, m), 3.81-3.92 (1H, m),	482

	piro[4.5]decane-2-carboxamide		6.69-6.74 (1H, m), 6.84 (1H, dt, *J* = 3.6, 8.6 Hz), 6.97-7.04 (1H, m), 7.16 (2H, d, *J* = 8.6 Hz), 7.50 (2H, d, *J* = 8.6 Hz).	
II-33	8-(quinoline-5-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	96	1.43-1.57 (1H, m), 1.61-1.80 (3H, m), 1.88-1.98 (2H, m), 3.12-3.48 (4H, m), 3.49-3.77 (3H, m), 3.92-4.03 (1H, m), 6.16 (1H, s), 7.14 (2H, d, *J* = 8.6 Hz), 7.26-7.35 (3H, m), 7.39-7.45 (3H, m).	499
II-34	8-(1H-benzo[d]imidazole-4-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	75	1.52-1.80 (4H, br), 1.95 (2H, t, *J* = 6.3 Hz), 3.35 (2H, s), 3.38-3.80 (6H, m), 7.16 (2H, d, *J* = 9.1 Hz), 7.36 (2H, dd, *J* = 1.4, 8.6 Hz), 7.50 (2H, d, *J* = 9.1 Hz), 7.66-7.76 (2H, br), 8.28 (1H, s).	488
II-35	8-(5-methylpyrazine-2-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	94	1.58-1.70 (2H, t, *J* = 5.9 Hz), 1.71-1.78 (2H, m), 1.95 (2H, td, *J* = 2.7, 7.0 Hz), 2.63 (3H, s), 3.38-3.73 (7H, m) 3.91-4.01 (1H, m), 6.18 (1H, s), 7.15 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz), 8.41 (1H, brs), 8.83 (1H, brs).	464
II-36	8-(5-methylisoxazole-3-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	30	1.63-1.72 (4H, m), 1.95 (2H, t, *J* = 7.7 Hz), 2.48 (3H, s), 3.38-3.45 (2H, m), 3.53-3.73 (4H, m), 3.89-4.01 (2H, m), 6.16 (1H, s), 6.28 (1H, s), 7.15 (2H, d, *J* = 8.6 Hz), 7.44 (2H, d, *J* = 8.6 Hz).	452
II-37	8-(5-cyclopropylisoxazole-4-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	19	1.12-1.20 (2H, m), 1.21-1.27 (2H, m), 1.60-1.75 (4H, br), 1.96 (2H, t, *J* = 7.3 Hz), 2.24-2.32 (1H, m), 3.44 (2H, s), 3.47-4.00 (6H, br), 6.16 (1H, s), 7.15 (2H, d, *J* = 9.1 Hz), 7.42 (2H, d, *J* = 9.1 Hz), 8.19 (1H, s).	479
II-38	8-(2-methylfuran-3-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	71	1.55-1.70 (4H, br), 1.94 (2H, t, *J* = 7.3 Hz), 2.39 (3H, s), 3.42 (2H, s), 3.44-3.95 (6H, m), 6.16 (1H, s), 6.34 (1H, d, *J* = 1.8 Hz), 7.15 (2H, d, *J* = 8.6 Hz), 7.27 (1H, brs), 7.43 (2H, d, *J* = 8.6 Hz).	452
II-40	8-(tetrahydrofuran-3-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	69	1.52-1.68 (4H, m), 1.91 (2H, t, *J* = 7.3 Hz), 2.02-2.14 (1H, m), 2.12-2.30 (1H, m), 3.20-3.30 (1H, m), 3.40 (2H, s), 3.41-3.50 (1H, m), 3.52-3.62 (3H, m), 3.76-3.92 (5H, m), 4.01 (1H, t, *J* = 8.2 Hz), 6.26 (1H, s), 7.14 (2H, d,	442

			J = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	
II-41	8-(tetrahydro-2H-pyran-4-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	50	1.51-1.68 (6H, m), 1.87-1.98 (4H, m), 2.74 (1H, tt, *J* = 3.6, 11 Hz), 3.36-3.51 (6H, m), 3.51-3.63 (3H, m), 3.75-3.87 (1H, br), 4.03 (2H, dq, *J* = 2.3, 11 Hz), 6.16 (1H, s), 7.15 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	456
II-43	8-(4-hydroxycyclohexanecarbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	29	1.47-1.80 (8H, m), 1.82-2.02 (6H, m), 2.52 (1H, tt, *J* = 3.2, 11 Hz), 3.32-3.48 (4H, m), 3.55 (2H, t, *J* = 6.8 Hz), 3.60-3.69 (1H, m), 3.71-3.84 (1H, m), 4.02 (1H, brs), 6.29 (1H, s), 7.14 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	470
II-44	N-(4-(trifluoromethoxy)phenyl)-8-(1-(trifluoromethyl)cyclopropanecarbonyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	37	1.16 (2H, t, *J* = 5.9 Hz), 1.34 (2H, t, *J* = 5.9 Hz), 1.55-1.70 (4H, m), 1.93 (2H, t, *J* = 7.3 Hz), 3.41 (2H, s), 3.46-3.62 (4H, m), 3.81 (2H, dt, *J* = 5.0, 13 Hz), 6.17 (1H, s), 7.15 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	480
II-45	8-(2,2-difluorocyclopropanecarbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	48	1.16 (2H, t, *J* = 5.9 Hz), 1.34 (2H, t, *J* = 5.9 Hz), 1.55-1.70 (4H, m), 1.93 (2H, t, *J* = 7.3 Hz), 3.41 (2H, s), 3.46-3.62 (4H, m), 3.81 (2H, dt, *J* = 5.0, 13 Hz), 6.17 (1H, s), 7.15 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	448
II-47	8-(2-cyclopropylacetyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	87	0.15-0.22 (2H, m), 0.53-0.61 (2H, m), 0.94-1.10 (1H, M), 1.50-1.73 (4H, m), 1.90 (2H, t, *J* = 7.1 Hz), 2.29 (2H, d, *J* = 6.8 Hz), 3.32-3.63 (7H, m), 3.70-3.84 (1H, m), 6.19 (1H, s), 7.14 (2H, d, *J* = 8.0 Hz), 7.43 (2H, d, *J* = 8.0 Hz).	426
II-49	8-(2-ethylbutanoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	9	0.82-0.91 (6H, m), 1.41-1.53 (1H, m), 1.54-1.71 (7H, m), 1.92 (2H, t, *J* = 6.8 Hz), 2.49-2.58 (1H, m), 3.40 (2H, s), 3.43-3.71 (5H, m), 3.80-3.91 (1H, br), 6.20 (1H, s), 7.14 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	442
II-51	8-(3-(3-hydroxyphenyl)propanoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	71	1.34-1.42 (2H, br), 1.50-1.60 (2H, br), 1.81-1.90 (2H, m), 2.62 (2H, td, *J* = 1.7, 7.8 Hz), 2.93 (2H, t, *J* = 7.8 Hz), 3.22-3.56 (7H, m), 3.71-3.80 (1H, m), 5.59 (1H, s), 6.19 (1H, s), 6.66-6.79 (3H, m), 7.12-7.18 (3H, m), 7.43	492

			(2H, d, *J* = 9.0 Hz).	

Starting from corresponding acyl halides and-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide (**II-55**) compounds in Table 2-7 were obtained following the procedure described for (2-benzyl-2,8-diazaspiro[4.5]decan-8-yl)(2,6-difluorophenyl)methanone (**II-13B1**).

Table 2-7. Data of compunds.

Compound	Name	yield (%)	^{1}H NMR ($CDCl_3$, 400MHz)	MS(ESI) $[M+H]^+$
II-32	8-(2-chlorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	90	1.43-1.57 (1H, m), 1.61-1.80 (3H, m), 1.88-1.98 (2H, m), 3.12-3.48 (4H, m), 3.49-3.77 (3H, m), 3.92-4.03 (1H, m), 6.16 (1H, s), 7.14 (2H, d, *J* = 8.6 Hz), 7.26-7.35 (3H, m), 7.39-7.45 (3H, m).	482
II-39	8-(furan-2-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	94	1.64-1.72 (4H, m), 1.95 (2H, t, *J* = 7.3 Hz), 3.43 (2H, s), 3.57 (2H, t, *J* = 7.3 Hz), 3.59-3.71 (2H, br), 3.87-3.97 (2H, m), 6.17 (1H, s), 6.49 (1H, dd, *J* = 1.8, 3.6 Hz), 7.00 (1H, d, *J* = 3.2 Hz), 7.15 (2H, d, *J* = 8.2 Hz), 7.44 (2H, d, *J* = 8.2 Hz), 7.48 (1H, brs).	438
II-42	8-(cyclohexanecarbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	41	1.20-1.30 (4H, m), 1.45-1.72 (8H, m), 1.77-1.84 (2H, m), 1.91 (2H, t, *J* = 7.3 Hz), 2.47 (1H, tt, *J* = 3.2, 11.8 Hz), 3.35-3.50 (4H, m), 3.51-3.62 (3H, m), 3.71-3.84 (1H, br), 6.15 (1H, s), 7.14 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	454
II-48	8-pivaloyl-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	28	1.29 (9H, s), 1.57-1.63 (4H, m), 1.91 (2H, t, *J* = 7.3 Hz), 3.39 (2H, s), 3.44-3.60 (4H, m), 3.72-3.82 (2H, br), 6.15 (1H, s), 7.14 (2H, d, *J* = 8.6 Hz), 7.43 (2H, d, *J* = 8.6 Hz).	428
II-50	8-pentanoyl-*N*-(4-(trifluoromethoxy)phenyl)-2,8-diazaspiro[4.5]decane-2-carboxamide	65	0.93 (3H, t, *J* = 7.7 Hz), 1.32-1.42 (2H, m), 1.52-1.65 (6H, m), 1.91 (2H, t, *J* = 7.3 Hz), 2.34 (2H, t, *J* = 7.7 Hz), 3.33-3.62 (7H, m), 3.73-3.84 (1H, m), 6.17 (1H, s), 7.14 (2H, d, *J* = 8.2 Hz), 7.43 (2H, d, *J* = 8.2 Hz).	428

Measurement of *in vitro* sEH inhibitory activity.

The sEH inhibition assays were performed as described by Jones, P. D.; Wolf, N. M.; Morisseau, C.; Whetstone, P.; Hock, B.; Hammock, B. D.(*Anal. Biochem.* **2005**, *343*, 66.). A solution of recombinant sEH from human or

mouse (the enzymes were purchased from Cayman Chemical Company) or rat (the enzyme was expressed in Sf9 insect cells using baculovirus) in buffer (BisTris–HCl, 25 mM, pH 7.0, containing 0.1 mg/ml BSA) was incubated with a inhibitor at room temperature for 30 min. To the resultant solution, cyano(6-methoxy-naphthalen-2-yl)methyl trans-[(3-phenyloxiran-2-yl)methyl] carbonate (purchased from Cayman Chemical Company) was added, and the mixture was incubated at room temperture for 20-45 min. $ZnSO_4$ was added, and the resultant solution of fluorescence intensity (excitation filter 330 nm, emission filter 465 nm) was measured. The reduction rate of enzyme activity by inhibitors were calculated using the fluorescence intensity, and IC_{50} values were determined. In these assays IC_{50} values of a representative sEH inhibitor 12-(3-adamantan-1-ylureido)-dodecanoic acid (AUDA) were 3 nM (human), 10 nM (murine), 10 nM (rat).

Protocol of Docking Studies

In order to get accurate results, all the docking experiments were performed with the default parameters.

Protein was prepared by fred_receptor 2.2.5 software (Openeye Scientific Software, Santa Fe, NM) using Xray co-crystal structure(PDB code 1VJ5). Each compound was converted into the set of low-energy 3D multi-conformers by Omega 2.3.2 (Openeye Scientific Software Santa Fe, NM) in order to perform docking studies with FRED 2.2.4 software (Openeye Scientific Software, Santa Fe, NM) which uses multi-conformer docking algorithm, and then do rigid docking for each conformer. Up to 400 conformers were generated for each compound.

Docking studies of sEH protein with the compounds conformers were performed by using FRED with a Gaussian type fitting scoring function Chemgauss3 to obtain the most reliable docking pose accoording to the core scoring fuction.

Chemgauss3 uses the potentials between the chemically matched positions around the docked ligand. These chemical positions are complementary to the surrounding specific groups in the protein. The interactions which can be scored by Chemgauss3 functions are: steric, acceptor, donors, coordinating groups, metals, lone pairs, polar hydrogens and chelator coordinating groups*.

* *Nature Reviews Drug Discovery* 3, 935-949

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4. a) Shen, H. C.; Ding, F.-X.; Deng, Q.; Xu, S.; Chen, H.; Tong, X.; Tong, V.; Mitra, K.; Kumar, S.; Zhang, X.; Chen, Y.; Zhou, G.; Pai, L.-Y.; Alonso-Galicia, M.; Chen, X.; Berger, J. P.; Zhang, B.; Tata, J. R.; Colletti, S. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5314. b) Shen, H. C.; Ding, F.-X.; Deng, Q.; Xu, S.; Chen, H.; Tong, X.; Tong, V.; Mitra, K.; Kumar, S.; Zhang, X.; Chen, Y.; Zhou, G.; Pai, L.-Y.; Alonso-Galicia, M.; Chen, X.; Berger, J. P.; Zhang, B.; Tata, J. R.; Colletti, S. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3398. c) Shen, H. C.; Ding, F.-X.; Wang, S.; Deng, Q.; Zhang, X.; Chen, Y.; Zhou, G.; Xu, S.; Chen, H.; Tong, X.; Tong, V.; Mitra, K.; Kumar, S.; Tsai, C.; Stevenson, A. S.; Pai, L.-Y.; Alonso-Galicia, M.; Chen, X.; Soisson, S. M.; Roy, S.; Zhang, B.; Tata, J. R.; Berger, J. P.; Colletti, S. L. *J. Med. Chem.* **2009**, *52*, 5009.

Chapter 3

Discovery of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives as highly potent soluble epoxide hydrolase inhibitors and orally active drug candidates for treatment of chronic kidney diseases

Abstract: The identification of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted ureas as highly potent soluble epoxide hydrolase (sEH) inhibitors and orally active agents for treating chronic kidney diseases is described. **III-22** exhibited excellent inhibitory activity against sEH and excellent bioavailability. When administered orally at 30 mg/kg, **III-22** lowered serum creatinine in a rat model of anti-glomerular basement membrane (GBM) glomerulonephritis but 2,8-diazaspiro[4.5]decane-based trisubstituted ureas did not. These results suggest that **III-22** is an orally active drug candidate for treating chronic kidney diseases.

Introduction

As described in Chapter 2, I found that 2,8-diazaspiro[4.5]decane-based trisubstituted ureas are highly potent sEH inhibitors and orally active drug candidates for treating hypertension (Figure 3-1, **II-22**). I expected that these derivatives would show renal protective effects derived from the beneficial effects of sEH inhibitors described in Chapter 1. Contrary to my expectation, oral administration of these derivatives failed to reduce serum creatinine in a rat model of anti-GBM glomerulonephritis[1]. I found that the solubility and microsomal stability of 2,8-diazaspiro[4.5]decane-based trisubstituted ureas can be conveniently modified by changing the substituent on the amide group. On the basis of these findings, I hypothesized that spirocyclic diamine-based trisubstituted ureas could be developed as sEH inhibitors and I aimed to find other such ureas that inhibited sEH as strongly as 2,8-diazaspiro[4.5]decane-based trisubstituted ureas did and that had different pharmacokinetic profiles.

Design

As shown in Figure 3-1, I designed 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivative **III-1**. Because of its introduced oxygen atom, **III-1** had lower cLogP than **II-22** and thus had higher aqueous solubility. A docking study of **III-1** with human sEH (Figure 3-2) suggested that the two carbonyl oxygen atoms of **III-1** bind to the catalytic pocket in the same manner as **II-22**. Working under this hypothesis, I began to explore 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives. In light of the docking study results (Figure 3-2), I also designed **III-18**, which has a carbon atom in place of the oxygen atom in the 1-oxa-4,9-diazaspiro[5.5]undecane scaffold of **III-1** (Figure 3-1); my expectation was that it would have sEH inhibitory activity as high as that of **II-22**.

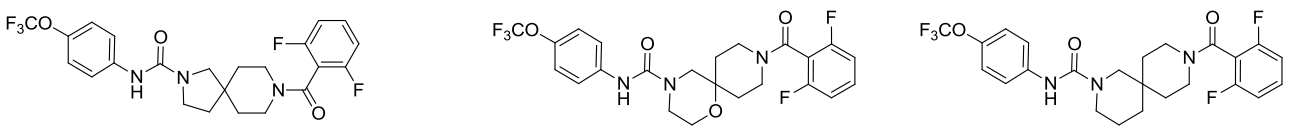


Figure 3-1. Structures of **II-22**, **III-1** and **III-18**.

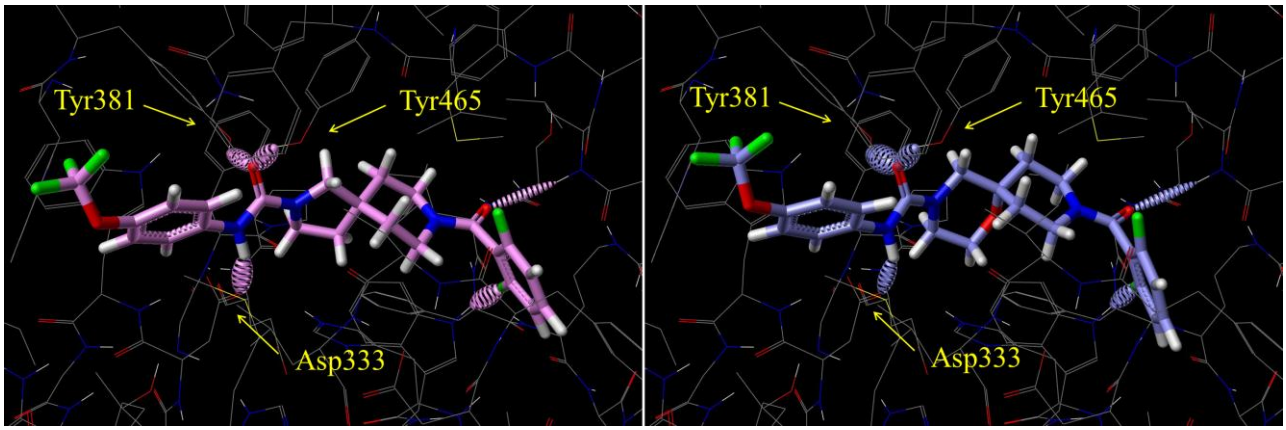
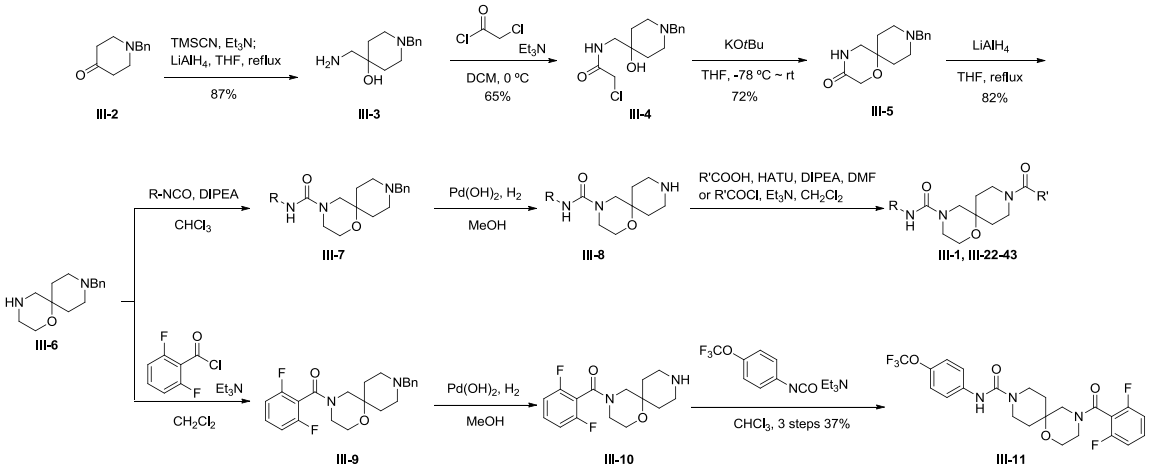


Figure 3-2. Docking studies of human sEH (PDB code: 1VJ5) with **II-22** (left) and **III-1** (right).

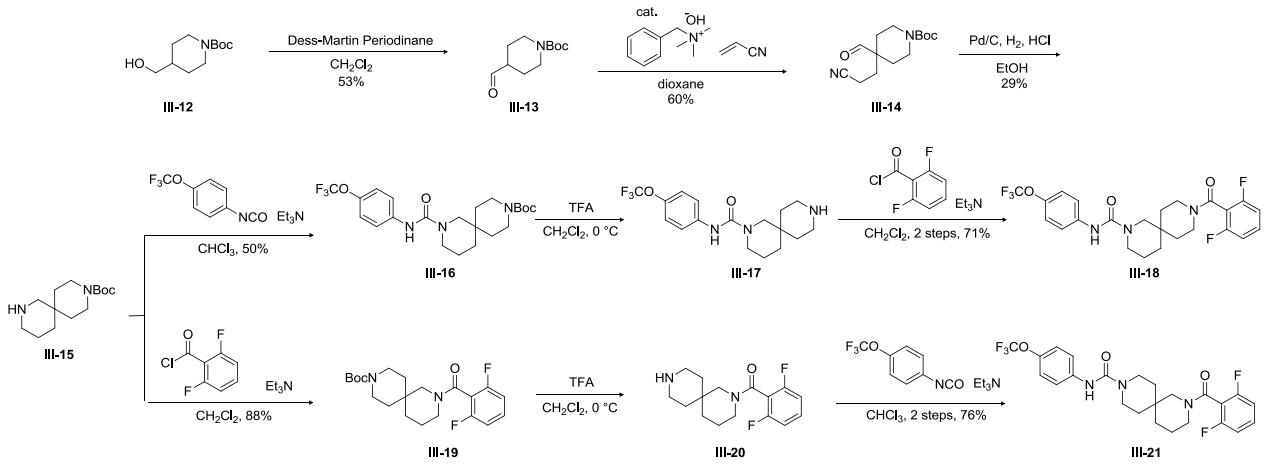
Chemistry

The general procedure for the synthesis of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives **III-1**, **III-22** to **III-43**, and **III-11** is shown in Scheme 3-1. Starting material **III-6** was synthesized by a reported method.[2] **III-2** was treated with trimethysilyl cyanide and Et_3N, then the reaction mixture was added dropwise to a solution of $LiAlH_4$. The obtained product, **III-3**, was treated with 2-chloroacetyl chloride affording **III-4**. Intramolecular cyclization of **III-4** gave **III-5**. This step was detailed in Chapter 4. Reduction of the amide group in **III-5** provided **III-6**. Treatment of **III-6** with isocyanate afforded **III-7**. Removal of the benzyl protecting group by $Pd(OH)_2$-catalyzed hydrogenation provided **III-8**. Then, condensation with carboxylic acid or acyl chloride produced **III-1** and **III-22** through **III-43**. Treatment of **III-6** with benzoyl chloride afforded **III-9**. Removal of the benzyl protecting group by $Pd(OH)_2$-catalyzed hydrogenation provided **III-10**. Then, treatment with isocyanate gave **III-11**. The synthesis of 2,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives **III-18** and **III-21** is shown in Scheme 3-2. Oxidation of alcohol **III-12** with Dess-Martin periodinane afforded **III-13**. Michael addition to acrylonitrile provided **III-14**. Then, hydrogenation of the nitrile group was accompanied by cyclization via reductive amination to give 2,9-diazaspiro[5.5]undecane **III-15**. Treatment of **III-15** with isocyanate, removal of the Boc protecting group by TFA, and then treatment with benzoyl chloride led to **III-18**. Treatment of **III-15** with benzoyl chloride, removal of the Boc protectiong group by TFA, and then

treatment with isocyanate afforded **III-21**.



Scheme 3-1. Synthesis of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives.



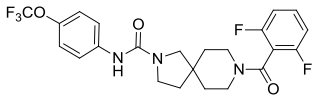
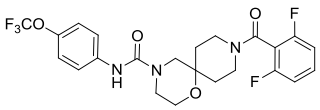
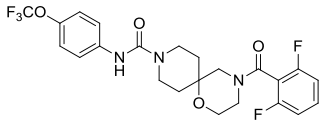
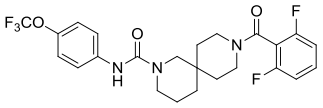
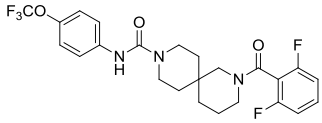
Scheme 3-2. Synthesis of 2,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives.

Results and discussion

The diazaspiro-based urea derivatives listed in Table 3-1 to 3-4 had 1 or 2 hydrogen bond donors, 6-9 hydrogen bond acceptors, molecular mass of 447-519 Da, and an octanol-water partition coefficient log P of 0.90-3.89. **III-28**, **III-37**, **III-41**, and **III-42** had molecular mass greater than 500. Other derivatives satisfied Lipinski's rule. I performed structure–activity relationship (SAR) and structure–property relationship (SPR) studies on the

diazaspiro scaffolds (Table 3-1). 4-(Trifluoromethoxy)phenyl and 2,6-difluorobenzoyl groups were selected as the left- and right-hand substituents, respectively. As I expected, **III-1** had moderate inhibitory activity against human sEH and better solubility than **II-22**. However, the microsomal stability of **III-1** was lower than that of **II-22**. Lower sEH inhibitory activity was found in **III-11**, whose diazaspiro scaffold was constructed with the left- and right-hand substituents swapped. In **III-18**, which had a carbon atom in place of the oxygen atom in the 1-oxa-4,9-diazaspiro[5.5]undecane scaffold of **III-1**, sEH inhibitory activity was improved but microsomal stability and solubility became problematically low. Replacing the oxygen atom of **III-11** with a carbon atom also led to lower solubility (**III-21**). Considering that **III-1** showed higher solubility, I next focused on derivatives with a 1-oxa-4,9-diazaspiro[5.5]undecane scaffold.

Table 3-1. SAR and SPR of diazaspiro scaffolds.

Structure	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability[a]	cLogP
		Human	Rat	JP1[b]	JP2[c]		
	**II-22**	0.4	4.5	24	21	0	3.33
	**III-1**	0.7	18.5	44	39	0.086	2.84
	**III-11**	5.4	N.D.[d]	52	46	N.D.	2.86
	**III-18**	0.125	3.3	9	9	0.602	3.89
	**III-21**	2.96	N.D.	0	0	N.D.	3.89

[a] Units: mL/min/mg protein.

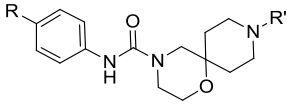
[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia.

[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia.

[d] N.D.: Not determined.

The SAR and SPR results for derivatives of **III-1** with benzamide moieties are shown in Table 3-2. The removal of the fluorine atom from **III-1** improved inhibitory activity against rat sEH, and also improved solubility and microsomal stability (**III-22**). Replacing the trifluoromethoxy group of **III-1** with a trifluoromethyl group (**III-23**) made little difference in activity or other properties. The effect of substituent position on the benzamide ring was investigated (**III-24** to **III-27**). Removing the substituent reduced human sEH inhibitory activity (**III-24**). Installing a chloro substituent at the *ortho* (**III-25**), *meta* (**III-26**), and *para* (**III-27**) positions gave the same human sEH inhibitory activity as **III-23**, and only **III-25** showed improved inhibitory activity against rat sEH. **III-25** and **III-27** exhibited sufficient microsomal stability. These results suggest that *ortho* substitution on the aromatic benzamide ring is particularly favorable for rat sEH inhibitory activity but *meta* or *para* substitution is not. In general, lower lipophilicity corresponds to better solubility and microsomal stability.[3] Aiming to achieve sufficient sEH inhibitory activity, solubility, and microsomal stability concurrently, I examined an *ortho*-substituted derivative with reduced lipophilicity (**III-28**); it had higher sEH inhibitory activity, solubility, and microsomal stability. Further investigation revealed that only a cyano group was tolerated at the *para* position on the benzamide ring: **III-29** exhibited good sEH inhibitory activity, solubility, and microsomal stability.

Table 3-2. SAR and SPR of 1-oxa-4,9-diazaspiro[5.5]undecane-based urea derivatives: substituents in benzene rings.



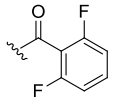
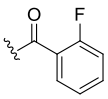
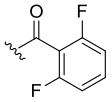
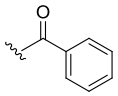
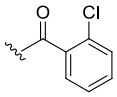
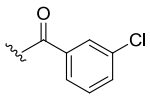
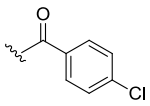
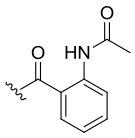
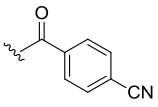
R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability	cLogP
			Human	Rat	JP1[b]	JP2[c]		
OCF_3		**III-1**	0.7	18.5	44	39	0.086	2.84

Table 3-2. SAR and SPR of 1-oxa-4,9-diazaspiro[5.5]undecane-based urea derivatives: substituents in benzene rings.

R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability	cLogP
			Human	Rat	JP1[b]	JP2[c]		
OCF_3		**III-22**	1.1	9.3	71	62	0.03	2.67
CF_3		**III-23**	1.3	19.1	43	38	0.093	3.04
CF_3		**III-24**	3.2	N.D.[d]	62	63	N.D.	2.66
CF_3		**III-25**	1.4	13	10	12	0.048	3.44
CF_3		**III-26**	1.2	36.9	26	21	0.1	3.44
CF_3		**III-27**	1	24.9	29	23	0.021	3.44
CF_3		**III-28**	0.8	6.8	92	93	0.039	2.14
CF_3		**III-29**	1.9	15	85	82	0.003	2.26

[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia.

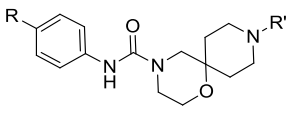
[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia.

[d] N.D.: Not determined.

Derivatives with a heteroaromatic ring as the amide substituent were explored in order to evaluate the effectiveness of reduced lipophilicity for improving solubility and microsomal stability. As expected, almost all

compounds in Table 3-3 exhibited improved solubility and microsomal stability. However, I observed decreased human sEH inhibitory activity in **III-30** which contained an unsubstituted five-membered ring. The derivatives with five-membered rings bearing a methyl or cyclopropyl substituent (**III-31** to **III-33**) exhibited increased human sEH inhibitory activity. Remarkably, pyrazole derivative **III-33**, which contained an NH moiety, was tolerated in humans and exhibited sEH inhibitory activity, but was not tolerated in rats. **III-34** with an unsubstituted pyridine ring showed lower activity, and **III-35** with a chloro substituent on the pyridine ring effectively inhibited sEH in humans and rats. A pyrazine ring was also an applicable substituent (**III-36**). Although **III-37** exhibited moderate human sEH inhibitory activity, its rat sEH inhibitory activity was greatly reduced.

Table 3-3. SARs and SPRs of 1-oxa-4,9-diazaspiro[5.5]undecane-based urea derivatives: heteroaromatics.



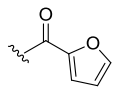
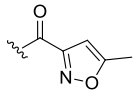
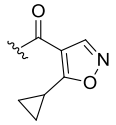
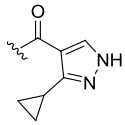
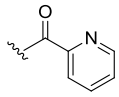
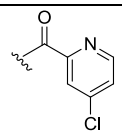
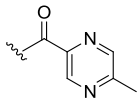
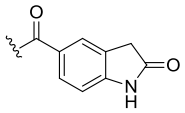
R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability	cLogP
			Human	Rat	JP1[b]	JP2[c]		
CF_3		**III-30**	5.4	N.D.	75	75	N.D.[d]	1.83
OCF_3		**III-31**	1.4	25.7	84	83	0.047	1.22
OCF_3		**III-32**	0.6	22.2	86	89	0.087	1.67
OCF_3		**III-33**	1.1	96.9	93	92	0.035	1.91
CF_3		**III-34**	4.2	N.D.	85	83	N.D.	1.56

Table 3-3. SARs and SPRs of 1-oxa-4,9-diazaspiro[5.5]undecane-based urea derivatives: heteroaromatics.

R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability	cLogP
			Human	Rat	JP1[b]	JP2[c]		
OCF_3		**III-35**	0.6	11.6	90	89	0.062	2.10
OCF_3		**III-36**	0.9	8.8	91	92	0.012	0.90
OCF_3		**III-37**	1.8	>20	96	93	0.029	1.49

[a] Units: mL/min/mg protein.

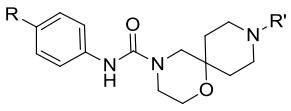
[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia.

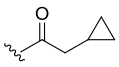
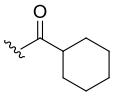
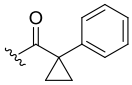
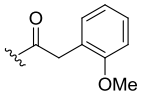
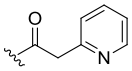
[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia.

[d] N.D.: Not determined.

Table 3-4 shows the results for derivatives with an aliphatic amide. **III-38** with a pivalamide moiety had high inhibitory activity against human and rat sEH, and **III-39** also showed increased human sEH inhibitory activity, but its rat sEH inhibitory activity was 0.4-fold that of **III-38**. **III-40** exhibited potent sEH inhibitory activity but was labile to CYP-mediated metabolism. The derivatives with bulky substituents (**III-41** to **III-43**) had moderate human sEH inhibitory activity, but showed decreased rat sEH inhibitory activity.

Table 3-4. SARs and SPRs of 1-oxa-4,9-diazaspiro[5.5]undecane-based urea derivatives: aliphatic amides.



R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability	cLogP
			Human	Rat	JP1[b]	JP2[c]		
CF_3		**III-38**	2.9	12	76	73	0.098	2.07
CF_3		**III-39**	2.1	31.4	79	79	0.074	1.81
OCF_3		**III-40**	0.6	14.5	69	63	0.357	2.67
OCF_3		**III-41**	0.4	28	37	33	0.228	3.25
OCF_3		**III-42**	0.5	71.6	66	59	0.461	2.82
OCF_3		**III-43**	0.7	>20	92	90	0.132	1.41

[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with the Japanese Pharmacopoeia.

[c] Simulated intestinal fluid compliant with the Japanese Pharmacopoeia.

[d] N.D.: Not determined.

Considering sEH inhibitory activity, solubility and stability to CYP-mediated metabolism, I selected compounds **III-22**, **III-28**, and **III-36** and evaluated their pharmacokinetic profiles (Table 3-5). **III-22** had good bioavailability, whereas **III-36** had modest bioavailability, which was attributed to low membrane permeability resulting from low lipophilicity (cLogP: 0.90).

Table 3-5. Pharmacokinetic profiles of **III-22**, **III-28**, and **III-36** in rat. [a]

Compound	i.v. (0.2 mg/kg)					p.o. (1 mg/kg)			
	C_{5min} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	$t_{1/2}$ (hr)	CL_{tot} (mL/hr/kg)	V_{dss} (mL/kg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	t_{max} (hr)	F (%)
III-22	71	173	2.63	1157	3562	20.4	306	1	35.4
III-28	77.7	90.2	1.17	2216	3315	9.58	123	2	24.1
III-36	134	111	1.53	1808	1825	8.64	61.3	1	11.1

[a] i.v.: Intravenous. p.o.: Oral administration. C: Concentration. AUC: Area under the blood concentration time curve. $t_{1/2}$: Half-life. CL_{tot}: Total body clearance. V_{dss}: Volume of distribution. F: Oral bioavailability.

Next the effect of **III-22** on serum creatinine levels in a rat model of anti-GBM glomerulonephritis was investigated [1] (Figure 3-4). Serum creatinine levels were significantly higher than in normal rat and increased in a time-dependent manner. Oral administration of **III-22** at 30 mg/kg significantly reduced serum creatinine in the rat model. The result indicates that **III-22** prevented the progression of glomerulonephritis. I do not yet know why 2,8-diazaspiro[4.5]decane-based trisubstituted ureas failed to reduce serum creatinine levels in the rat model, but I speculate that these derivatives did not sufficiently penetrate into renal tissue.

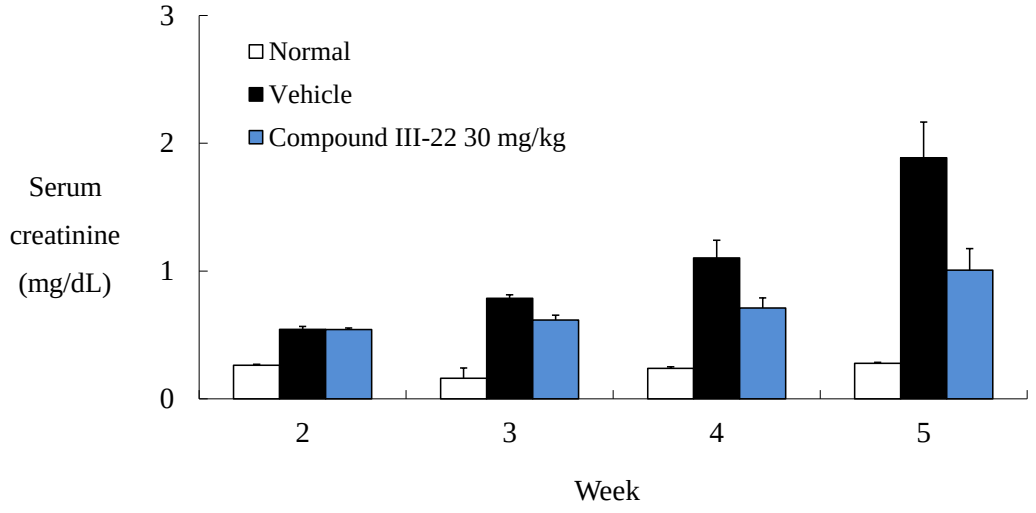


Figure 3-4 Effect of **III-22** on serum creatinine in a rat model of anti-GBM glomerulonephritis. **III-22** (30 mg/kg) was orally administered once daily for 3 weeks starting at 2 weeks after injection of anti-GBM antibody.

Summary

In conclusion, I identified 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted ureas as highly potent sEH inhibitors and orally active agents for treating chronic kidney diseases. **III-22** exhibited excellent inhibitory activity against sEH and excellent bioavailability, as well as a renal protective effect in a rat model of anti-GBM glomerulonephritis. These results suggest that **III-22** is an orally active drug candidate for treating chronic kidney diseases.

Experimental Section

General Information

All reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. ^{1}H NMR spectra were recorded on JNM-AL400 at 400 MHz and are referenced to an internal standard of tetramethylsilane (TMS, δ=0). Chemical shifts are given in ppm. Coupling constants (*J*) are given in Hz. Multiplicities are abbreviated as singlet (s), doublet (d), triplet (t), quartet (q), doublet‐doublet (dd), multiplet (m), and broad (br). Mass spectra were recorded with electron-spray ionization (ESI) on a Waters ZQ-2000.Thin layer chromatography was performed using Merck Kieselgel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV-light at 254 nm and color reagents. Flash chromatography was performed using Yamazen HI-FLASH COLUMNS (Particle Size：40 μm). Solvents were removed by rotary evaporation.

4-(Aminomethyl)-1-benzylpiperidin-4-ol (III-3).

Under argon atmosphere, to a mixture of 1-benzylpiperidin-4-one (**III-2**) (20 g, 106 mmol) and triethylamine (3.7 ml, 26.4 mmol) was added trimethylsilyl cyanide (15.6 g, 116 mmol). The solution was stirred at rt for 10 min. To a suspension of $LiAlH_4$ (2.1 g, 55 mmol) in THF (100 ml) cooled at 0 °C the reaction mixture was added. The solution was refluxed for 3 h. To the solution cooled at 0 °C water (5.2 ml) was added 1M aqueous NaOH (5.2 ml) and water (10.4 ml). The solution was stirred at 0 °C for 10 min. The mixture was filtered through a pad of Celite. The solvent was evaporated under reduced pressure to afford **III-3** in 87% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50-1.70 (4H, m), 2.30-2.42 (2H, m), 2.60 (2H, s), 2.60-2.70 (2H, m), 3.53 (2H, s), 7.21-7.35 (5H, m).

***N*-((1-Benzyl-4-hydroxypiperidin-4-yl)methyl)-2-chloroacetamide (III-4).**

To a solution of 4-(aminomethyl)-1-benzylpiperidin-4-ol (**III-3**) (0.50 g, 2.3 mmol) and triethylamine (3.7 ml, 26.4 mmol) in dichloromethane (10 ml) was added 2-chloroacetyl chloride (0.22 mL, 2.7 mmol). The solution was stirred at rt for 10 min. 1M aqueous HCl (9 ml) was added. The solution was stirred at rt for 10 min. 1M aqueous NaOH (10 ml) was added and the aqueous layer was extracted with dichloromethane. The combined organic

layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by amine silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-4** in 64% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.55-1.71 (4H, m), 2.31-2.40 (2H, m), 2.58-2.66 (2H, m), 3.36 (2H, d, *J* = 5.9 Hz), 3.53 (2H, s), 4.08 (2H, s), 6.94 (1H, brs), 7.21-7.35 (5H, m).

9-Benzyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one (III-5).

To a suspension of potassium *tert* –butoxide in THF (5.0 ml) was added *N*-((1-benzyl-4-hydroxypiperidin-4-yl)methyl)-2-chloroacetamide (**III-4**) (0.50 g, 1.7 mmol) in THF (5.0 ml). The solution was warmed up to rt and stirred for 35 min. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (methanol/chloroform) to afford **III-5** in 72% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.65-1.70 (2H, m), 1.87-1.95 (2H, m), 2.32-2.40 (2H, m), 2.57-2.64 (2H, m), 3.24 (2H, d, *J* = 2.7 Hz), 3.53 (2H, s), 4.16 (2H, s), 7.22-7.35 (5H, m).

MS (ESI) m/z 261 $[M+H]^+$.

9-Benzyl-1-oxa-4,9-diazaspiro[5.5]undecane (III-6).

To a suspension of $LiAlH_4$ (0.17 g, 4.6 mmol) in THF (10 ml) cooled at 0 °C was added 9-benzyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one (**III-5**) (0.60 g, 2.3 mmol). The solution was stirred at rt for 30 min. The solution was refluxed for 10 min. To the solution cooled at 0 ℃ water (0.17 ml) was added 1M aqueous NaOH (0.17 ml) and water (0.35 ml). The solution was stirred at 0 °C for 10 min. The mixture was filtered through a pad of Celite. The solvent was evaporated under reduced pressure to afford **III-6** in 82% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49-1.55 (2H, m), 1.88-1.95 (2H, m), 2.31-2.39 (2H, m), 2.49-2.57 (2H, m), 2.69 (2H, s), 2.81-2.83 (2H, m), 3.51 (2H, s), 3.63-3.67 (2H, m), 7.21-7.33 (5H, m).

9-Benzyl-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-7-1).

To a solution of 9-benzyl-1-oxa-4,9-diazaspiro[5.5]undecane (**III-6**) (2.3 g, 9.3 mmol) in chloroform (20 ml) cooled at 0 °C was added 4-(trifluoromethoxy)phenyl isocyanate (1.9 g, 9.3 mmol). The solution was

stirred at rt for 30 min. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-7-1** in 90% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57-1.67 (2H, m), 1.83-1.92 (2H, m), 2.36-2.44 (2H, m), 2.50-2.57 (2H, m), 3.48 (2H, t, *J* = 5.1 Hz), 3.52 (2H, s), 3.76 (2H, t, *J* = 5.1 Hz), 6.33 (1H, s), 7.14 (2H, d, *J* = 8.3 Hz), 7.22-7.34 (5H, m), 7.36 (2H, d, *J* = 8.3 Hz).

MS (ESI) m/z 450 $[M+H]^+$.

_N_-(4-(Trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-8-1).

To a solution of 9-benzyl-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (**III-7-1**) (3.8 g, 8.4 mmol) in methanol (50 ml) was added 20% $Pd(OH)_2$ on carbon (1.8 g). Under hydrogen atmosphere, the mixture was stirred at rt for 16 h and filtered through a pad of Celite. The solvent was removed under reduced pressure to afford **III-8-1** in 88% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47-1.57 (2H, m), 1.78-1.87 (2H, m), 1.97 (1H, brs), 2.75-2.84 (2H, m), 2.85-3.00 (2H, m), 3.35 (2H, s), 3.45-3.51 (4H, m), 3.77 (2H, t, *J* = 5.2 Hz), 6.44 (1H, s), 7.15 (2H, d, *J* = 9.1 Hz), 7.38 (2H, d, *J* = 9.1 Hz).

MS (ESI) m/z 360 $[M+H]^+$.

9-Benzyl-_N_-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-7-2).

Starting from 4-(trifluoromethyl)phenyl isocyanate (1.7 g, 8.9 mmol) the title compound was obtained following the procedure described for 9-benzyl-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide **(III-7-1)** in 72% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.55-1.74 (2H, m), 1.85-1.93 (2H, m), 2.37-2.46 (2H, m), 2.51-2.57 (2H, m), 3.35 (2H, s), 3.50 (2H, t, *J* = 5.0 Hz), 3.53 (2H, s), 3.77 (2H, t, *J* = 5.0 Hz), 6.44 (1H, s), 7.22-7.33 (5H, m), 7.46 (2H, d, *J* = 8.8 Hz), 7.54 (2H, d, *J* = 8.8 Hz).

MS (ESI) m/z 434 $[M+H]^+$.

9-Benzyl-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-8-2).

Starting from 9-benzyl-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide **(III-7-2)** (2.0 g, 4.6 mmol) the title compound was obtained following the procedure described for *N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide **(III-8-1)** in 88% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.70-1.57 (2H, m), 1.94 (1H, brs), 1.95-2.14 (2H, m), 3.00-3.05 (4H, m), 3.42 (2H, s), 3.49 (2H, m), 3.52 (2H, t, *J* = 5.0 Hz), 3.76 (2H, t, *J* = 5.0 Hz), 6.85 (1H, s), 7.52-7.65 (4H, m).

9-(2-Fluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-22).

To a solution of-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (**III-8-1**) (1.6 g, 4.6 mmol) and triethylamine (1.9 ml, 14 mmol) in dichloromethane (15 ml) cooled at 0 °C was added 2-fluorobenzoyl chloride (0.87 g, 5.5 mmol). The solution was stirred at 0 °C for 5 min. A saturated aqueous solution of $NaHCO_3$ was added and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-22** in 84% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52-1.63 (2H, m), 1.82-1.91 (1H, m), 2.03-2.10 (1H, m), 3.18-3.56 (7H, m), 3.71-3.85 (2H, m), 4.41-4.49 (1H, m), 6.66 (1H, s), 7.01-7.15 (3H, m), 7.16-7.22 (1H, m), 7.32-7.44 (4H, m).

MS (ESI) m/z 482 $[M+H]^+$.

9-(5-Methylisoxazole-3-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-31).

To a solution of-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (**III-8-1**) (0.40 g, 1.1 mmol), 5-methylisoxazole-3-carboxylic acid (0.14 g, 1.1 mmol) and DIPEA (0.49 ml, 2.8 mmol) in DMF (4.2 ml) was added HATU (0.66 g, 1.7 mmol). The solution was stirred at rt for 63 h. A saturated aqueous solution of $NaHCO_3$ was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Th

e crude product was purified by amine silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-31** in 92% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.51-1.65 (2H, m), 1.94-2.08 (2H, m), 2.46 (3H, s), 3.19-3.27 (1H, m), 3.35 (2H, s), 3.45-3.53 (3H, m), 3.74-3.84 (2H, m), 4.09-4.15 (1H, m), 4.36-4.43 (1H, m), 6.24 (1H, s), 6.50 (1H, s), 7.14 (2H, d, *J* = 8.3 Hz), 7.37 (2H, d, *J* = 8.3 Hz).

MS (ESI) m/z 469 $[M+H]^+$.

Following the procedure described for 9-(2-fluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (**III-22**) using corresponding acyl halides or the procedure described for 9-(5-methylisoxazole-3-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (**III-31**) using corresponding aminoacids compounds in Table 3-6 were obtained.

Table 3-6. Data of compunds.

Compound	Name	^{1}H NMR ($CDCl_3$, 400MHz)	MS(ESI) $[M+H]^+$
III-23	9-(2,6-difluorobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.50-1.65 (2H, m), 1.86-1.93 (1H, m), 2.08-2.16 (1H, m), 3.20-3.62 (7H, m), 3.73-3.85 (2H, m), 4.47-4.55 (1H, m), 6.88-6.97 (2H, m), 7.11 (1H, s), 7.27-7.33 (1H, m), 7.45 (2H, d, *J* = 9.3 Hz), 7.48 (2H, d, *J* = 9.3 Hz).	484
III-24	9-benzoyl-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.38-1.66 (2H, m), 1.81-1.89 (1H, m), 1.98-2.09 (1H, m), 3.39-3.70 (7H, m), 3.71-3.81 (2H, m), 4.31-4.45 (1H, m), 7.07 (1H, s), 7.35-7.51 (9H, m).	448
III-25	9-(2-chlorobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.35-1.55 (2H, m), 1.80-1.92 (1H, m), 2.01-2.14 (1H, m), 3.19-3.62 (7H, m), 3.71-3.85 (2H, m), 4.39-4.53 (1H, m), 6.82 (1H, s), 7.21-7.56 (8H, m).	482
III-26	9-(3-chlorobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.40-1.68 (2H, m), 1.84-1.97 (1H, m), 1.98-2.12 (1H, m), 3.22-3.62 (7H, m), 3.72-3.83 (2H, m), 4.27-4.42 (1H, m), 6.85 (1H, s), 7.21-7.41 (4H, m), 7.45 (2H, d, *J* = 8.8 Hz), 7.51 (2H, d, *J* = 8.8 Hz).	482

III-27	9-(4-chlorobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.49-1.69 (2H, m), 1.82-2.13 (2H, m), 3.22-3.65 (7H, m), 3.72-3.85 (2H, m), 4.26-4.42 (1H, m), 6.77 (1H, s), 7.32 (2H, d, *J* = 8.5 Hz), 7.38 (2H, d, *J* = 8.5 Hz), 7.45 (2H, d, *J* = 8.8 Hz), 7.52 (2H, d, *J* = 8.8 Hz).	482
III-28	9-(2-acetamidobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.42-1.65 (2H, m), 1.76-2.09 (2H, m), 2.13 (3H, s), 3.25-3.68 (7H, m), 3.73-3.82 (2H, m), 4.12-4.45 (1H, m), 6.74 (1H, s), 7.06-7.12 (1H, m), 7.16-7.22 (1H, m), 7.35-7.43 (1H, m), 7.47 (2H, d, *J* = 8.8 Hz), 7.53 (2H, d, *J* = 8.8 Hz), 8.12-8.17 (1H, m), 8.87 (1H, s).	506
III-29	9-(4-cyanobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.42-1.78 (2H, m), 1.85-1.95 (1H, m), 2.02-2.11 (1H, m), 3.25-3.55 (7H, m), 3.76-3.88 (2H, m), 4.32-4.45 (1H, m), 6.46 (1H, s), 7.46 (2H, d, *J* = 8.5 Hz), 7.50 (2H, d, *J* = 8.5 Hz), 7.55 (2H, d, *J* = 8.5 Hz), 7.71 (2H, d, *J* = 8.5 Hz).	473
III-30	9-(furan-2-carbonyl)-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.43-1.55 (2H, m), 1.91-2.05 (2H, m), 3.19-3.60 (6H, m), 3.80 (2H, t, *J* = 4.9 Hz), 4.10-4.39 (2H, m), 6.46 (1H, dd, *J* = 1.7, 3.4 Hz), 6.92 (1H, d, *J* = 3.4 Hz), 7.34 (1H, s), 7.43 (1H, d, *J* = 1.7 Hz), 7.50 (2H, d, *J* = 9.0 Hz), 7.54 (2H, d, *J* = 9.0 Hz).	438
III-32	9-(5-cyclopropylisoxazole-4-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.09-1.19 (2H, m), 1.20-1.27 (2H, m), 1.51-1.63 (2H, m), 1.95-2.04 (2H, m), 2.22-2.29 (1H, m), 3.21-3.61 (6H, m), 3.81 (2H, t, *J* = 4.8 Hz), 4.10-4.40 (2H, m), 6.36 (1H, s), 7.16 (2H, d, *J* = 8.8 Hz), 7.36 (2H, d, *J* = 8.8 Hz), 8.18 (1H, s).	495
III-34	9-picolinoyl-*N*-(4-(trifluoromethyl)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.53-1.68 (2H, m), 1.84-1.91 (1H, m), 2.00-2.10 (1H, m), 3.19-3.50 (6H, m), 3.67-3.82 (3H, m), 4.42-4.50 (1H, m), 7.05 (1H, s), 7.30-7.36 (1H, m), 7.48 (2H, d, *J* = 8.8 Hz), 7.52 (2H, d, *J* = 8.8 Hz), 7.57-7.62 (1H, m), 7.75-7.81 (1H, m), 8.54-8.60 (1H, m).	449
III-35	9-(4-chloropicolinoyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.55-1.69 (2H, m), 1.88-1.97 (1H, m), 2.01-2.11 (1H, m), 3.21-3.55 (6H, m), 3.65-3.83 (3H, m), 4.36-4.44 (1H, m), 6.56 (1H, s), 7.14 (2H, d, *J* = 8.8 Hz), 7.32-7.39 (3H, m), 7.62 (1H, d, *J* = 1.7 Hz), 8.46 (1H, d, *J* = 5.4 Hz).	499
III-36	9-(5-methylpyrazine-2-carbo	1.52-1.69 (2H, m), 1.91-1.99 (1H, m), 2.01-2.09	480

	nyl)-*N*-(4-(trifluoromethoxy) phenyl)-1-oxa-4,9-diazaspiro [5.5]undecane-4-carboxamid e	(1H, m), 2.62 (3H, s), 3.22-3.53 (6H, m), 3.72-3.84 (3H, m), 4.40-4.49 (1H, m), 6.49 (1H, s), 7.14 (2H, d, *J* = 8.0 Hz), 7.37 (2H, d, *J* = 8.0 Hz), 8.39 (1H, d, *J* = 1.5 Hz), 8.80 (1H, d, *J* = 1.5 Hz).	
III-37	9-(2-oxoindoline-5-carbonyl) -*N*-(4-(trifluoromethoxy)phe nyl)-1-oxa-4,9-diazaspiro[5. 5]undecane-4-carboxamide	1.42-1.63 (2H, m), 1.79-2.01 (2H, m), 3.21-3.55 (9H, m), 3.73-3.82 (2H, m), 4.09-4.51 (1H, m), 6.67 (1H, s), 6.83 (1H, d, *J* = 8.0 Hz), 7.12 (2H, d, *J* = 8.3 Hz), 7.22-7.38 (4H, m), 8.20 (1H, s).	519
III-38	9-pivaloyl-*N*-(4-(trifluoromet hyl)phenyl)-1-oxa-4,9-diazas piro[5.5]undecane-4-carboxa mide	1.28 (9H, s), 1.43-1.56 (2H, m), 1.89-1.97 (2H, m), 3.23-3.32 (2H, m), 3.39 (2H, s), 3.52 (2H, t, *J* = 5.0 Hz), 3.79 (2H, t, *J* = 5.0 Hz), 4.03-4.12 (2H, m), 6.86 (1H, s), 7.48 (2H, d, *J* = 8.8 Hz), 7.53 (2H, d, *J* = 8.8 Hz).	428
III-39	9-(2-cyclopropylacetyl)-*N*-(4 -(trifluoromethyl)phenyl)-1- oxa-4,9-diazaspiro[5.5]undec ane-4-carboxamide	0.11-0.19 (2H, m), 0.51-0.59 (2H, m), 0.97-1.09 (1H, m), 1.40-1.55 (2H, m), 1.89-1.98 (2H, m), 2.23-2.29 (2H, m), 3.01-3.11 (1H, m), 3.30-3.43 (3H, m), 3.45-3.63 (3H, m), 3.80 (2H, t, *J* = 5.2 Hz), 4.21-4.31 (1H, m), 6.87 (1H, s), 7.50 (2H, d, *J* = 8.8 Hz), 7.54 (2H, d, *J* = 8.8 Hz).	426
III-41	9-(1-phenylcyclopropanecarb onyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspir o[5.5]undecane-4-carboxami de	1.01-1.49 (6H, m), 1.59-1.69 (1H, m), 1.84-1.97 (1H, m), 3.09-3.25 (2H, m), 3.28 (2H, s), 3.45 (2H, t, *J* = 5.0 Hz), 3.66-3.83 (3H, m), 4.12-4.26 (1H, m), 6.63 (1H, s), 7.10-7.36 (9H, m).	505
III-42	9-(2-(2-methoxyphenyl)acet yl)-*N*-(4-(trifluoromethoxy)p henyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide	1.25-1.60 (2H, m), 1.75-1.82 (1H, m), 1.86-1.95 (1H, m), 3.05-3.15 (1H, m), 3.26 (2H, s), 3.30-3.39 (1H, m), 3.40-3.51 (2H, m), 3.59-3.78 (5H, m), 3.78 (3H, s), 4.21 -4.29 (1H, m), 6.69 (1H, s), 6.82-6.86 (1H, m), 6.85-6.76 (1H, m), 7.13 (2H, d, *J* = 9.0 H), 7.09-7.16 (2H, m), 7.36 (2H, d, *J* = 9.0 Hz).	509
III-43	9-(2-(pyridin-2-yl)acetyl)-*N*- (4-(trifluoromethoxy)phenyl) -1-oxa-4,9-diazaspiro[5.5]un decane-4-carboxamide	1.29-1.43 (2H, m), 1.79-1.84 (2H, m), 3.03-3.12 (1H, m), 3.28 (2H, s), 3.36-3.52 (3H, m), 3.75 (2H, t, *J* = 5.1 Hz), 3.78-3.96 (1H, m), 3.89 (1H, d, *J* = 14.6 Hz), 3.95 (1H, d, *J* = 14.6 Hz), 4.21-4.27 (1H, m), 6.73 (1H, s), 7.11-7.18 (3H, m), 7.27-7.32 (1H, m), 7.35-7.37 (2H, m), 7.60-7.66 (1H, m), 8.49-8.51 (1H, m).	479

9-(2-Fluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (III-11).

To a solution of 9-benzyl-1-oxa-4,9-diazaspiro[5.5]undecane (**III-6**) (18 mg, 0.071 mmol) and triethylamine (0.015 ml, 0.11 mmol) in dichloromethane (1 ml) was added 2,6-difluorobenzoyl chloride (14 mg, 0.079 mmol). The solution was stirred at rt for 5 min. 1M aqueous NaOH was added and and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford (9-benzyl-1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)(2,6-difluorophenyl)methanone (32.4 mg). To a solution of (9-benzyl-1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)(2,6-difluorophenyl)methanone (32.4 mg) in methanol (1 ml) was added 20% $Pd(OH)_2$ on carbon (10 mg). Under hydrogen atmosphere, the mixture was stirred at rt for 12 h and filtered through a pad of Celite. The solvent was removed under reduced pressure to afford (2,6-difluorophenyl)(1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)methanone (23.2 mg). To a solution of (2,6-difluorophenyl)(1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)methanone (10.8 mg) and triethylamine (0.008 ml, 0.058 mmol) in chloroform (1 ml) was added 4-(trifluoromethoxy)phenyl isocyanate (8.5 mg, 0.042 mmol). The solution was stirred at rt for 5 min. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (methanol/chloroform) to afford **III-11** in 3steps 37% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20-1.35 (1H, m), 1.61-1.75(1H, m), 1.91-1.99 (2H, m), 3.11-3.41 (4H, m), 3.68-3.89 (6H, m), 6.37 (0.40H, s), 6.47 (0.60H, s), 6.93-7.01 (2H, m), 7.10-7.18 (2H, m), 7.27-7.42 (4H, m).

MS (ESI) m/z 500 $[M+H]^+$.

***tert*-Butyl 4-formylpiperidine-1-carboxylate (III-13).**

To a solution of *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (**III-12**) (0.50 g, 2.3 mmol) in dichloromethane (10 ml) cooled at 0 °C was added Dess-Martin periodinane (1.1 g, 2.6 mmol). The solution was stirred at rt for 2 h. A aqueous solution of $Na_2S_2O_3$ and a saturated aqueous solution of $NaHCO_3$ were added. The

solution was stirred at rt for 5 min. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-13** in 53% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (9H, s), 1.52-1.65 (2H, m), 1.82-1.95 (2H, m), 2.93 (2H, brt, *J* = 10.6 Hz), 3.98-4.11 (2H, m), 9.67 (1H, s).

***tert*-Butyl 4-(2-cyanoethyl)-4-formylpiperidine-1-carboxylate (III-14).**

To a solution of *tert*-butyl 4-formylpiperidine-1-carboxylate (**III-13**) (0.26 g, 1.2 mmol) in 1,4-dioxane (0.50 ml) cooled at 0 °C were added acrylonitrile (88 μl, 1.3 mmol) and benzyltrimethylammonium hydroxide (20 μl, 10 % aqueous solution). The solution was stirred at rt for 10 h. A saturated aqueous solution of NH_4Cl was added. The aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-14** in 60% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39-1.51 (11H, m), 1.92 (2H, t, *J* = 7.9 Hz), 2.26 (2H, t, *J* = 7.9 Hz), 3.02 (2H, brt, *J* = 10.9 Hz), 3.70-3.89 (2H, m), 9.51 (1H, s).

***tert*-Butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate (III-15).**

To a solution of *tert*-butyl 4-(2-cyanoethyl)-4-formylpiperidine-1-carboxylate (**III-14**) (0.13 g, 0.49 mmol) in ethanol (6.5 ml) were added 10% Pd on carbon (0.11 g) and a HCl in methanol (0.15 ml). Under hydrogen atmosphere, the solution was stirred at rt for 25 h and filtered through a pad of Celite. The solvent was removed under reduced pressure. 1M aqueous HCl was added. The aqueous layer was washed with ethyl acetate. The aqueous layer was neutralized with 1M aqueous NaOH and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford **III-15** in 29% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41-1.75 (17H, m), 2.63 (2H, s), 2.78 (2H, brt, *J* = 5.2 Hz), 2.26 (4H, brt, *J* = 6.0 Hz).

MS (ESI) m/z 255 $[M+H]^+$.

***tert*-Butyl 2-((4-(trifluoromethoxy)phenyl)carbamoyl)-2,9-diazaspiro[5.5]undecane-9-carboxylate (III-16).**

To a solution of *tert*-butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate (**III-15**) (188 mg, 0.759 mmol) were added 4-(trifluoromethoxy)phenyl isocyanate (0.13 ml, 0.80 mmo) and triethylamine (120 μl, 0.87 mmol). The solution was stirred at rt for 5 min. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-16** in 50% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35-1.75 (17H, m), 3.11-3.52 (8H, m), 6.36 (1H, s), 7.14 (2H, d, *J* = 8.0 Hz), 7.36 (2H, d, *J* = 8.0 Hz).

9-(2,6-Difluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,9-diazaspiro[5.5]undecane-2-carboxamide (III-18).

To a solution of *tert*-butyl 2-((4-(trifluoromethoxy)phenyl)carbamoyl)-2,9-diazaspiro[5.5]undecane-9-carboxylate (**III-16**) (30.5 mg, 0.0667 mmol) in dichloromethane (2 ml) cooled at 0 °C was added TFA (0.50 ml). The solution was stirred at 0 °C for 30 min. The solvent was evaporated under reduced pressure. The mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. To solution of the crude product in dichloromethane (2 ml) cooled at 0 °C were added triethylamine (0.0702 ml, 0.504 mmol) and 2,6-difluorobenzoyl chloride (0.0151 ml, 0.121 mmol). The solution was stirred at 0 °C for 10 min. A saturated aqueous solution of $NaHCO_3$ and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-18** in 2steps 71% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36-1.73 (8H, m), 3.21 (1H, d, *J* = 13.2 Hz), 3.23-3.71 (6H, m), 3.80-4.04 (1H, m), 6.52 (1H, s), 6.92 (2H, brt, *J* = 8.0 Hz), 7.12 (2H, d, *J* = 8.3 Hz), 7.28-7.38 (3H, m).

MS (ESI) m/z 498 $[M+H]^+$.

***tert*-Butyl 2-(2,6-difluorobenzoyl)-2,9-diazaspiro[5.5]undecane-9-carboxylate (III-19).**

Starting from *tert*-butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate (**III-15**) (36.7 mg, 0.144 mmol) and 2,6-difluorobenzoyl chloride (0.0181 ml, 0.12 mmol) the title compound was obtained following the procedure described for 9-(2-fluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-1-oxa-4,9-diazaspiro[5.5]undecane-4-carboxamide (**III-22**) in 30% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32-1.63 (17H, m), 2.98-3.78 (8H, m), 6.85-6.99 (2H, m), 7.29 -7.40 (1H, m).

MS (ESI) m/z 395 $[M+H]^+$.

2-(2,6-Difluorobenzoyl)-*N*-(4-(trifluoromethoxy)phenyl)-2,9-diazaspiro[5.5]undecane-9-carboxamide (III-21).

To a solution of *tert*-butyl 2-(2,6-difluorobenzoyl)-2,9-diazaspiro[5.5]undecane-9-carboxylate (**III-19**) (17.1 mg, 0.043 mmol) in dichloromethane (1 ml) cooled at 0 °C was added TFA (0.50 ml). The solution was stirred at 0 °C for 1 h. The solvent was evaporated under reduced pressure. The mixture was neutralized with 1M aqueous NaOH and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. To solution of the crude product in chloroform (1 ml) cooled at 0 °C were added triethylamine (0.0078 ml, 0.056 mmol) and 4-(trifluoromethoxy)phenyl isocyanate (0.006 ml, 0.041 mmol). The solution was stirred at 0 °C for 5 min. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate-*n*-hexane) to afford **III-21** in 2steps 76% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35-1.70 (8H, m), 3.11-3.82 (8H, m) 6.50 (0.18H, s), 6.78 (0.82H, s), 6.81-6.99 (2H, m), 7.05-7.15 (2H, m), 7.30-7.41 (3H, m).

MS (ESI) m/z 498 $[M+H]^+$.

Measurement of *in vitro* sEH inhibitory activity.

The sEH inhibition assays were performed as described by Jones, P. D.; Wolf, N. M.; Morisseau, C.; Whetstone, P.; Hock, B.; Hammock, B. D.(*Anal. Biochem.* **2005**, *343*, 66.). A solution of recombinant sEH from human or mouse (the enzymes were purchased from Cayman Chemical Company) or rat (the enzyme was expressed in Sf9 insect cells using baculovirus) in buffer (BisTris–HCl, 25 mM, pH 7.0, containing 0.1 mg/ml BSA) was incubated with a inhibitor at room temperture for 30 min. To the resultant solution cyano(6-methoxy-naphthalen-2-yl)methyl trans-[(3-phenyloxiran-2-yl)methyl] carbonate (purchased from Cayman Chemical Company) was added and incubated at room temperture for 20-45 min. $ZnSO_4$ was added and the resultant solution of fluorescence intensity (excitation filter 330 nm, emission filter 465 nm) was measured. The reduction rate of enzyme activity by inhibitors were calculated using the fluorescence intensity, and IC_{50} values were determined. In these assays IC_{50} values of a representative sEH inhibitor 12-(3-adamantan-1-ylureido)-dodecanoic acid (AUDA) were 3 nM (human), 10 nM (murine), 10 nM (rat).

Protocol of Docking Studies

The docking studies in this chapter were effected by the same method as in chapter 2.

References and Notes

1. Krakower, C. A.; Nicholes, B. K.; Greenspon, S. A. *Proc. Soc. Exp. Biol. Med.* **1978**, *159*, 324.
2. Connors, R.V.; Dai, K.; Eksterowicz, J.; Fan, P.; Fisher, B.; Fu, J.; Li, K.; Li, Z.; McGee, L.R.; Sharma, R.; Wang, X.; McMinn, D.; Mihalic, J.; Deignan, J. PCT Int. Appl., WO 2009085185, 2009.
3. Nassar, A.-E. F.; Kamel, A. M.; Clarimont, C. *Drug Discov. Today* **2004**, *9*, 1020.

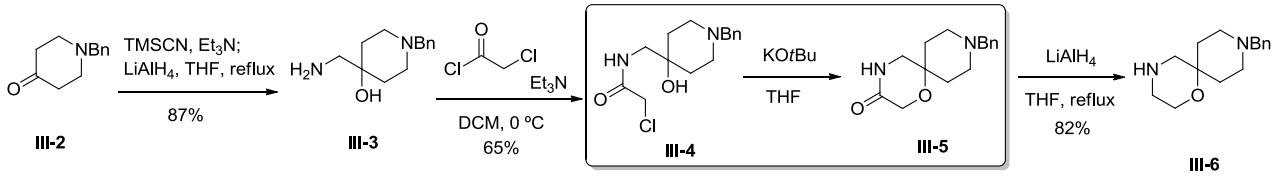
Chapter 4

Studies on synthesis of 1-oxa-4,9-diazaspiro[5.5]undecane scaffolds

Abstract: In the synthesis of **III-5**, which is a precursor of the 1-oxa-4,9-diazaspiro[5.5]undecane scaffold (**III-6**) described in Chapter 3, I faced a challenge dealing with the generation of by-products. I investigated the reaction conditions for the synthesis of **III-5** and found that reaction temperature and the order of addition of reagents affected the yield of **III-5** relative to by-products. **III-5** was isolated in 72% yield by conducting the reaction at -78 °C following a procedure where **III-4** was added to a solution of *t*-BuOK.

Introduction

As described in Chapter 3 I discovered 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted ureas as highly potent soluble epoxide hydrolase inhibitors and orally active agents for treating chronic kidney diseases. The synthesis of the derivatives was described in Scheme 3-1 of Chapter 3. I synthesized 1-oxa-4,9-diazaspiro[5.5]undecane and 2,9-diazaspiro[5.5]undecane scaffold **III-6**, a precursor of the derivatives, by following the procedure described in a patent[1] (Scheme 4-1). In the synthesis of **III-5**, I encountered the problem of reaction by-products. For studies on the physiological activities of the 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives, several grams of material are required. In addition the synthesized samples must not contain any impurities such as by-products or by-product derivatives. Aiming to synthesize several grams of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives in high yield and to reduce the generation of by-products, I investigated the reaction conditions for the synthesis of **III-5**, which was the most problematic step in the synthesis of the derivatives.



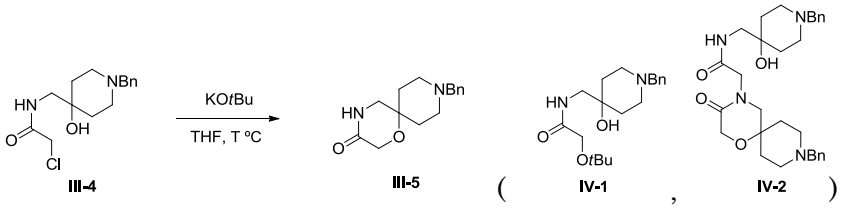
Scheme 4-1. Synthesis of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives.

Results and discussion

The reaction shown in entry 1 of Table 4-1 resulted in generation of a by-product (**IV-2**). This result led me to investigate the reaction conditions in order to improve the yield of **III-5** relative to by-products. Although the structure of **IV-2** has not been determined exactly, the postulated structure of **IV-2** suggested by ^{1}H-NMR spectra is shown in the figure of Table 4-1. Running the reaction at a lower temperature reduced the generation of **IV-2** (entries 2-4). At -78 °C (entry 4), the reaction proceeded slowly. **IV-2** was speculated to be a dimer of **III-5**, so I

expected that diluting the reaction medium would reduce the production of **IV-2**. Because dilution of reaction media would lower the efficiency of reaction, I adopted method b (entry 5 and 6). At room temperature (entry 5), the generation of by-product **IV-1**, which was the adduct of *tert*-butyl alcohol and **III-4** was observed. At lower temperature (entry 6), generation of by-product **IV-1** was reduced. Despite the reduced generation of **IV-2** in entry 4, those conditions were not selected because the reproducibility was insufficient due to the low solubility of *t*-BuOK in THF. I performed the reaction following the condition shown in entry 6 and isolated **III-5** in 72% yield. By developing these reaction conditions, I was able to prepare several grams of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives. I posited that in order to increase the yield of **III-5** and reduce the generation of by-products, the use of other bases and solvents should be investigated. The use of a non-nucleophilic base would prevent the generation of an adduct like **IV-I**. The use of other solvents would alter the reactivity of the alkoxide toward **III-4**.

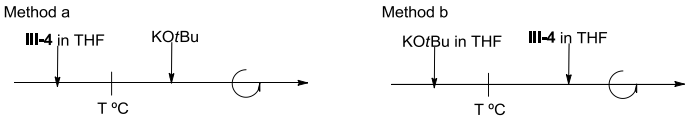
Table 4-1. Reaction conditions for synthesis of **III-5**.



entry	method [b]	T (°C)	t (min.)	ratio(mol)[a]		
				III-5	**IV-1**	**IV-2**
1	a	rt	10	1	-	1
2	a	0	10	4.6	-	1
3	a	-18	10	17	-	1
4	a	-78~rt	30	19	-	1
5	b	0	10	1	1	-
6	b	-78~rt	30	14	1	-

[a] Determined by ^{1}H-NMR spectral analysis.

[b] Method a and b are described below.



Summary

In the synthesis of **III-5**, which is a precursor of the scaffold **III-6**, I faced a challenge dealing with the generation of by-products. I investigated the reaction conditions for the synthesis of **III-5** and found that performing the reaction at lower temperature improved the yield of **III-5** relative to by-products **IV-1** and **IV-2**. Moreover, the order of addition of reagents had an effect in this regard. The procedure that *t*-BuOK was added to a solution of **III-4** gave by-product **IV-2**. A procedure where **III-4** was added to a solution of *t*-BuOK afforded by-product **IV-1**. **III-5** was isolated in 72% yield by conducting the reaction at -78°C and adding **III-4** to a solution of *t*-BuOK.

References and Notes

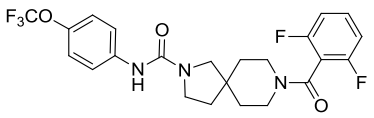
1. Connors, R.V.; Dai, K.; Eksterowicz, J.; Fan, P.; Fisher, B.; Fu, J.; Li, K.; Li, Z.; McGee, L.R.; Sharma, R.; Wang, X.; McMinn, D.; Mihalic, J.; Deignan, J. PCT Int. Appl., WO 2009085185, 2009.
2. The experimental data of synthesis of **III-5** was described in Chapter 3.

Chapter 5

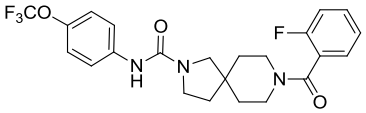
Summary

In this research, I was able to identify orally active soluble epoxide hydrolase (sEH) inhibitors for the treatment of hypertension and chronic kidney disease.

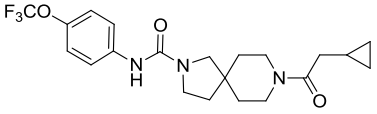
As described in Chapter 2, I identified 2,8-diazaspiro[4.5]decane-based trisubstituted ureas as highly potent sEH inhibitors and orally active agents for treating hypertension. In a structure–activity relationship study of the left-hand side of the ureas, I found that the potent human sEH inhibitor **II-21** was a poor mouse sEH inhibitor. Using X-ray crystal structures, I conducted docking studies of human and mouse sEHs with **II-21** and found steric hindrance around the side chain of Phe406 in mouse sEH. On the basis of the docking study results, I adopted a trifluoromethoxy moiety instead of a trifluoromethyl moiety in order to prevent such steric hindrance and succeed in improving mouse sEH inhibitory activity. The oral administration of **II-22**, **II-30**, and **II-47** at a dose of 30 mg/kg reduced blood pressure in spontaneously hypertensive rat, but had little effect on blood pressure in the normotensive rat.



II-22

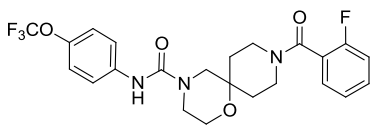


II-30



II-47

In Chapter 3, I described the identification of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted ureas as highly potent sEH inhibitors and orally active agents for treatment of chronic kidney diseases. **III-22** exhibited excellent inhibitory activity against sEH and excellent bioavailability, as well as a renal protective effect in a rat model of anti-glomerular basement membrane glomerulonephritis. These results suggest that **III-22** is an orally active drug candidate for treatment of chronic kidney diseases.



III-22

Chapter 4 presents my investigation of the reaction conditions for synthesizing **III-5**, a precursor of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted ureas, in order to prepare sufficient amounts of material for use in biological tests. I found that reaction temperature and the order of addition of reagents affected the yield of **III-5**. **III-5** was isolated in 72% yield by conducting the reaction at -78°C by a procedure where **III-4** was added to a solution of *t*-BuOK.

This research provided orally active sEH inhibitors for the treatment of hypertension with little effect on blood pressure and for the treatment of chronic kidney diseases. The toxicity, physical properties, and other properties of the derivatives should be evaluated in preclinical studies.

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Finally, I would like to dedicate this dissertation to my family.

Yuko Kato

List of publications and patents included in this thesis

Kato,Y.; Fuchi, N.; Saburi, H.; Nishimura, Y.; Watanabe, A.; Yagi, M.; Nakadera, Y.; Higashi, E.; Yamada, M.; Aoki, T. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5975.

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List of publications and patents not included in this thesis

A Homodinuclear $Mn(III)_2$-Schiff Base Complex for Catalytic Asymmetric 1,4-Additions of Oxindoles to Nitroalkenes

Kato, Y.; Furutachi, M.; Chen, Z.; Mitsunuma, H.; Matsunaga, S.; Shibasaki, M. *J. Am. Chem. Soc.* **2009**, *131*, 9168.

Catalytic Asymmetric Synthesis of Nitrogen-Containing *gem*-Bisphosphonates Using a Dinuclear Ni_2–Schiff Base Complex

Kato, Y.; Chen, Z.; Matsunaga, S.; Shibasaki, M. *Synlett* **2009**, 1635.

Stereodivergent Catalytic Doubly Diastereoselective Nitroaldol Reactions Using Heterobimetallic Complexes

Sohtome, Y.; Kato, Y.; Handa, S.; Aoyama, N.; Nagawa, K.; Matsunaga, S.; Shibasaki, M. *Org. Lett.* **2008**, 10, 2231.

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Chen, Z.; Furutachi, M.; Kato, Y.; Matsunaga, S.; Shibasaki, M. *Angewante Chemie., Int. Ed.* **2009**, *48*, 2218.

Nipecotic acid derivative and use thereof for medical purposes (sEH inhibitors)

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Cyclohexanediamide derivative and use thereof for medical purposes (sEH inhibitors)

Kurosawa, S.; Nishimura, Y. ; Kato,Y.; Fuchi, N.; Aoki, T.; Yamada, M.; Yamada, N. PCT Int. Appl., WO 2013161980, 2013.