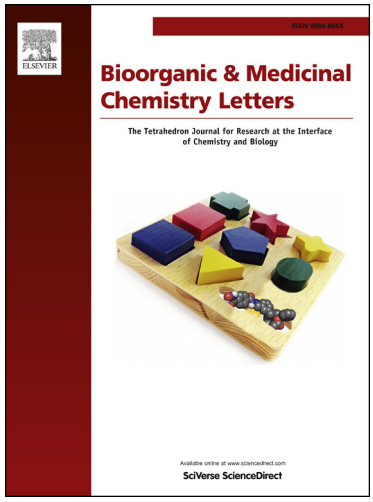

Discovery of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives as highly potent soluble epoxide hydrolase inhibitors and orally active drug candidates for treating of chronic kidney diseases

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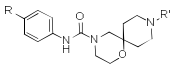

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Discovery of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives as highly potent soluble epoxide hydrolase inhibitors and orally active drug candidates for treating of chronic kidney diseases

Yuko Kato[a,b,*], Nobuhiro Fuchi[a], Yutaka Nishimura[a], Ayano Watanabe[a], Mai Yagi[a], Yasuhito Nakadera[a], Eriko Higashi[a], Masateru Yamada[a], Takumi Aoki[a], and Hideo Kigoshi[b]

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ABSTRACT

We identified 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. **19** exhibited excellent sEH inhibitory activity and bioavailability. When administered orally at 30 mg/kg, **19** lowered serum creatinine in a rat model of anti-glomerular basement membrane glomerulonephritis but 2,8-diazaspiro[4.5]decane-based trisubstituted ureas did not. These results suggest that **19** is an orally active drug candidate for treating chronic kidney diseases.

Soluble epoxide hydrolase (sEH) is an enzyme that catalyzes the conversion of epoxyeicosatrienoic acids (EETs) into dihydroxy eicosatrienoic acids (DHETs) (Figure 1),[1] and is mainly expressed in liver, kidney, and vascular tissue.[2] EETs, which are synthesized by CYP2J- and CYP2C-mediated epoxidation of arachidonic acid, are associated with physiologically beneficial effects such as vasodilatation, vasoprotection, and anti-inflammation.[3] Inhibition of sEH produces effects expected from an increase in EET level.[4]

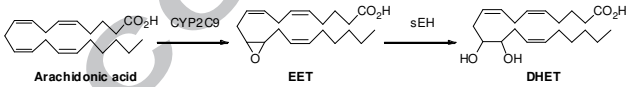

Figure 1. Role of sEH.

According to a recent report,[5] 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 level, 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.

We previously reported that 2,8-diazaspiro[4.5]decane-based trisubstituted ureas are highly potent sEH inhibitors and orally active drug candidates for treating hypertension (Figure 2, **1**).[6] Contrary to our expectation, oral administration of these derivatives failed to reduce serum creatinine in a rat model of anti-glomerular basement membrane (GBM) glomerulonephritis[7]. We found that 2,8-diazaspiro[4.5]decane-based trisubstituted ureas had high sEH inhibitory activity and oral availability. The solubility and microsomal stability of these compounds can be conveniently modified by changing the substituent on the amide group. On the basis of these findings, we hypothesized that spirocyclic diamine-based trisubstituted ureas could be developed as sEH inhibitors and we aimed to find other such ureas that inhibited sEH as strongly as 2,8-diazaspiro[4.5]decane-based trisubstituted ureas and had different pharmacokinetic profiles. Here we report that 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted ureas are highly potent sEH inhibitors and are available for oral administration in a rat model of anti-GBM glomerulonephritis.

The hydrolase catalytic pocket of sEH consists of Tyr381, Tyr465, and Asp333, which are responsible for its enzymatic activity. Amide or urea derivatives bind to the pocket via hydrogen bonds between the carbonyl oxygen of the amide or urea moiety and the hydroxyl group of Tyr381 or Tyr465, and

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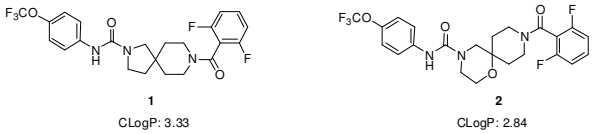


Figure 2. Structure of **1** (previously reported) and **2** (newly designed).

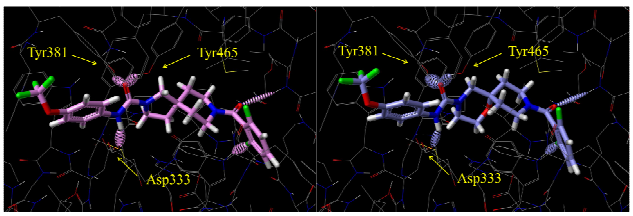
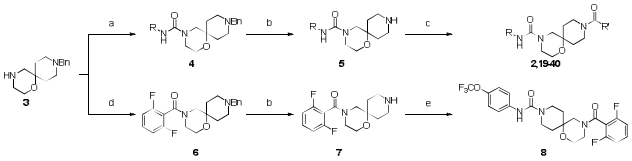


Figure 3. Docking studies of human sEH (PDB code: 1VJ5) with **1** (left) and **2** (right).

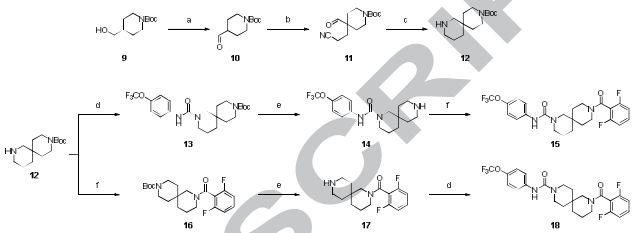
between the NH of the amide or urea moiety and the carboxylate of Asp333.[8] We previously reported that **1** (Figure 2) exhibits highly potent sEH inhibitory activity. A docking study of **1** with human sEH indicated that the oxygen atom of the compound's urea moiety binds to Tyr381 and Tyr465 in the hydrolase catalytic pocket of sEH (Figure 3). We then designed 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivative **2**. Because of its introduced oxygen atom, **2** has lower cLogP than **1** and thus would have higher aqueous solubility (Figure 2). A docking study of **2** with human sEH (Figure 3) suggested that the two carbonyl oxygen atoms of **2** bind to the hydrolase catalytic pocket in the same manner as **1**. Under this hypothesis, we began to explore 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives.

The general procedure for the synthesis of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives **2**, **19-40** and **8** is shown in Scheme 1. Starting material **3** was synthesized by a reported method.[9] Treatment of **3** with isocyanate afforded **4**. Removal of the benzyl protecting group by $Pd(OH)_2$-catalyzed hydrogenation provided **5**. Then, condensation with carboxylic acid or acyl chloride led to **2** and **19-40**. Treatment of **3** with benzoyl chloride afforded **6**. Removal of the benzyl protecting group by $Pd(OH)_2$-catalyzed hydrogenation provided **7**. Then, treatment with isocyanate led to **8**. The synthesis of 2,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives **15** and **18** is shown in Scheme 2. Oxidation of alcohol **9** with Dess-Martin periodinane afforded **10**. Michael addition to acrylonitrile provided **11**. Then, sequential reduction of nitrile group and cyclization via reductive amination gave 2,9-diazaspiro[5.5]undecane **12**. Treatment of **12** with isocyanate, removal of the Boc protecting group by TFA, then, treatment with benzoyl chloride led to **15**. Treatment of **12** with benzoyl chloride, removal of the Boc protectiong group by TFA, then, treatment with isocyanate led to **18**.

We performed structure–activity relationship (SAR) and structure–property relationship (SPR) studies on the diazaspiro scaffolds (Table 1).[10] 4-(Trifluoromethoxy)phenyl and 2,6-difluorobenzoyl groups were selected as the left- and right-hand substituents, respectively. As we expected, **2** had moderate inhibitory activity against human sEH and better solubility than **1**. However, the microsomal stability of **2** was lower than that of **1**. Lower sEH inhibitory activity was found in **8**, whose diazaspiro scaffold was constructed with the left- and right-hand substituents swapped. In **15**, which had a carbon atom in place of the oxygen atom in the 1-oxa-4,9-diazaspiro[5.5]undecane scaffold of **2**, sEH inhibitory activity was improved but microsomal stability and solubility became problematically low.



Scheme 1. Synthesis of 1-oxa-4,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives **2**, **19-40** and **8**. Reagents and conditions: (a) R-NCO, DIPEA, $CHCl_3$; (b) H_2, $Pd(OH)_2$, MeOH; (c) R'COOH, HATU, DIPEA, DMF or R'COCl, Et_3N, CH_2Cl_2. (d) 2,6-difluorobenzoyl chloride, Et_3N, CH_2Cl_2; (e) 4-(trifluoromethoxy)phenyl isocyanate, DIPEA, $CHCl_3$, 37% for 3steps.



Scheme 2. Synthesis of 2,9-diazaspiro[5.5]undecane-based trisubstituted urea derivatives **15** and **18**. Reagents and conditions: (a) Dess-Martin Periodinane, CH_2Cl_2, 53%; (b) trimethylbenzylammonium hydroxide 40% in water, acrylonitrile, 1,4-dioxane, 60%; (c) H_2, Pd/C, HCl, EtOH, 29%; (d) 4-(trifluoromethoxy)phenyl isocyanate, Et_3N, $CHCl_3$; (e) TFA, CH_2Cl_2, 0 °C; (f) 2,6-difluorobenzoyl chloride, Et_3N, CH_2Cl_2.

Replacing the oxygen atom of **8** also led to the lower solubility (**18**). Considering that **2** showed higher solubility, we next focused on derivatives with a 1-oxa-4,9-diazaspiro[5.5]undecane scaffold.

The SAR and SPR results for derivatives of **2** with benzamide moieties are shown in Table 2.[10] The removal of the fluorine atom from **2** improved rat sEH inhibitory activity, and also improved solubility and microsomal stability (**19**). Replacing the trifluoromethoxy group of **2** with a trifluoromethyl group (**20**) made little difference in activity or other properties. The effect of substituent position on the benzamide ring was investigated (**21**-**24**). Removing the substituent reduced human sEH inhibitory activity (**21**). Installing a chloro substituent at the *ortho* (**22**), *meta* (**23**), and *para* (**24**) positions gave the same human sEH inhibitory activity as **20**, and only **22** showed improved rat sEH inhibitory activity. **22** and **24** 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.[11] To achieve sufficient sEH inhibitory activity, solubility, and microsomal stability concurrently, we examined an *ortho*-substituted derivative with reduced lipophilicity (**25**); it had higher sEH inhibitory activity, solubility, and microsomal stability. Further investigation revealed that only a cyano group was acceptable at the *para* position on the benzamide ring: **26** exhibited good sEH inhibitory activity, solubility and microsomal stability.

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 [10] exhibited improved solubility and microsomal stability. However, we observed decreased human sEH inhibitory activity in **27** which contained an unsubstituted five-membered ring. The derivatives with five-membered rings bearing a methyl or

cyclopropyl substituent (**28-30**) exhibited increased human sEH inhibitory activity. Remarkably, pyrazole derivative **30**, which contained an NH moiety, was tolerated in humans and exhibited sEH inhibitory activity, but was not tolerated in rats. **31** with an unsubstituted pyridine ring showed lower activity, and **32** with a chloro substituent on the pyridine ring effectively inhibited sEH in humans and rats. A pyrazine ring was also an applicable substituent (**33**). Although **34** exhibited moderate human sEH inhibitory activity, its rat sEH inhibitory activity was low.

Table 4 [10] shows the results for derivatives with an aliphatic amide. **35** with a pivalamide moiety had high sEH inhibitory activity, and **36** also showed increased human sEH inhibitory activity, but its rat sEH inhibitory activity was 0.4-fold that of **35**. **37** exhibited potent sEH inhibitory activity but was labile to CYP-mediated metabolism. The derivatives with bulky substituents (**38**-**40**) had moderate human sEH inhibitory activity, but showed decreased rat sEH inhibitory activity.

We selected compounds **19**, **28**, and **33** and evaluated their pharmacokinetic profiles (Table 5). **19** had good bioavailability, whereas **33** had modest bioavailability, which was attributed to low permeability resulting from low lipophilicity (cLogP: 0.90).

We next investigated the effect of **19** on serum creatinine level in a rat model of anti-GBM glomerulonephritis[7] (Figure 4). The serum creatinine level was significantly higher than that of the normal rat and increased in a time-dependent manner. Oral administration of **19** at 30 mg/kg significantly reduced serum creatinine in the rat model. The result indicate that **19** prevented the progression of glomerulonephritis. We do not yet know why 2,8-diazaspiro[4.5]decane-based trisubstituted ureas failed to reduce serum creatinine in the rat model, but we speculate that these derivatives did not sufficiently penetrate into renal tissue. Currently, we are investigating the exposure of renal tissue to these ureas and **19** in order to elucidate the reason behind the difference in renal protective effect.

In conclusion, we 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. **19** exhibited excellent sEH inhibitory activity and bioavailability, as well as a renal protective effect in a rat model of anti-GBM glomerulonephritis. These results suggest that **19** is an orally active drug candidate for treating chronic kidney diseases. We are currently investigating further derivatization of **19**.

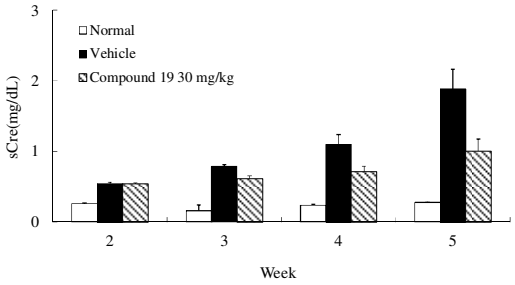
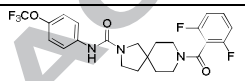
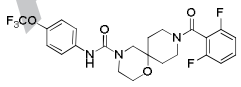
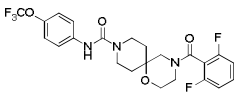
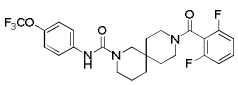
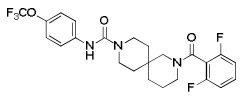


Figure 4 Effect of **19** on serum creatinine (sCre) in a rat model of anti-GBM glomerulonephritis. **19** (30 mg/kg) was orally administered once daily for 3 weeks from 2 weeks after injection of anti-GBM antibody.

Table 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]		
	**1**	0.4	4.5	24	21	0	3.33
	**2**	0.7	18.5	44	39	0.086	2.84
	**8**	5.4	N.D.[d]	52	46	N.D.	2.86
	**15**	0.125	3.3	9	9	0.602	3.89

	**18**	2.96	N.D.	0	0	N.D.	3.89

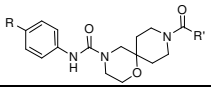
[a] Units: mL/min/mg protein.

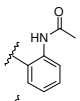
[b] Simulated gastric fluid compliant with Japanese Pharmacopeia.

[c] Simulated intestinal fluid compliant with Japanese Pharmacopeia.

[d] N.D.: Not determined.

Table 2. SAR and SPR of 1-oxa-4,9-diazaspiro[5.5]undecane-based urea derivatives.



R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability[a]	cLogP
			Human	Rat	JP1[b]	JP2[c]		
OCF_3		**2**	0.7	18.5	44	39	0.086	2.84
OCF_3		**19**	1.1	9.3	71	62	0.03	2.67
CF_3		**20**	1.3	19.1	43	38	0.093	3.04
CF_3		**21**	3.2	N.D.[d]	62	63	N.D.	2.66
CF_3		**22**	1.4	13	10	12	0.048	3.44
CF_3		**23**	1.2	36.9	26	21	0.1	3.44
CF_3		**24**	1.0	24.9	29	23	0.021	3.44
CF_3		**25**	0.8	6.8	92	93	0.039	2.14
CF_3		**26**	1.9	15	85	82	0.003	2.26

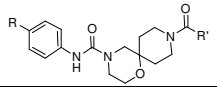
[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with Japanese Pharmacopeia.

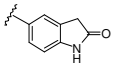
[c] Simulated intestinal fluid compliant with Japanese Pharmacopeia.

[d] N.D.: Not determined.

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



R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability[a]	cLogP
			Human	Rat	JP1[b]	JP2[c]		
CF_3		**27**	5.4	N.D.	75	75	N.D.[d]	1.83

OCF_3		**28**	1.4	25.7	84	83	0.047	1.22
OCF_3		**29**	0.6	22.2	86	89	0.087	1.67
OCF_3		**30**	1.1	96.9	93	92	0.035	1.91
CF_3		**31**	4.2	N.D.	85	83	N.D.	1.56
OCF_3		**32**	0.6	11.6	90	89	0.062	2.10
OCF_3		**33**	0.9	8.8	91	92	0.012	0.90
OCF_3		**34**	1.8	>20	96	93	0.029	1.49

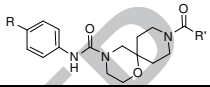
[a] Units: mL/min/mg protein.

[b] Simulated gastric fluid compliant with Japanese Pharmacopeia.

[c] Simulated intestinal fluid compliant with Japanese Pharmacopeia.

[d] N.D.: Not determined.

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



R	R'	Compound	sEH IC_{50}(nM)		Solubility (μg/mL)		Human liver microsomal stability[a]	cLogP
			Human	Rat	JP1[b]	JP2[c]		
CF_3		**35**	2.9	12	76	73	0.098	2.07
CF_3		**36**	2.1	31.4	79	79	0.074	1.81
OCF_3		**37**	0.6	14.5	69	63	0.357	2.67
OCF_3		**38**	0.4	28	37	33	0.228	3.25
OCF_3		**39**	0.5	71.6	66	59	0.461	2.82
OCF_3		**40**	0.7	>20	92	90	0.132	1.41

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

[b] Simulated gastric fluid compliant with Japanese Pharmacopeia.

[c] Simulated intestinal fluid compliant with Japanese Pharmacopeia.

Table 5. Pharmacokinetic profiles of **19**, **28,** and **33** in rat.

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 (%)
19	71	173	2.63	1157	3562	20.4	306	1	35.4
28	77.7	90.2	1.17	2216	3315	9.58	123	2	24.1
33	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.

References and notes

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Supplementary Material

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