1-(1-Acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea (AR9281) as a potent, selective, and orally available soluble epoxide hydrolase inhibitor with efficacy in rodent models of hypertension and dysglycemia

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Abstract

1-(1-Acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea **14a** (AR9281), a potent and selective soluble epoxide hydrolase inhibitor, was recently tested in a phase 2a clinical setting for its effectiveness in reducing blood pressure and improving insulin-resistance in pre-diabetic patients. In a mouse model of diet induced obesity, AR9281 attenuated the enhanced glucose excursion following an intraperitoneal glucose tolerance test. AR9281 also attenuated the increase in blood pressure in angiotensin-II-induced hypertension in rats. These effects were dose-dependent and well correlated with inhibition of the sEH activity in whole blood, consistent with a role of sEH in the observed pharmacology in rodents.

Soluble epoxide hydrolase (sEH, *Epxh2*) has attracted considerable interest over the last decade as a novel pharmaceutical target primarily for cardiovascular and inflammatory disease as well as a number of other indications. Considerable effort has been expended both in academia and the pharmaceutical industry to develop novel, drug-like inhibitors.[1] We wish to report the chemical details and rationale for the selection of the sEH inhibitor, 1-(1-acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea[2] **14a** (AR9281), as a candidate for human clinical trials in hypertension and metabolic syndrome.[3]

sEH is responsible for the conversion of C18, C20 and C22 epoxy lipids, including epoxyeicosatrienoic acids (EETs), which are formed by the monooxygenase epoxidation of arachidonic acid by CYP2J and CYP2C epoxygenases, to the corresponding dihydroxyeicosatrienoic acids (DHETs). EETs are recognized modulators of biological function eliciting a range of pharmacological effects, while the corresponding DHETs are less active.[4] Inhibition of sEH is proposed to stabilize EETs, and effectively increase EET

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concentrations in relevant compartments to produce biological effects. sEH attracted attention as a potential pharmaceutical target based on reports of lipid modulatory, anti-hypertensive, cardioprotective, organ protective, and anti-inflammatory effects in a variety of animal models.[5–17] The observed profile of effects suggest that sEH inhibition should have a beneficial impact on the component processes and symptoms of metabolic syndrome.[5]

Prototypical sEH inhibitors, dicyclohexyl urea (DCU) **1** and adamantyl ureido dodecanoic acid (AUDA) **2**, are potent enzyme inhibitors (DCU $IC_{50} = ca.$ 52 nM, AUDA $IC_{50} = ca.$ 3 nM),[18] but suffer from less than optimal physical properties and lack appropriate pharmacokinetic behavior for drug development.[19] Subsequent efforts have involved modification of the lipophilic aliphatic chain of AUDA on the right hand side (RHS) of the urea primary pharmacophore and introduction of rigid cyclic moieties on both the right and left hand side (LHS) resulting in compounds with improved potency and drug-like properties.[1] Most of the potent sEH inhibitors published to date contain urea as the primary pharmacophore. However, Boehringer Ingelheim and Merck have recently reported potent sEH inhibitors with improved pharmacokinetic behavior with amide as the primary pharmacophore utilizing rigid, biaryl and/or spirocyclic moieties as the LHS/RHS portions. Boehringer reports optimization of its amide-based diphenylmethane series for metabolic stability and reports compound **3** as an optimal compound showing excellent potency and *in vitro* microsomal stability resulting in good plasma exposure in rat.[20] A second, amide-based series, exemplified by compound **4**, is also reported.[21] No animal pharmacology studies are reported for either series. Merck has disclosed three structural classes of potent, urea-based inhibitors exemplified by compounds **5**, **6**, and **7**, as well as a series of non-urea, non-amide based inhibitors.[22, 23] However, despite desirable PK and demonstrated target engagement, compounds **5**, **6**, and **7** failed to show efficacy in an SHR hypertensive model.[22] No results for AngII-hypertensive rat models were reported by Merck in spite of this being one of the standard paradigms used in previous sEH hypertension studies.[6, 7, 9]

One of the many structural variations on sEH inhibitors published by the Hammock laboratory involved incorporation of a solubilizing group into one of the cyclohexane rings of DCU **1**. The resulting 4-piperidinyl ureas were reported to be potent sEH inhibitors.[2] We focused on expanding the SAR around the piperidinyl urea based sEH inhibitors **14** and wish to report the details of the chemistry SAR and the rationale for the selection of one member of this chemical series, 1-(1-acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea[2] **14a** (AR9281), as a clinical candidate in hypertension and metabolic syndrome.[3]

The synthesis of **14a** and its analogs *via* key intermediates **10** and **11** is shown in Scheme 1. This library approach allows facile preparation of various LHS and RHS combinations around the piperidinyl urea nucleus. An additional advantage of this approach is that it allows urea formation using a nucleophilic amine *via* intermediate **11** rather than *via* an isocyanate.[24] In some cases, especially with UV transparent amines such as adamantylamine and cyclohexylamine, the corresponding symmetrical ureas, *e.g.*, dicyclohexyl- and bisadamantyl urea, which are unavoidable impurities in the isocyanates, can be almost impossible to remove efficiently from a reaction product and are potent inhibitors of sEH in their own right. Urea formation with aryl isocyanates is less problematic.

The readily available N-Boc protected piperidone **8** is reacted with hydroxyl amine to obtain the corresponding oxime **9** which is subsequently reduced with Raney nickel to afford the N-Boc aminopiperidine **10**. Treatment of N-Boc-4-piperidineamine **10** with *p*-nitrobenzene chloroformate affords key intermediate **11**. At this point amine **10** can be treated with an isocyanate to provide the Boc protected urea **12**. This sequence was typically used with aromatic isocyanates. An alternate procedure was employed for certain more nucleophilic,

UV transparent amines. Reaction of intermediate **11** with nucleophilic amines at room temperature affords LHS substituted products **12**. Less nucleophilic amines, *e.g.*, aromatic amines can also be reacted with **11** but require elevated reaction temperatures. The Boc group on **12** can then be removed with TFA to give the corresponding amine intermediate **13**. Amine **13** can be treated with various reagents, *e.g.,* activated carboxylic acids or sulfonyl chlorides, to afford the desired substituted piperidinyl urea **14.** Representative compounds are shown in Table 1. An alternate preparation of **14a** useful for large scale preparations has been disclosed in the patent literature.[25]

Piperidinyl urea **14a** exhibited excellent enzyme and cell-based assay IC_{50} values, but the pharmacokinetic profile in rat was less than ideal. The poor pharmacokinetic behaviour was identified as due to rapid CYP mediated metabolic oxidation at the adamantyl group *in vivo*.[26] Introduction of electronegative fluorine(s) on the LHS to circumvent CYP oxidation was investigated, but analogues with mono fluoro or trifluoro substitution on the adamantyl ring[27] (Table 1: **14b**, **14c**) afforded reduced enzyme potency. A similar drop in potency with fluorine introduction was also observed with a cyclohexyl LHS, *e.g.,* **14f** and 4, 4-difluorocyclohexyl analogue **14j**. Replacement of the adamantyl group with a 4-trifluoromethylphenyl, *e.g.,* **14k**, or 4-trifluoromethoxyphenyl, *e.g.,* **14m**, group gave comparable enzyme and cell-based assay potency and afforded a dramatic improvement in the pharmacokinetic profile. Unfortunately, replacing the adamantyl group on the LHS with substituted phenyl had a decidedly negative effect on hERG channel liability. While **14a** showed only 8% hERG channel block at 100 µM, both **14k** and **14o** showed *ca.* 35% inhibition at 30 µM, which was judged to be unacceptable. A large number of analogues with various electron withdrawing groups, *e.g.*, F, Cl, Br, CF_3, CF_3O, CHF_2O, and CF_3CH_2, substituted independently at one or two of the 3, 4, 5 positions on the aromatic ring to alter the electronic nature and/or steric requirements of the LHS aryl system afforded no significant improvement in hERG liability with the exception of the 3-trifluoromethylphenyl analogue **14l** which shows a significant loss in cell potency. Use of one or more electron donating groups on the LHS aryl ring or use of a LHS heteroaryl ring also gave no improvement in hERG liability and typically resulted in less effective sEH enzyme inhibition (data not shown). While most of the examples given in the table contain N-acetyl as the RHS functionality, additional analogues with an N-sulfonyl RHS were also prepared. These analogues were, in general, more potent than the N-acetyl analogues (*e.g.*, **14n**, **o** *vs.* **14a**, **k**) but the increase in potency was compromised by decreased oral exposure. While the methyl sulfonamide analogue **14o** had very similar hERG liability compared to the acetamide analogue **14k**, the isopropyl sulphonamide **14r** was found to block the hERG channel by three-fold less. Introduction of aryl or pyridyl rings in the RHS along with aryl LHS substitution, *e.g.*, **14p**, gave increased hERG liability. The hERG liability with an aryl RHS series could be ameliorated by substituting cycloalkyl for aryl on the LHS as shown in example **14q**. While cycloalkyl replacements for adamantyl LHS (**14g**–**14i**) afforded reduced hERG liability, no significant improvement in pharmacokinetic profile was observed.

Piperidinyl urea compounds with a cycloalkyl LHS including adamantyl, *e.g.,* **14a**, typically showed an oral pharmacokinetic profile that indicated a rapid absorption with a high C_{max}, but rapid metabolism manifested by a short half life and relatively high oral clearance. Reduction of metabolic liability in non-adamantyl LHS cycloalkyl groups was attempted by introducing steric hindrance or electron withdrawing fluorine groups. None of these compounds afforded the desired improvement in pharmacokinetic profile, although all gave significant reduction in hERG inhibition. Replacement of the adamantyl group with aryl did result in potent sEH inhibitors with the desired pharmacokinetic profile but they all suffered from increased inhibition of hERG activity. Since the targeted disease states, *i.e.,*

hypertension and metabolic syndrome are chronic indications, it was decided to advance compounds having reasonable PK and extended inhibition of sEH with minimal risk of cardiovascular toxicity. Compound **14a** was the candidate of choice based on scalable PK, target engagement, and its consistent efficacy in animal models of hypertension and diet induced obesity.

Compound **14a** is a potent inhibitor with a human sEH enzyme IC_{50} value of 8 nM and mouse sEH enzyme IC_{50} value of 3 nM. The cell-based human sEH IC_{50} value was found to be 57 nM consistent with a *ca.*10 fold decrease in potency seen for most of the sEH inhibitors between isolated enzyme and cell-based assays. In murine PK/PD experiments, the plasma concentrations of **14a** were directly correlated with the inhibition of sEH activity in the whole blood, as measured by the hydrolysis rate of 14,15-EET. Plasma exposure was also directly associated with increases in ratios of endogenous epoxy-to-dihydroxy lipids. Total EET/DHET and EpOME/DiHOME (9,10- and 12,13-octadecenoic acid) ratios in **14a** treated wild type mice, where blood sEH activity was inhibited 90%, were similar to those in *Ephx* deficient mice. An oral dosing schedule of 100 mg/kg BID in mice resulted in an extended period of 90% or greater inhibition of blood sEH activity. Compound **14a** was found to be highly selective with no inhibitory activity against microsomal epoxide hydrolase or an extended panel of *ca.*150 other enzyme and receptor targets when tested at 10 μM. No significant inhibition of drug metabolizing CYP enzymes such as CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4, as well as CYP2J and CYP2C, which are essential for the production of EETs was noted with **14a**. The plasma protein binding of the compound was modest, ranging from 58–61% across multiple species. The compound was highly permeable across a Caco-2 monolayer. When administered orally, **14a** was well absorbed in various species with oral bioavailability ranging from 25% in cynomolgus monkey to 100% in rat. The oral exposure, as measured by the area under the plasma concentration-time curve, increased in approximate proportion to dose up to 2000 mg/kg in rats, 500 mg/kg in dogs, and 180 mg/kg in cynomolgus monkeys. The compound had a relatively moderate to high clearance and short half life, and is eliminated primarily by the oxidative modifications on the adamantyl ring, in the form of hydroxylated adamantyl metabolites.

Following the establishment of potency and pharmacokinetic properties, several compounds from different series as well as **14a** were evaluated in an AngII-infused rat model of hypertension. This model was selected for early pharmacological evaluation due to the published efficacy of AUDA.[7] Compounds were tested at 50 mg/kg, dosed orally twice daily in a suspension in 0.5% carboxymethylcellulose, 0.1% Tween 80 in Sprague-Dawley rats that were induced to be hypertensive by a continuous infusion of 65 ng/min of AngII. The blood pressure was assessed continuously by telemetric recording. Of the compounds tested, **14a** showed superior efficacy in reducing systolic and diastolic blood pressure. Relative to vehicle control treatment, systolic blood pressure was decreased by 8–10% (or 14–16 mm Hg) with a more modest effect on diastolic pressure. This result was confirmed independently in a second laboratory.[28]

Compound **14a** was also tested in an SHR rat model of hypertension[8] and was found to be moderately efficacious at a dose of 300 mg/kg with a 14% decrease in mean blood pressure, but no effect was noted at 100 mg/kg. These data are consistent with the recent report[22] showing a lack of correlation between potency and exposure with blood pressure lowering in the SHR model. sEH inhibitors exert antihypertensive effects in some but not all animal models of hypertension.[28] Collectively, the lack of a robust relationship between target engagement, exposure, and the pharmacological endpoint was insufficient to support solely a human hypertension indication for clinical evaluation of sEH inhibitors.

The hypertension models utilized were based on the literature precedence for a blood pressure lowering effect of sEH inhibitors both in AngII-induced hypertension,[7] and in SHR.[8] Compound **14a** was subsequently evaluated in a diet induced-obesity (DIO) mouse model of aberrant glucose regulation.[29] The DIO mouse model tested the pharmacology of these inhibitors in a model of insulin resistance with anticipated activity due in part to the vasodilator, lipid modulatory and anti-inflammatory effects of EETs.[14] EETs have been shown, to play a role in endothelial cell function and vascular inflammatory processes, involving modulation of NF-κB-mediated transcription.[30] Vascular inflammation has been implicated as an important factor in insulin sensitivity.[31] EETs are regulated by sEH,[32] and EETs have been shown to modulate PPARγ[33] and eNOS.[34] The enzymes, PPARγ and eNOS, are regulators in insulin resistance.[35] Thus, sEH inhibition was proposed as a mechanism by which insulin resistance could be improved in a model of diet-induced obesity.

The efficacy of **14a** was evaluated in DIO male C57Bl/6 mice with emphasis on the impact of administration of **14a** on glucose excursions following a bolus administration of glucose. C57BL/6 mice were fed a high fat, high fructose diet using Research Diets 45% fat diet, D12451, with administration of high fructose corn syrup in the liquid portion of the diet at 0.42 kcal/mL for 6 weeks prior to initiation of AR9281 administration and throughout AR9281 administration.[29] Accordingly, **14a** or vehicle was administered *via* oral gavage at a dose of 100 mg/kg twice a day for an additional 12 weeks. After 4 weeks treatment, a glucose tolerance test was conducted by intraperitoneal injection of glucose (2 g/kg) at 4 hours after the dose of **14a**. Blood glucose measurements were taken with a glucometer at time intervals up to 2 hours following glucose administration. The vehicle-treated mice had an impaired glucose tolerance evidenced by the blood glucose excursions being in excess of mice fed normal chow and that the blood glucose concentration was still not restored to the baseline at 2 hour after glucose load (Figure 1, left). However, in comparison to vehicle-treated animals, the **14a**-treated mice had a lower glucose AUC as well as lower maximal glucose excursion. At the end of the study, blood samples were taken at specific times after the last dose and processed to plasma for **14a** concentration measurements using LC/MS/MS and blood sEH activity. Blood sEH activity was defined as the rate of 14, 15 EET hydrolysis, corrected for non-specific hydrolysis in the presence of 5 mM AUDA.[29] sEH activity was undetectable in whole blood from animals receiving **14a** for up to 7 hours post administration. The sEH-catalyzed EET hydrolysis activity continued to be inhibited by ~70% for 12 hours after the last dose, suggesting that at trough there continued to be significant target inhibition (Figure 1, right).[29] These results indicate that **14a** inhibited sEH activity and significantly improved glucose tolerance in a DIO mouse model.

In conclusion we have shown that piperidinyl urea **14a** (AR9281) is a potent sEH inhibitor with excellent selectivity against other targets. AR9281 was well tolerated in early animal and human dose range finding toxicology studies.[29] AR9281 affords an acceptable half-life in mouse for pharmacology testing with whole blood sEH activity essentially completely inhibited for up to 7 hours post administration and still inhibited by ~70% for twelve hours after the last 100 mg/Kg dose. In humans it was shown that greater than 90% inhibition of blood sEH was possible with once a day and twice a day dosing.[36] Based on the blood pressure lowering activity in the AngII-induced hypertension and SHR models and the more robust activity in the DIO mouse, **14a** (AR9281) was selected for proof of concept studies in a human clinical trial involving obese patients with stage 1 hypertension and impaired glucose tolerance.

Acknowledgments

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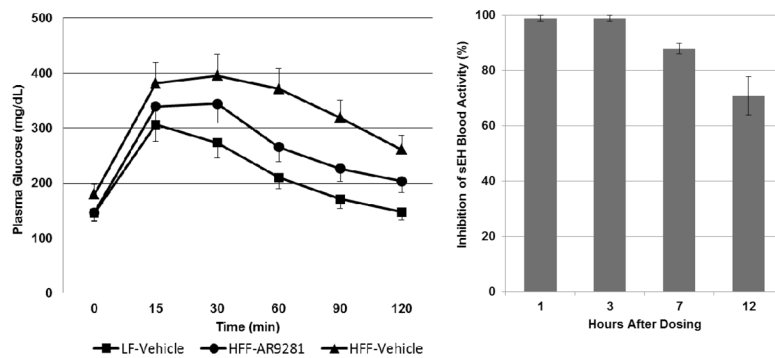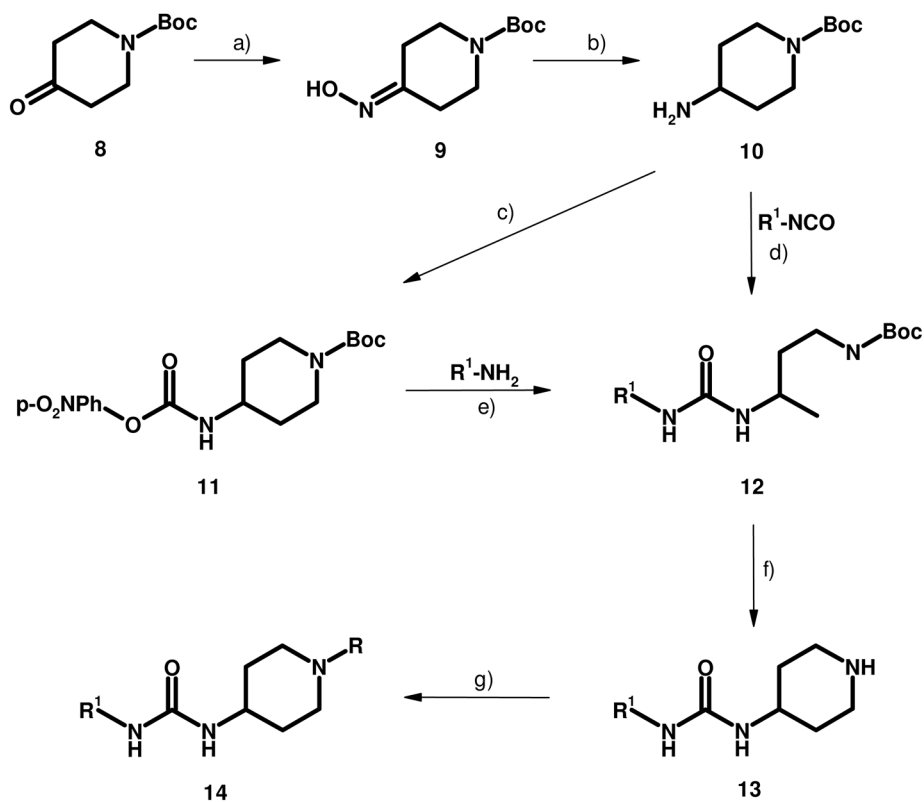
Figure 1.
Evaluation of **14a** (AR9281) in DIO mouse model. Left is the blood glucose levels over time following an intraperitoneal administration in a typical experiment. Right is the whole blood sEH activity following the last dose of the study.

Scheme 1.
Preparation of piperidinyl ureas **14:** a) NH$_2$OH·HCl, EtOH, Pyridine, reflux, 1h, 82% b) RaNi, MeOH, rt, 4 h, 83% c) *p*-nitrophenyl chloroformate, DCM, 2 h, 87% d) DCM, rt, 12 h, 82% e) THF, reflux, 12 h, 85% f) TFA, DCM, rt, 12 h, 88% g) Ac$_2$O or MeSO$_2$Cl, DCM, rt, 18 h, 70–90%
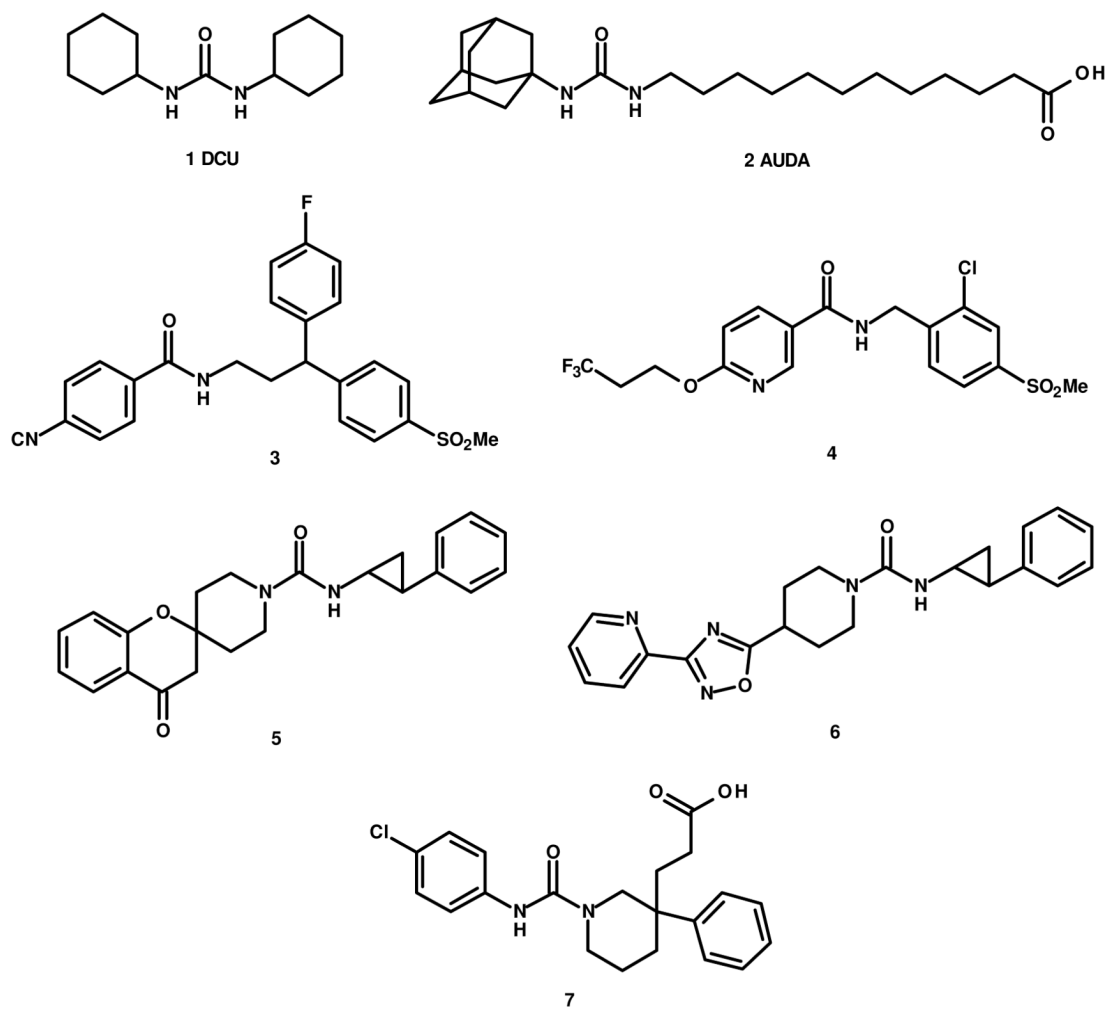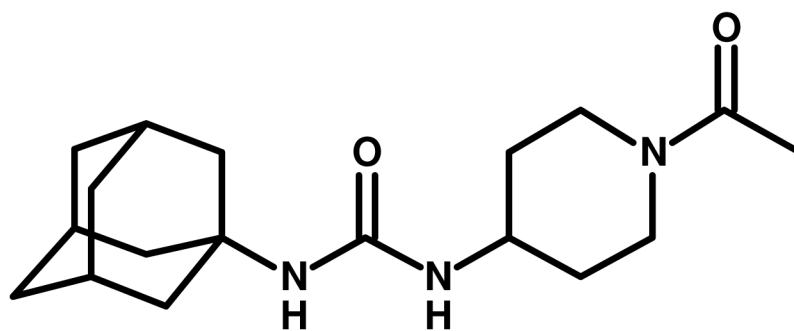
Figure 3.
Structures 1–7

14a (AR9281)

Figure 4.
Structure 14a

Table 1

Enzyme and cell IC$_{50}$ values, hERG inhibition and oral exposure for selected sEH inhibitors

Cmpd	R^1	R	hsEH[a] IC$_{50}$ (nM)	Cell[b] IC$_{50}$ (nM)	hERG[c] % Inhibition at 30 μM	Rat AUC$_{24h}$ (ng h/mL)[e]
14a	Adamantyl	COCH$_3$	8	57	< 8[d]	720
14b	3-Fluoroadamantyl	COCH$_3$	58	-	-	-
14c	3,5,7-Trifluoroadamantyl	COCH$_3$	120	-	-	-
14d	4-Methyl[2,2,2]bicyclooctyl	COCH$_3$	25	300	-	1480
14e	[2,2,1]Cycloheptyl	COCH$_3$	14	120	-	-
14f	Cyclohexyl	COCH$_3$	29	-	-	-
14g	Cycloheptyl	COCH$_3$	0.8	27	9	7840
14h	4-t-Butylcyclohexyl	COCH$_3$	13	270	7	1780
14i	4,4-Dimethylcyclohexyl	COCH$_3$	6.0	38	0.8	f
14j	4,4-Difluorocyclohexyl	COCH$_3$	185	-	-	-
14k	4-CF$_3$Phenyl	COCH$_3$	11	98	35	13100
14l	3-CF$_3$Phenyl	COCH$_3$	10	226	10	19600
14m	4-CF$_3$OPhenyl	COCH$_3$	12	59	-	-
14n	Adamantyl	SO$_2$CH$_3$	1.9	3.9	−2.8	640
14o	4-CF$_3$Phenyl	SO$_2$CH$_3$	3.7	26	34	6850
14p	4-CFO$_3$Phenyl	CO(3-pyridyl)	1.6	6.7	57	11300
14q	Cyclohexyl	CO(2-pyridyl)	6.4	67	3.4	8320
14r	4-CF$_3$Phenyl	SO$_2$CH(Me)$_2$	2.9	13	13	5890
14s	4-CF$_3$OPhenyl	SO$_2$CH(Me)$_2$	1.6	64	11	7300

[a] Enzyme activity was determined in duplicate.[18]

[b] Conversion of 14,15-EET to 14,15-DHET was measured in HUVEC cell.

[c] hERG activity inhibition was determined by a high throughput patch clamp method.

[d] 8% at 100 μM

[e] Data for PO dosing normalized to 10 mg/Kg.

[f] PO data not available; IV showed a very short half life.