Bioorganic & Medicinal Chemistry Letters 19 (2009) 5716–5721

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A strategy of employing aminoheterocycles as amide mimics to identify novel, potent and bioavailable soluble epoxide hydrolase inhibitors

Hong C. Shen [a,*], Fa-Xiang Ding [a], Qiaolin Deng [a], Suoyu Xu [b], Xinchun Tong [b], Xiaoping Zhang [c], Yuli Chen [c], Gaochao Zhou [c], Lee-Yuh Pai [d], Magdalena Alonso-Galicia [d], Sophie Roy [d], Bei Zhang [c], James R. Tata [a], Joel P. Berger [c], Steven L. Colletti [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA
[b] Department of Drug Metabolism, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA
[c] Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA
[d] Department of Cardiovascular Diseases, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA

ARTICLE INFO

Article history:
Received 13 July 2009
Revised 28 July 2009
Accepted 3 August 2009
Available online 7 August 2009

Keywords:
Amide mimics
Aminoheterocycles
Inhibitors
sEH
Soluble epoxide hydrolase

ABSTRACT

Distinct from previously reported urea and amide inhibitors of soluble epoxide hydrolase (sEH), a novel class of inhibitors were rationally designed based on the X-ray structure of this enzyme and known amide inhibitors. The structure–activity relationship (SAR) study was focused on improving the sEH inhibitory activity. Aminobenzisoxazoles emerged to be the optimal series, of which a potent human sEH inhibitor **7t** was identified with a good pharmacokinetics (PK) profile. The strategy of employing aminoheterocycles as amide replacements may represent a general approach to develop mimics of known hydrolase or protease inhibitors containing an amide moiety.

Soluble epoxide hydrolase, also referred to as sEH or *EPHX2*, is an enzyme that converts epoxyeicosatrienoic acids (EETs) to dihydroxy eicosatrienoic acids (DHETs).[1] As endogenous epoxides derived from arachidonic acid by cytochrome P450 epoxygenases,[2] EETs exhibit significant physiological effects leading to reduced blood pressure and myocardial perfusion in animal models.[3] These effects presumably result from the well-known roles of EETs to increase sodium renal excretion,[4] relax conduit vessels, and dilate renal afferent arterioles and coronary resistance vessels.[5] Furthermore, there are indications that EETs may be anti-atherosclerotic.[6] This hypothesis is based upon the observation that EETs modify leukocyte adhesion, platelet aggregation, vascular smooth muscle cell migration, and thrombolysis in preclinical animals.[7] Lastly, EETs are anti-inflammatory as they reduce cytokine-induced endothelial expression of several pro-inflammatory factors.[8]

Guided by the above observations, Arete Therapeutics recently developed an sEH inhibitor which is now entering phase II clinical trials for the metabolic syndromes.[9] The sEH inhibitor most extensively described in the literature is 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA), which appeared to have attained proof-of-concept with regard to blood pressure reduction and renal protection in a salt-sensitive and hypertensive animal model.[10] However, this class of disubstituted ureas is typically associated with poor pharmacokinetics and physical properties. Amide sEH inhibitors have also been reported. For example, a Taisho patent described an amide sEH inhibitor that lowered blood pressure in angiotensin II-induced hypertensive rats.[11] In addition to amides, chalcone oxides,[12] carbamates,[13] acyl hydrazones,[14] and *trans*-3-phenylglycidols[15] have also been reported as sEH inhibitors.

The X-ray crystallographic structures of human sEH and an inhibitor (4-(3-cyclohexyluriedo)-butyric acid) complex (PDB code: 1ZD3) revealed the catalytic pocket and key structural elements required to inhibit this enzyme. The hydrolase catalytic pocket of sEH consists of two tyrosine residues (Tyr381 and Tyr465) which act as hydrogen bond donors to facilitate the epoxide ring opening by Asp333.[16] It has been recognized that amide or urea groups fit well in the hydrolase catalytic pocket. Specifically, the carbonyl oxygen of the amide or urea is engaged in a hydrogen bond interaction with Tyr381 and Tyr465, and the N–H acts as a hydrogen bond donor to Asp333. Therefore, various ureas and amides have been developed as reversible sEH inhibitors.[17] However, novel sEH inhibitors beyond the scope of amides or ureas are uncommon.[12–15] Herein, we report our efforts in developing

* Corresponding author. Tel.: +1 732 594 1755; fax: +1 732 594 9473.
E-mail address: hong_shen@merck.com (H.C. Shen).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.08.006

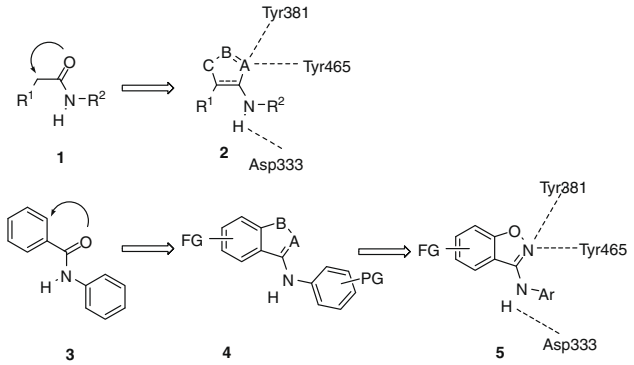


Figure 1. Pharmacophore of aminoheterocyclic sEH inhibitors.

aminobenzisoxazole derivatives as a novel class of sEH inhibitors. This bioisostere approach[18] may lead to potential advantages in intellectual property, target potency and pharmacokinetics (PK).

Based on known amide inhibitors of sEH such as general structures **1** and **3**,[17] the design of aminoheterocycle derivatives stemmed from the tethering strategy of the carbonyl of a benzamide with the adjacent side chain of **1** or benzene ring of **3** (Fig. 1). As a result, scaffolds **2** and **4** could be envisioned in which heteroatom A mimics the oxygen of the amide carbonyl, and the N–H group in **2** and **4** overlaps with the N–H of amides **1** and **3**, respectively. Therefore, the hydrogen bond interactions between amides and Tyr381, Tyr465 and Asp333 of sEH should be preserved in new scaffolds such as **2** and **4**. The structure–activity relationship (SAR) of such compounds was initially explored regarding different choices of monocyclic and fused bicyclic heterocycles. It was quickly demonstrated that aminobenzisoxazoles (**5**) appeared to be optimal and hence it was selected as a lead structure for further optimization. Herein we report the systematic and rational medicinal chemistry efforts that led to the discovery of a novel, potent, and bioavailable sEH inhibitor.

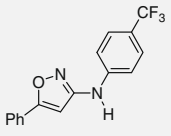
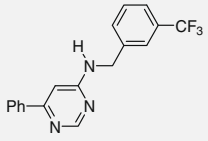
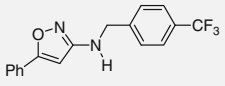
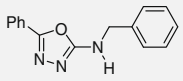
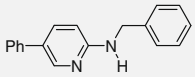
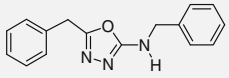
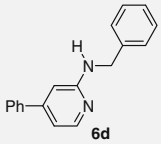
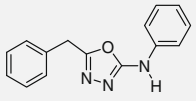
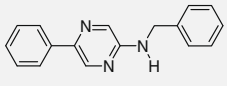
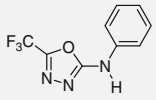
Initially, a scaffold hopping strategy[19] was employed to identify a hit from monocyclic aminoheterocycles shown in Table 1. The first round of screening of various amino-pyridine, pyrazine, oxadiazole and pyrimidine derivatives did not yield any potent inhibitors of the human sEH.

Subsequently, a reasonably comprehensive list of bicyclic aminoheterocyclic analogs was interrogated to identify a hit (Table 2). Among compounds including benzisoxazole **7a** and **7d**, benzisothiazole **7b**, indazole **7c**, benzothiazole **7e**, benzoxazole **7f**, and quinoxaline **7g**, only benzisoxazole **7a** provided good activity (IC_{50} = 25 nM) against human sEH. Therefore, **7a** was selectived as a hit for sEH and further SAR was based upon the aminobenzoxazole scaffold.

To introduce more polarity to this scaffold, pyridine-fused isoxazoles were prepared (**7h–k**). Distinct enzyme inhibitory activities were observed for the four pyridine-fused isoxazole regioisomers, of which **7h** and **7i** demonstrated better sEH inhibition than **7j** and **7k**. Aniline-derived analogs such as **7a** provided superior activity to benzylamine derivative **7d**. Other benzyl amine-derived analogs **7e–7g** were inactive against sEH.

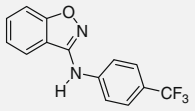
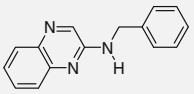
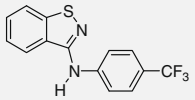
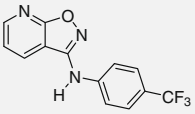
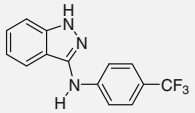
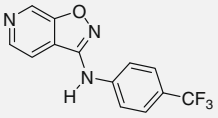
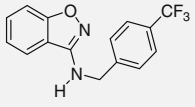
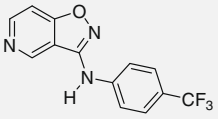
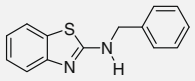
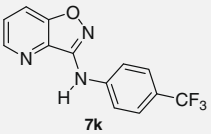
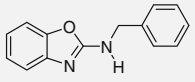
Taking benzisoxazole **7a** as the hit, the SAR was also explored in regard to the substitution of aniline (Table 3) and benzisoxazole (Table 4), respectively. Among various substitutions of the aniline, the *para*-CF_3 group gave the best sEH inhibition (**7a**). *ortho*- and *meta*-Substituted compounds were generally less active than the *para*-substituted analogs (**7a** vs **7l**, **7m** vs **7n**, and **7p** vs **7o**, **7q**). In addition, biphenyl or naphthylamine analogs **7p–s** were 8- to 50-fold less active against human sEH than compound **7a**.

Table 1
In vitro SAR of human sEH inhibition by monocyclic aminoheterocycles[a]

Compds	sEH IC_{50} (h) μM	Compds	sEH IC_{50} (h) μM
 **6a**	>10	 **6f**	>10
 **6b**	4.7	 **6g**	>10
 **6c**	>10	 **6h**	>10
 **6d**	>10	 **6i**	>10
 **6e**	>10	 **6j**	2

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.

Table 2
In vitro SAR of human sEH inhibition by bicyclic aminoheterocycles[a]

Compds	sEH IC_{50} (h), μM	Compds	sEH IC_{50} (h), μM
 **7a**	0.025	 **7g**	>10
 **7b**	2.5	 **7h**	0.027
 **7c**	>10	 **7i**	0.031
 **7d**	1.2	 **7j**	0.34
 **7e**	>10	 **7k**	1.1
 **7f**	>10		

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.

Substituting benzisoxazole with different functional groups effectively improved the sEH inhibitory activity of compounds. For example, CF_3-substituted analog **7t** had an IC_{50} of 4 nM for human sEH. The docking of **7t** in the binding pocket of sEH reveals a hydrogen bonding interaction between Tyr381 and the oxygen and nitrogen atoms of the benzisoxazole (Fig. 2). However, no direct hydrogen bond was identified between **7t** and Tyr465. The presence of a $-CF_3$ group dramatically enhanced its inhibitory activity against sEH versus **7a**, which is likely due to the additional favorable interaction between the $-CF_3$ fluoro atom and N–H of Gln382. In contrast, two other $-CF_3$ regioisomers, **7u** and **7v**, were less active. Regarding the other side of the scaffold, two aminopyridine-derived analogs (**7ee** and **7ff**) lost sEH inhibitory activity significantly.

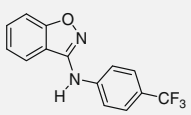
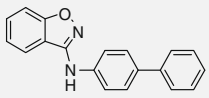
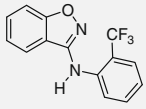
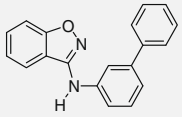
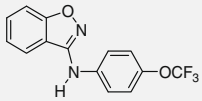
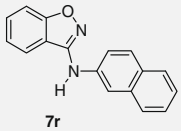
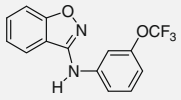
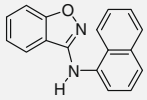
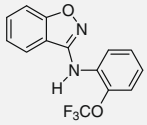
In further exploratory studies, various 6- and 7-aryl substituted benzisoxazoles were prepared. These compounds provided moderate to excellent sEH inhibitory activities (Table 4). Of particular interest were analogs **7w** with an IC_{50} of 8 nM, and **7z** and **7cc** with balanced human and rat enzyme activity (IC_{50} = 20–30 nM). With low nanomolar activities against human sEH, 7-arylbenzisoxazoles were typically more active than the corresponding 6-arylbenzisoxazoles (**7gg** vs **7w**, **7hh** vs **7x**, and **7ii** vs **7dd**). The introduction of a nitrogen atom into the ring in trifluoromethylpyridine analog **7jj** resulted in a fourfold loss of human sEH activity with respect to **7t**.

It is conceivable that by avoiding the potential in vivo amide hydrolysis by proteases, aminoheterocycle mimics of amides may improve PK properties especially bioavailability and clearance. Indeed, the rat PK profile of compound **7t** (Table 5) was characterized by excellent bioavailability (98%), good oral exposure (1.6 μM h kg/mg), moderate clearance (30 mL/min/kg), and good half-life (5.9 h).

It was later determined that the DHET inhibition of **7t** was shifted 300-fold in the presence of 10% human serum (IC_{50} = 1.2 μM) with respect to its human sEH enzyme inhibition (IC_{50} = 4 nM). Furthermore, **7t** was much less active against rat sEH (IC_{50} = 127 nM). Plasma protein binding data in human and rat plasma were also obtained. The free fraction of the radiolabeled **7t** was less than 1% in both species (0.9% for rat and 0.7% for human at compound concentrations of 0.5–25 μM). The collective data suggested that it was unlikely that **7t** would demonstrate robust sEH engagement in vivo, in contrast to several previously described sEH inhibitors.[17t]

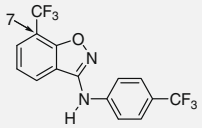
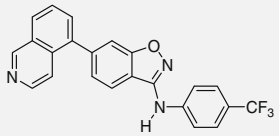
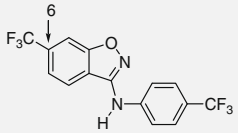
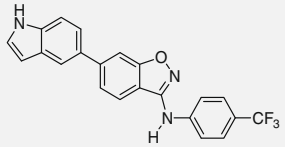
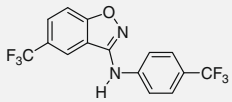
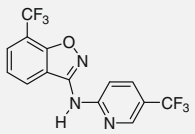
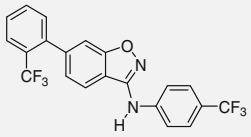
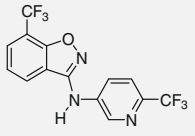
The synthesis of **7t** commenced with benzoic acid **8** (Scheme 1). The amide formation provided intermediate **9**, which then reacted with phosphorus pentachloride to produce chloroimine **10**. The subsequent reaction of compound **10** with trimethylsilyl hydroxyamine generated hydroxy amidine **11**, which upon a desilylation and a base-mediated cyclization, resulted in the formation of **7t** with excellent overall yield.[20] The same chemistry was readily applied to prepare all analogs described in Tables 3 and 4.

Table 3
In vitro SAR of human sEH inhibition by aminobenzisoxazoles[a]

Compds	sEH IC_{50} (h, r), μM	Compds	sEH IC_{50} (h, r), μM
 **7a**	0.025, 0.076	 **7p**	0.17, 0.74
 **7l**	0.96, —	 **7q**	0.96, —
 **7m**	0.06, 0.17	 **7r**	1.2, —
 **7n**	0.16, 0.16	 **7s**	0.84, 4.6
 **7o**	5.2, —		

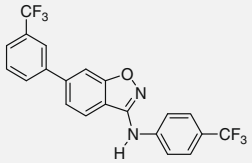
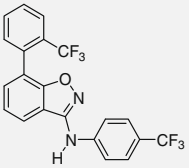
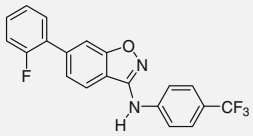
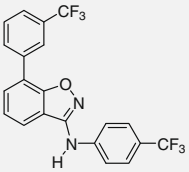
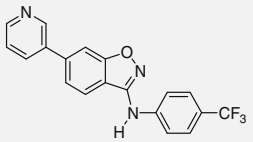
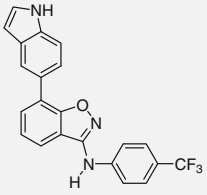
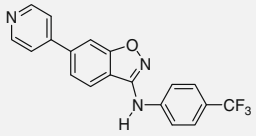
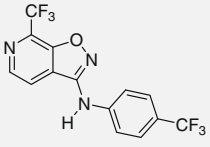
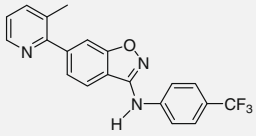
[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.

Table 4
In vitro SAR of human and rat sEH inhibition by substituted aminobenzisoxazoles[a]

Compds	sEH IC_{50} (h, r), μM	Compds	sEH IC_{50} (h, r), μM
 **7t**	0.004, 0.127	 **7cc**	0.019, 0.030
 **7u**	0.018, —	 **7dd**	0.07, 0.077
 **7v**	0.040, 1.5	 **7ee**	>10, >10
 **7w**	0.008, 0.07	 **7ff**	0.074, 3.4

(*continued on next page*)

Table 4 (*continued*)

Compds	sEH IC_{50} (h, r), μM	Compds	sEH IC_{50} (h, r), μM
 **7x**	0.47, 0.57	 **7gg**	0.004, 0.079
 **7y**	0.091, 0.055	 **7hh**	0.01, 0.13
 **7z**	0.023, 0.020	 **7ii**	0.008, 0.041
 **7aa**	0.058, 0.019	 **7jj**	0.019, 0.3
 **7bb**	0.07, 0.29		

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.

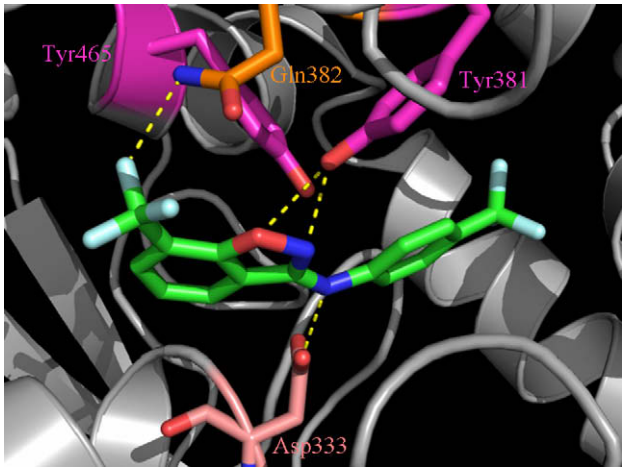


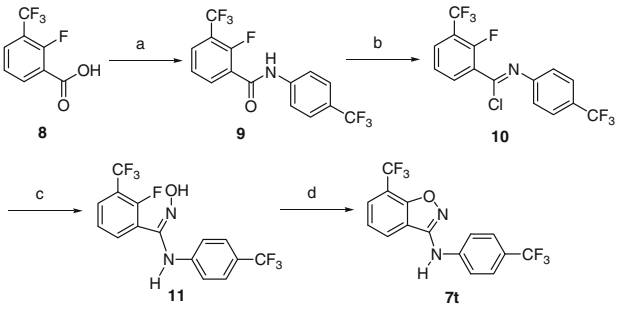
Figure 2. The minimized docking model of **7t** (green) in human sEH. The dashed lines illustrate atom pairs within hydrogen bond distance. The pictures were prepared by PyMOL (Delano Scientific LLC, South San Francisco, CA). The model is based on the x-ray crystallographic structure of 1ZD3 (PDB code): human soluble epoxide hydrolase 4-(3-cyclohexyluriedo)-butyric acid complex.

In conclusion, aminobenzisoxazole analogs were discovered as novel and potent sEH inhibitors. An optimized compound **7t** displayed a good PK profile and excellent human sEH inhibitory activity (IC_{50} = 4 nM). The success of this amide bioisostere approach may find utility in protease inhibitor programs in which the amide moiety of such inhibitors might be replaced with aminoheterocycles. In addition to intellectual property considerations, the obvious advantages include improved bioavailability and reduced clearance as the potentially metabolically labile amide is removed. Due to its modest rat sEH activity and significant serum shift, the sEH engagement of **7t** in rat is assumed to be rather poor. Future studies on the aminoheterocyclic inhibitor series should be direc-

Table 5
Rat PK of **7t**[a]

Compds	*F*%	Cl (mL/min/kg)	Vd_{ss} (L/kg)	$T_{1/2}$ (h)	$AUCN_{po}$ (μM h kg/mg)
7t	98	30	7	5.9	1.6

[a] Formulations: 1 mg/mL ethanol/PEG/water (20:40:40). IV dose: 1 mg/kg (n = 2). PO dose: 2 mg/kg (n = 3). Blood concentration was determined by LC/MS/MS following protein precipitation with acetonitrile.



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, Et_3N, DMF, then 4-trifluoromethylaniline, CH_2Cl_2, 1 h, 85%; (b) PCl_5, 65 °C, 2 h, 44%; (c) NH_2OTMS, THF, rt, 16 h, then 1 N HCl, 5 min, 61%; (d) KO*t*-Bu, NMP, 100 °C, 1.5 h, 95%.

ted towards reducing the impact of serum upon their potency by introducing polar groups. The potential indications of sEH inhibitors, including hypertension, diabetes, atherosclerosis, inflammation, and pain, are all worth pursuing once a potent sEH inhibitor in rat with good ex vivo (or in vivo) target engagement is identified.

References and notes

1. For a leading review: Newman, J. W.; Morisseau, C.; Hammock, B. D. *Prog. Lipid Res.* **2005**, *44*, 1.
2. (a) Yu, Z.; Xu, F.; Huse, L. M.; Morisseau, C.; Draper, A. J.; Newman, J. W.; Parker, C.; Graham, L.; Engler, M. M.; Hammock, B. D.; Zeldin, D. C.; Kroetz, D. L. *Circ. Res.* **2000**, *87*, 992; (b) Spector, A. A.; Fang, X.; Snyder, G. D.; Weintraub, N. L. *Prog. Lipid Res.* **2004**, *43*, 55.
3. (a) Capdevila, J. H.; Falck, J. R.; Harris, R. C. *J. Lipid Res.* **2000**, *41*, 163; (b) Nithipatikom, K.; Moore, J. M.; Isbell, M. A.; Falck, J. R.; Gross, G. J. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, *290*, H500; (c) Lin, W. K.; Falck, J. R.; Wong, P. Y. *Biochem. Biophys. Res. Commun.* **1990**, *167*, 977.
4. Maier, K. G.; Roman, R. J. *Curr. Opin. Nephrol. Hypertens.* **2001**, *10*, 81.
5. (a) Larsen, B. T.; Gutterman, D. D.; Hatoum, O. A. *Eur. J. Clin. Invest.* **2006**, *36*, 293; (b) Fisslthaler, B.; Popp, R.; Kiss, L.; Potente, M.; Harder, D. R.; Fleming, I.; Busse, R. *Nature* **1999**, *401*, 493.
6. Roman, R. J. *Physiol. Rev.* **2002**, *90*, 1028.
7. Spiecker, M.; Liao, J. K. *Arch. Biochem. Biophys.* **2005**, *433*, 413.
8. Node, K.; Huo, Y.; Ruan, X.; Yang, B.; Spiecker, M.; Ley, K., et al *Science* **1999**, *285*, 1276.
9. http://www.aretetherapeutics.com.
10. (a) Imig, J. D.; Zhao, X.; Zaharis, C. Z.; Olearczyk, J. J.; Pollock, D. M.; Newman, J. W.; Kim, I.-H.; Watanabe, T.; Hammock, B. D. *Hypertension* **2005**, *46*, 975; (b) Jung, O.; Brandes, R. P.; Kim, I.-H.; Schmidt, R.; Hammock, B. D.; Busse, R.; Fleming, I. *Hypertension* **2005**, *45*, 759.
11. (a) Ota, T.; Takahashi, H.; Kakinuma, H.; Busujima, T. WO2007043653, 2007.; (b) Ding, Y.; Longdregan, A. T.; Marino, J. P. WO2009049154, 2009.; (c) Ding, Y.; Thalji, R. K.; Marino, J. P. WO2009049157, 2009.; (d) Ding, Y.; Thalji, R. K.; Marino, J. P. WO2009049165, 2009.
12. Mullin, C. A.; Hammock, B. D. *Arch. Biochem. Biophys.* **1982**, *216*, 423.
13. Morisseau, C.; Goodrow, M. H.; Dowdy, D.; Zheng, J.; Greene, J. F.; Sanborn, J. R.; Hammock, B. D. *Proc. Natl. Acad. Sci.* **1999**, *96*, 8849–8854.
14. Cardozo, M. G.; Ingraham, R. H. WO2006121684, 2006.
15. Dietze, E. C.; Casas, J.; Kuwano, E.; Hammock, B. D. *Comp. Biochem. Physiol. B.* **1993**, *104*, 309–314.
16. Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. W. *Protein Sci.* **2006**, *15*, 58.
17. (a) Delombaert, S.; Eldrup, A. B.; Kowalski, J. A.; Mugge, I. A.; Soleymanzadeh, F.; Swinamer, A. D.; Taylor, S. J. WO2007106705, 2007.; (b) Ingraham, R. H. WO2007106706, 2007.; (c) Hammock, B. D.; Jones, P. D.; Morisseau, C.; Huang, H.; Tsai, H.-J.; Gless, R. WO2007106525, 2007.; (d) Hammock, B. D.; Kim, I.-H.; Morisseau, C.; Watanabe, T.; Newman, J. W. WO2006045119, 2006.; (e) Eldrup, A. B.; Farrow, N. A.; Kowalski. J. A.; Delombaert, S.; Mugge, I. A.; Soleymanzadeh, F.; Swinamer, A. D.; Taylor, S. J. WO2007098352, 2007.; (f) Takahashi, H.; Ota, T.; Kakinuma, H. WO2007043652, 2007.; (g) Ota, T.; Kakahashi, H.; Kakinuma, H.; Busujima, T. WO2007043653, 2007.; (h) Ingraham, R. H.; Proudfoot, J. R. WO2003002555, 2003.; (i) Cywin, C. L.; De Lombaert, S.; Eldrup, A. B.; Ingraham, R. H.; Taylor, S.; Soleymanzadeh, F. WO2006121719, 2006.; (j) Kroetz, D.; Zeldin, D. C.; Hammock, B. D.; Morisseau, C. US20030119900, 2003.; (k) Kroetz, D.; Zeldin, D. C.; Hammock, B. D.; Morisseau, C. US20040092487, 2004.; (l) Hammock, B. D.; Kim, I.-H.; Morisseau, C.; Watanabe, T.; Newman, J. W. US20050026844, 2005.; (m) Erickson, D.; Hoffman, A. F.; Warren, T. C. WO200023060, 2000.; (n) Hwang, S. H.; Tsai, H.-J.; Liu, J.-Y.; Morisseau, C.; Hammock, B. D. *J. Med. Chem.* **2007**, *50*, 3825; (o) Kim, I.-H.; Morrisseau, C.; Watanabe, T.; Hammock, B. D. *J. Med. Chem.* **2004**, *47*, 2110; (p) Kim, I.-H.; Heirtzler, F. R.; Morisseau, C.; Hishi, K.; Tsai, H.-J.; Hammock, B. D. *J. Med. Chem.* **2005**, *48*, 3621; (q) Morisseau, C.; Newman, J. W.; Tsai, H.-J.; Baecker, P. A.; Hammock, B. D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5439; (r) Li, H.-Y.; Jin, Y.; Morisseau, C.; Hammock, B. D.; Long, Y.-Q. *Bioorg. Med. Chem. Lett.* **2006**, *14*, 6586; (s) Shen, H. C.; Ding, F.-X.; Wang, S.; 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.; Zhang, B.; Tata, J. R.; Berger, J. P.; Colletti, S. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3398; (t) 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*, in press; (u) Shen, H. C.; Ding, F.-X.; Deng, Q.; Xu, S.; Chen. H.; Tong, X.; Tong, V.; Zhang, X.; Chen, Y.; Zhou, G.; Pai, L.-Y.; Alonso-Galicia, M.; Zhang, B.; Roy, S.; Tata, J. R.; Berger, J. P.; Colletti, S. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, in press.
18. Lima, L. M.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23.
19. Zhao, H. *Drug Discovery Today* **2007**, *12*, 149.
20. *The procedures of preparation of* **7t**: To the solution of **8** (500 mg, 2.4 mmol) in 20 mL DCM was added DMF (10 μL) and oxalyl chloride (3 mL, 2 M, 6 mmol) at 0 °C, and the resulting solution was stirred at rt for 1 h. After removing the solvent, the residue was dissolved in 20 mL DCM and added to a solution of 4-trifluromethyl aniline (387 mg, 2.4 mmol) and triethylamine (338 μL, 2.4 mmol) in 20 mL DCM at 0 °C. The resulting solution was stirred at rt for 1 h. The reaction mixture was washed with 3 N HCl (2 × 10 mL) and satd $NaHCO_3$ (100 mL), dried over Na_2SO_4, concentrated to give **9** (0.72 g) as white solid. The solution of **9** (0.72 g, 2.05 mmol) and PCl_5 (0.43 g, 2.05 mmol) in thionylchloride (3 mL) was heated at 65 °C for 2 h. After concentration, the residue was treated diethylether, the solid was filtered, and the filtrated was concentrated to give **10** (0.33 g, 44% yield) as a colorless oil. To the solution of **10** (0.33 g, 0.89 mmol) in THF (20 mL) was added trimethylsilyloxyamine (0.88 mL, 7.14 mmol) dropwise and the resulting solution was stirred at rt overnight. To the reaction solution was added 1 N HCl (10 mL) and stirred for 5 min before was neutralized to pH 8 by $NaHCO_3$. The solution was extracted with EtOAc (3 × 50 mL), the combined organic phase was washed with brine (2 × 100 mL) and dried over Na_2SO_4. After concentration, the residue was purified on silica gel using 35% EtOAc /hexane to give **11** (200 mg, 61% yield) as a white solid. The solution **11** (200 mg, 0.54 mmol)) and potassium *tert*-butoxide (74 mg, 0.63 mmol) in 8 mL of NMP was heated at 100 °C for 1.5 h. After cooling to rt, the solution was diluted with EtOAc (100 mL), washed with water (3 × 100 mL), dried over Na_2SO_4, purified on silica gel using 35% of EtOAc in hexanes to give **7t** (180 mg, 95% yield) as a white solid. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.90–7.87 (2H, m), 7.75 (d, 2H), 7.69 (d, 2H), 7.49 (t, 1H), 6.71 (s, 1H); LCMS m/z: 347 (M^++1).