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Exploration of secondary and tertiary pharmacophores in unsymmetrical *N,N′*-diaryl urea inhibitors of soluble epoxide hydrolase

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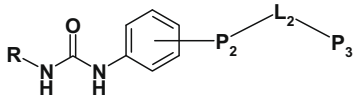
ABSTRACT

The impact of various secondary and tertiary pharmacophores on in vitro potency of soluble epoxide hydrolase (sEH) inhibitors based on the unsymmetrical urea scaffold **1** is discussed. *N,N′*-Diaryl urea inhibitors of soluble epoxide hydrolase exhibit subtle variations in inhibitory potency depending on the secondary pharmacophore but tolerate considerable structural variation in the second linker/tertiary pharmacophore fragment.

Epoxyeicosatrienoic acids (EETs), which elicit a range of biological effects,[1] are formed by the monooxygenase epoxidation of arachidonic acid by CYP2J and CYP2C epoxygenases. Soluble epoxide hydrolase (sEH) converts these epoxides to the corresponding, less active diols by addition of a molecule of water across the epoxide. Thus, inhibition of sEH is proposed to stabilize, and hence increase EET concentrations to produce biological effects. Soluble epoxide hydrolase inhibitors have been evaluated in a range of animal models, and lipid modulatory,[2] anti-hypertensive,[3] glucoregulatory,[4] cardioprotective,[5] organ protective,[6] and anti-inflammatory[7] effects have been reported. These pre-clinical observations suggest that sEH inhibition may be of therapeutic benefit in treating certain of the risk factors of metabolic syndrome.[8] An sEH inhibitor, AR9281, is currently completing a phase 2 clinical trial for the treatment of type 2 diabetes mellitus and hypertension.[9]

The prototypical sEH inhibitors contained a urea as the primary pharmacophore, which is proposed to mimic the endogenous epoxide ligand with a similar binding motif, flanked by two lipophilic groups.[10] These compounds, for example, dicyclohexyl urea (DCU), cyclohexyl dodecyl urea (CDU), adamantyl dodecyl urea (ADU), and adamantyl ureido dodecanoic acid (AUDA) are potent inhibitors of the sEH enzyme, but are less than optimal from a physical property standpoint.[11] Subsequent elaboration, primarily by the Hammock group, led to additional refinement of the pharmacophore model with the addition of secondary and tertiary pharmacophores, P_2 and P_3 (Fig. 1).[12–14] The details of the various aspects of this model have been well delineated,[15] and it is noteworthy that the great majority of published sEH inhibitors are structurally consistent with this model.[16]

Various secondary pharmacophores have been described in the sEH inhibitor literature, and an evaluation of both secondary and tertiary pharmacophores have been presented for *N*-adamantyl, *N′*-alkyl ureas derived from CDU and AUDA[12,14] as well as for *N*-adamantyl, *N′*-aryl ureas.[15] However, there is no systematic discussion of the impact of various secondary and tertiary pharmacophores on enzyme inhibitory potency in more rigid *N,N′*-diaryl urea systems. The aryl urea scaffold **1** with an amide as a secondary pharmacophore was selected as a convenient platform for the comparison of the impact of the second linker/tertiary pharmacophore combination L_2P_3 on enzyme inhibition.



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A series of *N*-adamantyl, *N′*-aryl ureas as well as the analogous *N,N′*-diaryl ureas were prepared to confirm the equivalency of adamantyl and trifluoromethylphenyl as the R group with respect to inhibitory potency prior to evaluation of various L_2P_3 combinations. The L_2P_3 region of the pharmacophore model is not necessarily required for activity and has been shown to be useful primarily as a 'solubilizing' group.[11,12] A variety of functional groups appear to be acceptable as the tertiary pharmacophore P_3 when it is sufficiently distant from P_1 since it may actually lie in the solvent sphere outside the sEH binding site.[10] Acidic, basic, and neutral fragments

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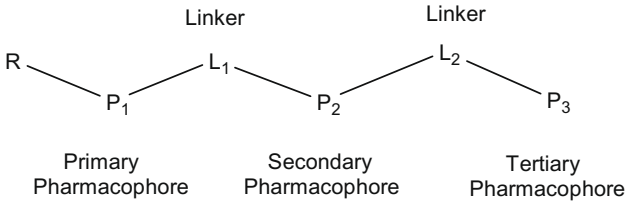


Figure 1. Pharmacophore model.

(Figs. 2–4) were explored as the second linker/tertiary pharmacophore L_2P_3 to determine their impact on inhibitory potency. The amide secondary pharmacophore was then replaced with a sulfonamide or sulfone functionality in selected examples, and the impact on enzyme inhibition of these secondary pharmacophores was compared with results obtained with an ether secondary pharmacophore in a similar system.[17]

The general synthetic approach used to prepare the desired inhibitors with an amide as the secondary pharmacophore is presented in Scheme 1. Accordingly, 3- or 4-nitrobenzoic acid chloride **2** was treated with the appropriate L_2P_3 amine to obtain the amide **3**. The nitro functionality of **3** was then reduced with iron and ammonium formate to the corresponding amine **4**. Treatment of amine **4** with isocyanate then afforded the corresponding urea **5.** Typically, urea formation was conducted in a polar solvent such as DMF at 60–85 °C. The corresponding fluoroaryl analogs **6** were obtained using a similar synthetic sequence as that employed for the preparation of the desfluoro analogs **5**.

The synthesis of unsymmetrical diaryl urea inhibitors with sulfonamide as the secondary pharmacophore was carried out according to Scheme 2 starting with 3- or 4-nitrobenzenesulfonyl chloride. The sulfonyl chloride **7,** on treatment with appropriate amine followed by hydrogenation of the nitro group using palladium on charcoal, afforded the required amine **8**. Treatment of amine **8** with various aryl isocyanates resulted in the desired sulfonamide analog **9**.

The general synthetic protocol for the preparation of sEH inhibitors with sulfone as the secondary pharmacophore is presented in Scheme 3. The 3- or 4-nitrobenzenethiol **10** was treated with 1-bromo-3-chloropropane followed by reduction of the nitro group to give the amino intermediate **11** which on treatment with various aryl isocyanates resulted in chloro intermediate **12**. Oxidation of the thio functionality of intermediate **12** followed by reacting with morpholine afforded the desired sulfone analogs **13**.

The general structure activity relationships observed for scaffold **1** are shown in Fig. 5. The relative impact of L_2P_3 fragments on potency against the sEH enzyme are color coded with green > yellow > orange > red. Fragments shown in green typically afforded more potent compounds, while fragments shown in red, the least. Enzyme IC_{50} values for selected examples from the exploration of various second linker/tertiary pharmacophore L_2P_3 are shown in Table 1.

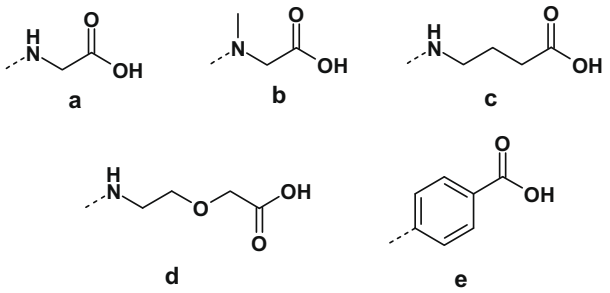


Figure 2. Acidic L_2P_3 fragments.

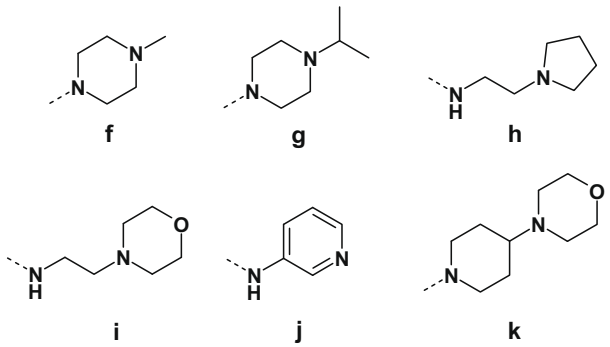


Figure 3. Basic L_2P_3 fragments.

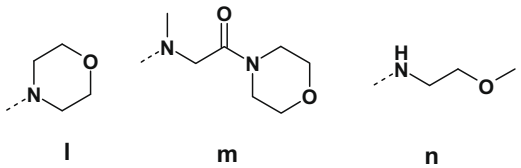
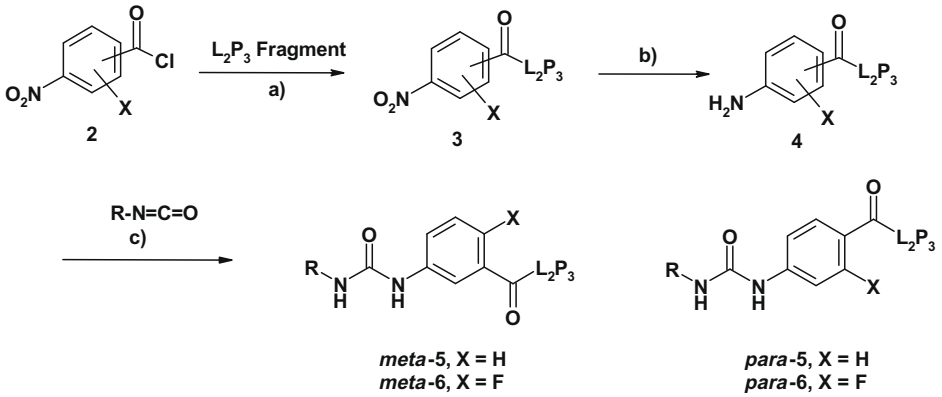


Figure 4. Neutral L_2P_3 fragments.

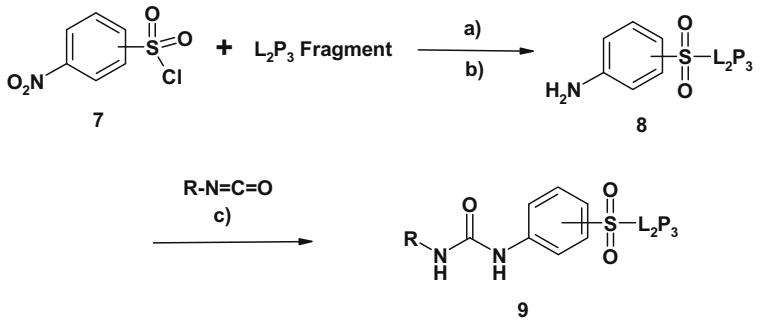
Examination of the enzyme IC_{50} values for compounds with amide as the secondary pharmacophore, P_2, shows consistent superiority in potency with *para* substitution on the aromatic L_1 linker. This effect is more pronounced with less potent compounds. The exceptions to this observation occur either with very potent compounds (e.g., **5h**) or with L_2P_3 fragments having rigid or bulky functionality (e.g., **5u**). R may be either cycloalkyl or a substituted aromatic ring. Cyclic and fused polycyclic rings as the R group afford potent inhibitors with adamantyl affording more potency than cyclohexyl (**5n** vs **5m**). Replacement of the potentially metabolically labile alkyl R group with substituted aromatic moieties confirmed the SAR seen previously,[17] namely, potency increasing with substitution at the *para* position of the aromatic ring with $CF_3 \approx CF_3O > Cl > F > H$ (**5o–s**, **5v–z**). A cyclohexyl ring as R exhibited potency comparable to 4-chlorophenyl (**5m** vs **5q**) while adamantyl afforded potency comparable to the 4-trifluoromethylphenyl group (**5f** vs **5h** and **5n** vs **5r**) especially with the *para* isomer.

For molecules containing an acidic L_2P_3 fragment (a–e), more lipophilic or extended L_2 linkers that displace the polar functionality further from the lipophilic P_1 binding site afford better potency. Even a subtle change such as replacing an amide NH with NMe (e.g., **5a** vs **5b**) or, assuming equivalent potency for the adamantyl and trifluoromethylphenyl groups as R, inserting oxygen in an alkyl chain (e.g., **5c** vs **5d**) has a negative impact on potency. The less acidic aromatic acid analogs **5e** were found to be generally more potent than the aliphatic acid analogs (**5a–d**).

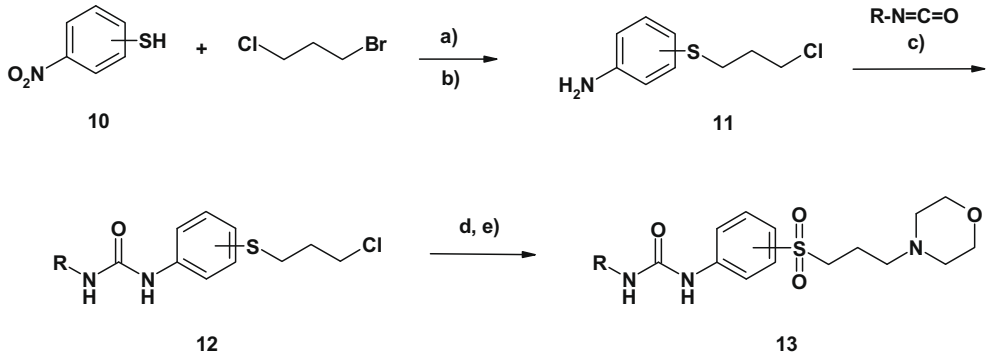
Molecules containing a basic L_2P_3 fragment (f–k) exhibit a similar trend to that noted with the acidic L_2P_3 fragments, namely longer or more lipophilic L_2 linkers which displace the amine functionality further from the lipophilic P_1 binding site in general afford better potency. Increasing lipophilicity also trends to somewhat more potent inhibitors, for example, **5h** versus **5j** and **5i** versus **5k**. Reducing the polarity (basicity) either by incorporating the amine nitrogen into aromatic ring (e.g., **5t**) or in a morpholine ring system (**5l** vs **5r**) affords more potent compounds. Molecules containing a neutral L_2P_3 fragment (l–n) exhibit similar trends to those noted with the acidic and basic L_2P_3 fragments with the more lipophilic moieties again affording better potency.



Scheme 1. Preparation of amides. Reagents and conditions: (a) K_2CO_3, ACN, 60–85 °C, 12 h, 70–90%; (b) Fe/$HCOONH_4$, toluene/H_2O, 70–80 °C, 6–12 h, 40–70%; (c) DMF, 60–80 °C, 12 h, 70–90%.



Scheme 2. Preparation of sulfonamides. Reagents and conditions: (a) TEA, DCM, 3 h, 30–50%; (b) Pd/C, MeOH, rt, 12 h, 75–80%; (c) toluene, 12 h, 60 °C, 60–70%.



Scheme 3. Preparation of sulfones. Reagents and conditions: (a) NaOH, EtOH, rt 12 h, 71%; (b) RaNi, MeOH, rt, 3 h, 77%; (c) DCM, rt, 3 h, 50%; (d) *m*-CPBA, DCM, rt, 1 h, 50%; (e) morpholine, ACN, 80 °C, 3 h, 40%.

Introduction of fluorine in the L_1 phenyl linker in either the 3 position (**6**-*para* series) or the 4 position (**6**-*meta* series) appears to afford a slight improvement in potency against the enzyme (Table 1). It is not clear whether this is due to an electronic effect on the urea NH or just an electronic effect decreasing the polarity of the adjacent P_2 amide moiety in the same fashion noted above in the replacement of a P_2 amide NH with an amide *N*-alkyl.[17]

Having explored the effect of structural variations in the L_2P_3 portion of the molecule with amide as the secondary pharmacophore, we then explored the effect of substituting the amide secondary pharmacophore with sulfonamide or sulfone in selected examples of scaffold **1**. Enzyme IC_{50} values for selected sulfonamides and sulfones are presented in Table 2.

Compounds containing an amide as the secondary pharmacophore at the *para* position on the L_1 aromatic ring consistently exhibit superior enzyme inhibitory potency in comparison to the corresponding *meta* substituted analogs (Table 1). This is in distinct contrast with compounds with ether as the secondary pharmacophore in which both the *para* substituted and *meta* substituted series give comparable enzyme potencies.[17] It would be reasonable to postulate that this difference can be ascribed to increased flexibility in the ether series or possibly the more polar character of an amide, especially given that tertiary amides generally appear to be more active than secondary amides. In the case of the *meta* amide series, the polar amide may be infringing on the lipophilic region in the enzyme near the P_1 binding site, while in the *para* amide series, the amide may be sufficiently distant.[12a] Although the data set is small, it appears that compounds with sulfonamide or sulfone as the secondary pharmacophore are significantly more potent than the compounds with amide as the secondary pharma-

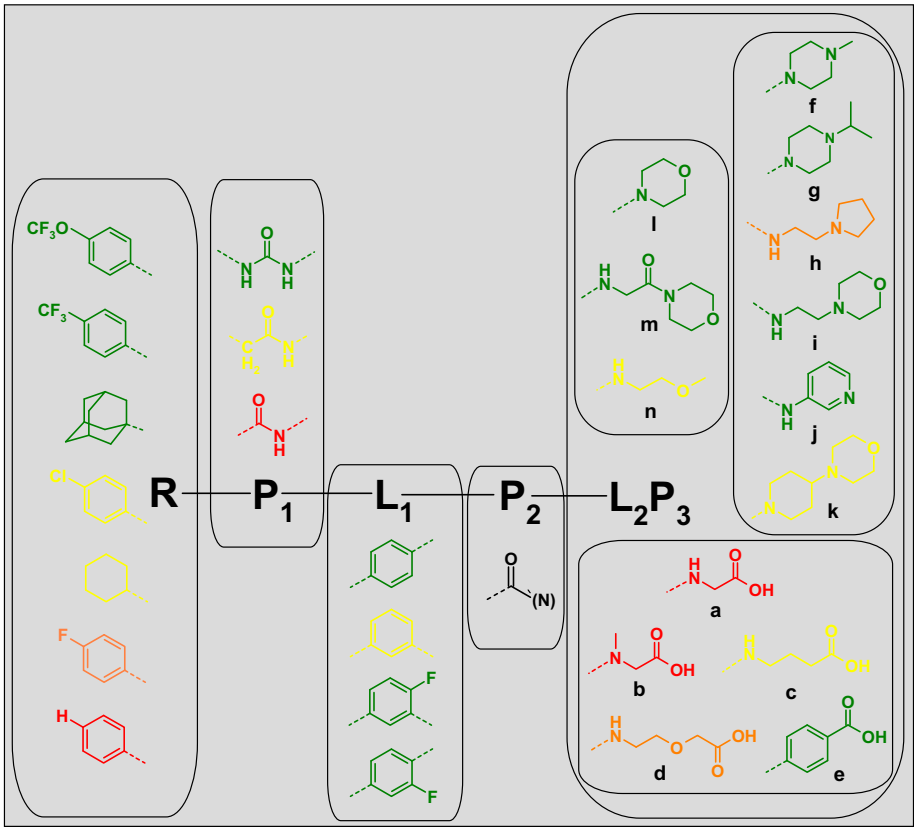


Figure 5. General SAR for P_1 ureas and amides.

Table 1
IC_{50} values[a] for sEH inhibitors with amide as the secondary pharmacophore

Entry	Compound	R	L_2P_3	*para* Isomer IC_{50} (nM)	*meta* Isomer IC_{50} (nM)
1	**5a**	Adamantyl	a	490	800
2	**5b**	Adamantyl	b	200	230
3	**5c**	4-Trifluoromethylphenyl	c	19	120
4	**5d**	Adamantyl	d	80	130
5	**5e**	4-Trifluoromethylphenyl	e	20	27
6	**5f**	Adamantyl	f	12	36
7	**5g**	4-Fluorophenyl	f	160	—
8	**5h**	4-Trifluoromethylphenyl	f	14	12
9	**5i**	4-Trifluoromethoxyphenyl	f	7	—
10	**5j**	4-Trifluoromethylphenyl	g	2	15
11	**5k**	4-Trifluoromethoxyphenyl	g	4	—
12	**5l**	4-Trifluoromethylphenyl	h	48	68
13	**5m**	Cyclohexyl	i	19	280
14	**5n**	Adamantyl	i	3	62
15	**5o**	Phenyl	i	320	2800
16	**5p**	4-Fluorophenyl	i	140	—
17	**5q**	4-Chlorophenyl	i	16	130
18	**5r**	4-Trifluoromethylphenyl	i	1	22
19	**5s**	4-Trifluoromethoxyphenyl	i	2	—
20	**5t**	4-Trifluoromethylphenyl	j	8	18
21	**5u**	4-Chlorophenyl	k	235	47
22	**5v**	Phenyl	l	251	—
23	**5w**	4-Fluorophenyl	l	170	—
24	**5x**	4-Chlorophenyl	l	18	49
25	**5y**	4-Trifluoromethylphenyl	l	5	9
26	**5z**	4-Trifluoromethoxyphenyl	l	4	—
27	**5aa**	4-Trifluoromethylphenyl	m	3	—
28	**5ab**	Adamantyl	n	54	110
29	**6a**	4-Trifluoromethylphenyl	f	4	16
30	**6b**	4-Chlorophenyl	h	—	760
31	**6c**	4-Trifluoromethylphenyl	h	19	—
32	**6d**	4-Chlorophenyl	i	—	38
33	**6e**	4-Trifluoromethylphenyl	i	2	18
34	**6f**	4-Chlorophenyl	l	11	—
35	**6g**	4-Trifluoromethylphenyl	l	2	8

[a] IC_{50} determinations were done using a fluorescence assay.[18]

Table 2
IC_{50} values[a] for sEH inhibitors with sulfonamide and sulfone as the secondary pharmacophore

Entry	Compound	R	L_2P_3	*para* Isomer IC_{50} (nM)	*meta* Isomer IC_{50} (nM)
1	**9a**	4-Trifluoromethylphenyl	g	5	3
2	**9b**	4-Trifluoromethylphenyl	i	3	1
3	**9c**	4-Trifluoromethylphenyl	l	1	1
4	**13a**	phenyl	—	54	100
5	**13b**	4-Trifluoromethylphenyl	—	1	1

[a] IC_{50} determinations were done using a fluorescence assay.[18]

cophore (Table 2). The examples prepared with sulfonamide as the secondary pharmacophore are sufficiently active that it is difficult to discern a significant difference in enzyme potency in the *para* and *meta* substituted series. However, the *para* and *meta* sulfone isomers appear to exhibit the same potency relationship as that noted with compounds with amide as the secondary pharmacophore. It is clear from these data that a variety of secondary pharmacophores can be employed to prepare very potent sEH inhibitors.

Potent *N*,*N*′-diaryl urea sEH inhibitors incorporating several secondary pharmacophores and a variety of second linker/tertiary pharmacophore L_2P_3 fragments have been prepared. Potent inhibitors with potentially metabolically stable, substituted aromatic R groups and potency equivalent to adamantyl analogs have been identified. Soluble epoxide hydrolase inhibitors containing urea and amide primary pharmacophores exhibit subtle variations in enzyme inhibitory potency based on the secondary pharmacophore but tolerate considerable structural variation in the second linker/tertiary pharmacophore L_2P_3 fragment. In general, more lipophilic, nonpolar fragments afford the most potent inhibitors. Further work is in progress to find optimum pharmacophore and linker combinations that afford the desired potency, solubility, selectivity, and appropriate pharmacokinetic properties.

References and notes

1. (a) Spector, A. A.; Fang, X.; Snyder, G. D.; Weintraub, N. L. *Prog. Lipid Res.* **2004**, *434*, 55; (b) Lason, B. T.; Gutterman, D. D.; Hatoum, O. A. *Eur. J. Clin. Invest.* **2006**, *36*, 293.
2. (a) Zhang, L.-N.; Vincelette, J.; Cheng, Y.; Mehra, U.; Chen, D.; Anandan, S.-K.; Gless, R.; Webb, H. K.; Wang, Y.-X. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 1265; (b) Ulu, A.; Davis, B. B.; Tsai, H.-J.; Kim, I.-H.; Morisseau, C.; Inceoglu, B.; Fiehn, O.; Hammock, B. D.; Weiss, R. H. *J. Cardiovasc. Pharm.* **2008**, *52*, 314.
3. (a) Jung, O.; Brandes, R. P.; Kim, I.; Schweda, F.; Schmidt, R.; Hammock, B. D.; Busse, R.; Fleming, I. *Hypertension* **2005**, *45*, 759; (b) 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; (c) 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.
4. Wong, K.; Zhang, L.-N.; Vincelette, J.; Chen, D.; Mehra, U.; Chen, Y.; Gless, R.; Anandan, S.-K.; Webb, H. K.; MacIntyre, E.; Wang, X.-Y. 'A Novel Inhibitor of Soluble Epoxide Hydrolase, AR9281, Improves Glucose Homeostasis in Diet-Induced Obese Mice' presented at the American Diabetes Association Meeting 69th Scientific Session, New Orleans, LA, June, 2009.
5. Xu, D.; Li, N.; He, Y.; Timofeyev, V.; Lu, L.; Tsai, H.-J.; Kim, I.-H.; Tuteja, D.; Mateo, R. K. P.; Singapuri, A.; Davis, B. B.; Low, R.; Hammock, B. D.; Chiamvimonvat, N. *Proc. Nat. Acad. Sci.* **2006**, *103*, 18733.
6. (a) Zhao, X.; Yamamoto, T.; Newman, J. W.; Kim, I.-H.; Watanabe, T.; Hammock, B. D.; Stewart, J.; Pollock, J. S.; Pollock, D. M.; Imig, J. D. *J. Am. Soc. Nephrol.* **2004**, *15*, 1244; (b) Imig, J. D. *Am. J. Physiol. Renal Physiol.* **2005**, *289*, F496; (c) Dorrance, A. M.; Rupp, N.; Pollock, D. M.; Newman, J. W.; Hammock, B. D.; Imig, J. D. *J. Cardiovasc. Pharm.* **2005**, *46*, 842; (d) Liu, Y.; Webb, H.; Kroetz, D. L. *FASEB J.* **2008**, *22*, 479.19; (e) Parrish, A. R.; Chen, G.; Burghardt, R. C.; Watanabe, T.; Morisseau, C.; Hammock, B. D. *Cell. Biol. Toxicol.* **2009**, *25*, 217.
7. (a) Node, K.; Huo, Y.; Ruan, X.; Yang, B.; Spiecker, M.; Ley, K.; Zeldin, D. C.; Liao, J. K. *Science* **1999**, *285*, 1276; (b) Schmelzer, K. R.; Kubala, L.; Newman, J. W.; Kim, I.-H.; Eiserich, J. P.; Hammock, B. D. *Proc. Nat. Acad. Sci.* **2005**, *102*, 9772; (c) Smith, K. R.; Pinkerton, K. E.; Watanabe, T.; Pedersen, T. L.; Ma, S. J.; Hammock, B. D. *Proc. Nat. Acad. Sci.* **2005**, *102*, 2186.
8. Govindarajan, G.; Whaley-Connell, A.; Mugo, M.; Stump, C.; Sowers, J. R. *Am. J. Med. Sci.* **2005**, *330*, 311.
9. http://www.aretetherapeutics.com.
10. Morisseau, C.; Goodrow, M. H.; Newman, J. W.; Wheelock, C. E.; Dowdy, D. L.; Hammock, B. D. *Biochem. Pharmacol.* **2002**, *63*, 1599.
11. Kim, I.-H.; Nishi, K.; Tsai, H.-J.; Bradford, T.; Koda, Y.; Watanabe, T.; Morisseau, C.; Blanchfield, J.; Toth, I.; Hammock, B. D. *Biorg. Med. Chem. Lett.* **2007**, *15*, 312.
12. (a) Kim, I.-H.; Morisseau, C.; Watanabe, T.; Hammock, B. D. *J. Med. Chem.* **2004**, *47*, 2110; (b) Kim, I.-H.; Heirtzler, F. R.; Morisseau, C.; Nishi, K.; Tsai, H.-J.; Hammock, B. D. *J. Med. Chem.* **2005**, *48*, 3621; (c) Li, H.-Y.; Jin, Y.; Morisseau, C.; Hammock, B. D.; Lon, Y.-Q. *Bioorg. Med. Chem.* **2006**, *14*, 6586; (d) Jones, P. D.; Tsai, H.-J.; Do, Z. N.; Morisseau, C.; Hammock, B. D. *Biorg. Med. Chem. Lett.* **2006**, *16*, 5212.
13. (a) Hwang, S. H.; Morisseau, C.; Do, Z.; Hammock, B. D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5773; (b) Hwang, S. H.; Tsai, H. –J.; Liu, J.-K.; Morisseau, C.; Hammock, B. D. *J. Med. Chem.* **2007**, *50*, 3825.
14. Kim, I.-H.; Tsai, H.-J.; Nishi, K.; Kasagami, T.; Morisseau, C.; Hammock, B. D. *J. Med. Chem.* **2007**, *50*, 5217.
15. Kasagami, T.; Kim, I.-H.; Tsai, H.-J.; Nishi, K.; Hammock, B. D.; Morisseau, C. *Bioorg. Med. Chem.* **2009**, *19*, 1784.
16. (a) Marino, J. P. *Curr. Top. Med. Chem.* **2009**, *9*, 452; (b) Shen, H. C.; Ding, F.-X.; Wang, S.; Xu, S.; Chen, H.-S.; 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.* **2009**, *19*, 3398; (c) Shen, H. C.; Ding, F.-X.; Wang, S.; Deng, Q.; Zhang, X.; Chen, Y.; Zhou, G.; Xu, S.; Chen, H.-S.; 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; (d) Eldrup, A. B.; Solewymanzadeh, F.; Taylor, S. J.; Muegge, I.; Farrow, N. A.; Joseph, D.; McKellop, K.; Man, C. C.; Kulkulka, A.; Lombaert, S. D. *J. Med. Chem.* **2009**, *52*, 5880.
17. Anandan, S.-K.; Webb, H. K.; Do, Z. N.; Gless, R. D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4259.
18. Fluorescence assays were carried out using 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) as a standard which afforded an average IC_{50} value of ca. 3 nM. Jones, P. D.; Wolf, N. M.; Morisseau, C.; Whetstone, P.; Hock, B.; Hammock, B. D. *Anal. Biochem.* **2005**, *343*, 66.