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Discovery of 3,3-disubstituted piperidine-derived trisubstituted ureas as highly potent soluble epoxide hydrolase inhibitors

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ABSTRACT

3,3-Disubstituted piperidine-derived trisubstituted urea ***ent*A-2b** was discovered as a highly potent and selective soluble epoxide hydrolase (sEH) inhibitor. Despite the good compound oral exposure, excellent sEH inhibition in whole blood, and remarkable selectivity, compound ***ent*A-2b** failed to lower blood pressure acutely in spontaneously hypertensive rats (SHRs). This observation further challenges the premise that sEH inhibition can provide a viable approach to the treatment of hypertensive patients.

Epoxyeicosatrienoic acids (EETs) are endogenous lipids derived from the metabolism of arachidonic acid by cytochrome P450 epoxygenases.[1] EETs exhibit important physiological effects. EETs can dilate conduit vessels, renal afferent arterioles and coronary resistance vessels.[2] In addition, EETs increase sodium renal excretion.[3] Therefore, EETs have been shown to lower blood pressure and reduce myocardial perfusion in animal models.[4] Furthermore, EETs elicit vascular protective effects by modifying vascular smooth muscle cell migration, leukocyte adhesion, platelet aggregation and thrombolysis.[5] EETs are also anti-inflammatory as they reduce cytokine-induced endothelial expression of the leukocyte adhesion molecules, vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin.[6] Thus, EETs may constitute an endogenous protective mechanism against atherosclerosis.[7] The clinical implications of short-term infusion of EETs were demonstrated by intracoronary infusion of EETs to reduce infarct size in a canine ischemia-reperfusion model,[8] and intra-arterial infusion of EETs to reduce blood pressure.[9]

To enhance the levels of EETs thereby providing potential therapeutic benefits, a straightforward approach is to inhibit soluble epoxide hydrolase, which is also referred to as sEH or *EPHX2*. sEH is an enzyme that converts EETs to dihydroxy eicosatrienoic acids (DHETs) via hydrolysis of EETs, whereby the biological effects of EETs are diminished or eliminated.[10] It has been postulated that sEH inhibition may lead to elevated levels of EETs, which could then have a significant beneficial impact on blood pressure, inflammation, and lipid and carbohydrate metabolism. In particular, the hypothesis on the blood pressure effect of sEH inhibition was strongly supported by the blood pressure lowering and renal protection effects of the sEH inhibitor 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA) in a salt-sensitive hypertensive animal model.[11] However, the lack of off-target activity data of AUDA does not allow for the formal conclusion that sEH is indeed a preclinically validated hypertension target. In addition, scientists at Arete developed an sEH inhibitor (structure unknown) that has recently entered phase II clinical trials specifically targeted for diabetes.[12]

The hydrolase catalytic pocket of sEH contains two tyrosine residues (Tyr381 and Tyr465) which act as hydrogen bond donors to activate the epoxide ring opening by Asp333.[13] Amide and urea groups fit in the pocket with the carbonyl oxygen being a hydrogen

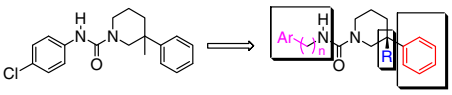

Figure 1. General scaffold of urea analogs.

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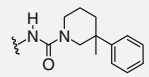
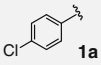
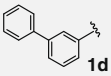
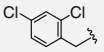
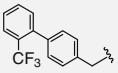
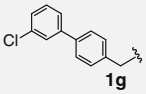
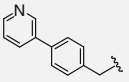
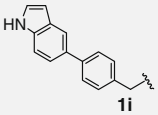
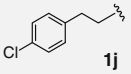
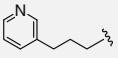
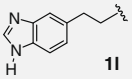
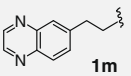
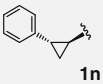
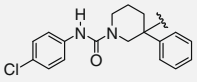
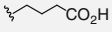
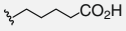
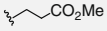
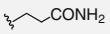
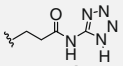
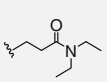
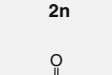
Table 1
In vitro SAR on sEH and mEH inhibition, DHET production, CYP and ion channel inhibition

Compd	sEH IC_{50} (h, r)[a] nM	DHET, IC_{50}[b] (nM)	mEH, IC_{50}[c] (μM)	CYP2C9, 2D6, 3A4 IC_{50} (μM)	DLZ, IKr $Na_v1.5$, IC_{50} (μM)
					
 **1a**	1, 5	<1	>100	>10, >10, <10	1.7, 12, >10
 **1b**	59, 207	—[d]	—	<10, >10, >10	19, 18, <10
 **1c**	550, 349	—	>100	>10, >10, <10	15, 5, >10
 **1d**	7, 6	—	—	<10, >10, >10	0.9, 21, <10
 **1e**	<1, 3	—	—	>10, <10, <10	2, 20, <10
 **1f**	8, 11	—	>100	<10, >10, <10	0.7, 0.2, >1
 **1g**	14, 7	—	22	<10, >10, <10	0.9, 2, ~10
 **1h**	1, 3	<1	47	<10, ~10, <10	0.5, 4, >10
 **1i**	7, 6	3	14	<10, ~10, >10	1, 0.8, >10
 **1j**	1, 5	—	54	>10, <10, <10	2, 9, —
 **1k**	7, 6	8	3	>10, >10, <10	—, 5, —
 **1l**	29, 26	13	>100	<10, <10, >10	—, 13, —
 **1m**	5, 19	3	>100	<10, ~10, <10	—, 9, —
 **1n**	1, 4	<1	>100	<10, —, <10	10, 26, >10

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.
[b] Studies were performed with HEK293 cells.
[c] Human mEH was used.
[d] Dashed lines indicate that compounds were not tested.

bond acceptor for Tyr381 and Tyr465, and the N–H of amide or urea being a hydrogen bond donor to Asp333. Therefore, various ureas and amides have been designed and prepared as selective and orally bioavailable sEH inhibitors.[14] Herein we report 3,3-disubstituted piperidine-derived trisubstituted ureas (Fig. 1) as highly potent and bioavailable sEH inhibitors, whose sEH and off-

Table 2
In vitro SAR on sEH and mEH inhibition, DHET production, CYP and ion channel inhibition

Compd	sEH IC_{50} (h, r)[a] (nM)	DHET, IC_{50}[b] (nM)	mEH, IC_{50}[c] (μM)	CYP2C9, 2D6, 3A4 IC_{50} (μM)	DLZ, IKr $Na_v1.5$, IC_{50} (μM)
					
 **2a**	52, 398	38	>100	>10, >10, >10	>30, >30, >10
 **2b**	5, 76	4	>100	>10, >10, >10	>30, >30, >10
 **2c**	12, 176	6	>100	>10, >10, >10	>30, >30, >10
 **2d**	5, 120	<1	>100	>10, >10, >10	>30, >30, >10
 **2e**	<1, 3	—[d]	—	—	—
 **2f**	3, 16	<1	>100	>10, >10, >10	7, 10, >10
 **2g**	1, 15	<1	>100	<10, ~10, >10	0.9, 0.07, >10
 **2h**	1.3, 10	<1	>100	<10, >10, <10	5, 6, >10
 **2i**	5,26	<1	>100	>10, >10, >10	—, 7, —
 **2j**	12, 119	1.3	>100	>10, <10, <10	0.1, 0.3, >10
 **2k**	5, 59	<1	>100	>10, ~10, <10	5, 3, >10
 **2l**	1.4, 34	1.4	>100	>10, >10, >10	22, 27, —
 **2m**	5, 32	<1	>100	~10, >10, ~10	4, 8, >10
 **2n**	3, 31	—	—	>10, >10, <10	1, 6, >10
 **2o**	8, 100	<1	—	>10, >10, >10	26, 29, >10

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.
[b] Studies were performed with HEK293 cells.
[c] Human mEH was used.
[d] Dashed lines indicate that compounds were not tested.

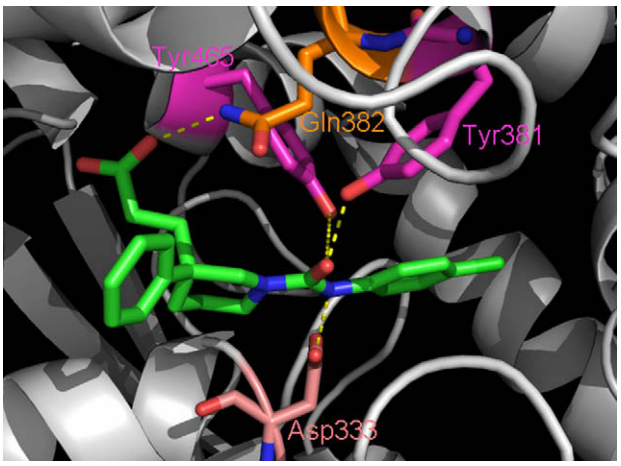
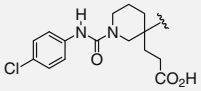
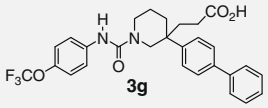


Figure 2. The minimized docking model of ***ent*A-2b** (green) in human sEH. The dashed lines illustrate atom pairs within hydrogen bond distance. The pictures were prepared by PyMOL (Delano Scientific LLC, South San Francisco, CA). The model is based on the X-ray crystallographic structure of 1ZD3 (PDB code): human soluble epoxide hydrolase 4-(3-cyclohexyluriedo)-butyric acid complex.[13]

target activities, ex vivo target engagement, pharmacokinetics (PK) and pharmacodynamics (PD) profiles are subject to detailed discussion.

Human and rat sEH enzyme inhibition assays established the intrinsic activity of our compounds. Potent compounds were further evaluated in a human whole cell 14,15-DHET production assay to assess their cellular activity. Major counterscreening targets included microsomal epoxide hydrolase (mEH), several CYP enzymes and selected ion channels. As mEH plays significant roles in xenobiotic detoxification[15] and steroid metabolism,[16] and certain ureas were reported to inhibit mEH,[17] mEH was selected as a major counterscreen target. Additionally, the inhibitory properties of compounds on CYP2C9, 2D6 and 3A4 were also measured. Of these three CYP enzymes, CYP2C9 is of particular importance due to its role in the production of EETs.[1b] Moreover, the activity of the compounds against potassium (IKr),[18] calcium (DLZ binding)[19] and sodium ($Na_v1.5$)[20] channels, was also examined due to their known blood pressure and/or cardiac effects. Importantly, to the best of our knowledge, all previous publications on sEH inhibitors by other research groups did not address whether they were effectors of mEH, CYP enzymes or ion channels. It is our belief that to determine whether sEH inhibitors can provide mechanism-based blood

Table 3
In vitro SAR on sEH and mEH inhibition, DHET production, CYP and ion channel inhibition

Compd	sEH IC_{50} (h, r)[a] (nM)	DHET, IC_{50}[b] (nM)	mEH, IC_{50}[c] (μM)	CYP2C9, 2D6, 3A4 IC_{50} (μM)	DLZ, IKr $Na_v1.5$, IC_{50} (μM)
					
 ***ent*A-2b**	5, 69	2	>100	>100, >100, >100 65 for CYP2C8	>30, >30, >10
 **3a**	16, 70	<1	>100	>10, >10, >10	>30, >30, >10
 **3b**	12, 176	—[d]	>100	>10, >10, >10	>30, >30, >10
 **3c**	5, 120	<1	>100	>10, >10, >10	>30, >30, >10
 **3d**	30, 467	—	>100	~10, >10, >10	>30, >30, >10
 **3e**	28, 331	—	—	>10, >10, >10	>30, >30, >10
 **3f**	19, 290	—	—	>10, >10, >10	>30, >30, >10
 **3g**	8, 413	<1	—	<10, >10, >10	>30, 21, <10

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat.
[b] Studies were performed with HEK293 cells.
[c] Human mEH was used.
[d] Dashed lines indicate that compounds were not tested.

pressure lowering efficacy, both high selectivity and excellent potency against sEH are imperative.

Starting with rationally designed trisubstituted urea **1a** as a lead structure, the structure–activity relationship (SAR) was explored by systematically modifying the three fragments of the general scaffold colored in purple, blue, and red respectively shown in Figure 1.

The SAR on the left-hand moiety of the scaffold was first examined with a 3-methyl-3-phenyl piperidine as the common right-hand motif (Table 1). Aniline-derived ureas such as **1a** in general gave excellent sEH inhibitory activity with various para- or meta-aniline substituents including chloro, CF_3, alkyl, and phenyl groups (data not shown). To minimize the potential oxidation of the aniline moiety of the ureas, electron-rich aniline-derived ureas were not prepared. Several bicyclic and monocyclic aminoheterocycles, however, gave inferior sEH potency as represented by **1b** and **1c**. 3-Biphenylaniline-derived urea **1d** also provided good sEH activity. Substituted benzylamine **1e** or 4-aryl benzylamine analogs **1f–i** led to potent analogs, of which **1h** was the most active for both human and rat sEH enzymes. Analogs bearing aryl or heteroaryl groups two or three carbon atoms away from the urea gave a wide range of activities (**1j–m**). It should be noted that with a few exceptions, this series of compounds provided better activity against human sEH than rat sEH. The major issues of these compounds (**1a–m**) were substantial ion channel or CYP inhibitory activity (IC_{50} <10 μM). In addition to its fairly clean ion channel profile, phenyl cyclopropyl amine-derived urea such as **1n** gave remarkable inhibitory activity against human and rat sEH enzymes, and the whole cell DHET production. With the exception of compounds **1g–k**, all tested analogs were completely inactive against mEH (IC_{50} >100 μM).

Next, the R substituent of the piperidine shown in Figure 1 was optimized (Table 2). With the ion channel issues of the compounds in Table 1, it was envisioned that by introducing a carboxylic acid moiety, the off-target profiles, in particular ion channel activity, would be minimized. As expected, compounds **2a–d** demonstrated neither ion channel nor CYP inhibitory activities. The linker length between the carboxylic acid and piperidine was crucial for the sEH potency of compounds. For example, analogs bearing a carboxylic acid two to four carbon atoms away from piperidine (**2b**, **2c** and **2d**) gave significantly more potent sEH inhibition than analog **2a** with one methylene linker. This observation may be explained by invoking our model of **2b** in the binding pocket of human sEH. In particular, a potential H-bond interaction between the acid moiety and Gln382 was identified that allowed for the presence of an acid group based on a docking model (Fig. 2). If the carboxylic acid linker was too short, as in the case of **2a**, the interaction between the enzyme and the inhibitor would be less favorable. On the other hand, longer linkers (**2c** and **2d**) could adopt certain conformation to allow the favorable interaction of the acid group with Gln382 (modeling results not shown) thereby providing better activity. The ester, cyanide, and alcohol analogs with a two-carbon linkder (**2e**–**g**) were even more potent against sEH than **2b** presumably as a result of H-bonds between these head groups with Gln382, but these compounds were accompanied with ion channel activity. Among four amides (**2i**, **2m**–**o**), **2o** was the least active for sEH, but was also the cleanest for CYP and ion channels. Interestingly, the tetrazole replacement of carboxylic acid led to more ion channel activity (**2k**). In contrast, a tetrazole amide (**2l**) was an effective replacement for the acid providing sEH inhibition and an off-target profile similar to **2b**. Moderately active against sEH, amine analog (**2j**) was a potent inhibitor of calcium and potassium channels. Correlating well with their enzyme inhibitory activity, low to subnanomolar inhibition of whole cell DHET production was observed for analogs **2d–o**.

As a potent and selective sEH inhibitor, racemic compound **2b** was further resolved to provide two enantiomers. The eutomer ***ent*A-2b** was 100- and 500-fold more active than the distomer ***ent*B-2b** in the sEH inhibition and DHET production assays respectively. The substituent effect of the phenyl group of **2b** was subsequently studied. Para-trifluoromethyl analog **3c** was more active against sEH than the meta- and ortho-trifluoromethyl analogs (**3a–b**). Furthermore, pyridine replacement of the phenyl group in **2b** yielded compounds with weaker sEH inhibition while maintaining excellent off-target profiles (**3d–f**). Biaryl compound **3g** was associated with good human but weak rat sEH activity, as well as substantial sodium channel and CYP2C9 inhibition (Table 3).

The rat PK data of several potent analogs are summarized in Table 4. Clearly, compound **2b** was unique in its excellent bioavailability, low clearance, and good oral exposure. In contrast, other analogs of **2b** (**1a**, **2i**–**k**) had much higher clearance and lower oral exposure.

To assess the serum shift of the sEH inhibition, the whole cell 14,15-DHET production assay of analog ***ent*A-2b** was conducted in the presence of 10% human serum. In this study, ***ent*A-2b** demonstrated a 2.5-fold serum shift (IC_{50} = 5 nM) with respect to its IC_{50} in this assay in the absence of serum (IC_{50} = 2 nM). Compound ***ent*A-2b** was further evaluated in an in vitro rat whole blood DHET production assay in which ***ent*A-2b** (1 μM) or vehicle and substrate (14,15-EET, 10 μM) were incubated for 0, 15, 30 and 60 min, and the DHET production rate was measured at each time point (Table 5). It should be noted that the data variation was small and reproducibility was quite good for this assay. Throughout the 60 min period, ***ent*A-2b** suppressed 14,15-DHET production by more than 90% relative to the vehicle control, consistent with the excellent sEH inhibitory activity of the compound in whole blood.

To ensure its selectivity for sEH, compound ***ent*A-2b** was tested against 167 other biological targets. Remarkably, the compound showed minimal activity in all cases (IC_{50} >10 μM).

With extraordinarily selective sEH inhibition, excellent DHET production inhibition in whole blood, and a good PK profile, ***ent*A-2b** was evaluated for its blood pressure effect in the spontaneously hypertensive rat (SHR) model (50 mpk, po), in which systolic and diastolic blood pressure, heart rate, and pulse pressure were measured via a telemetry method over 8 h post dose. No blood pressure effects were observed in this study for ***ent*A-2b** (Fig. 3). In addition, several other compounds with significantly

Table 4
Rat PK[a]

Compds	*F* (%)	Cl (mL/min/kg)	Vd_{ss} (L/kg)	$T_{1/2}$ (h)	$AUCN_{po}$ (μM h kg/mg)
1a	13	43	12	3.9	0.15
2b	84	4	0.8	3.2	10
2i	13	116	0.88	4.4	0.05
2j	52	59	1.4	0.66	0.37
2k	0.17	124	1.4	0.39	~0

[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.

Table 5
Inhibition of in vitro 14,15-DHET production in whole rat blood by ***ent*A-2b**[a]

Incubation time (min)	0	15	30	60
DHET production rate (% of control)	6.3	3.4	5.3	8.1
Deviation (%)	0.1	0.4	0.0	0.8

[a] 14,15-DHET production was measured at the indicated time point post dosing.

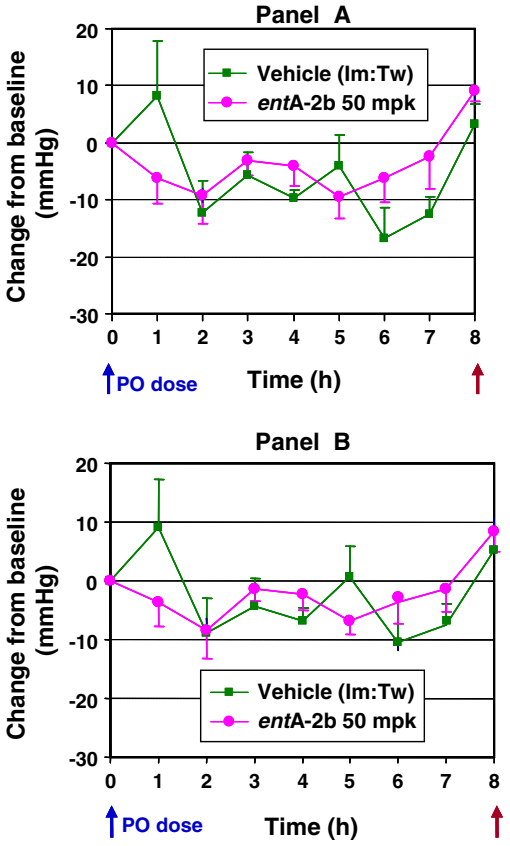
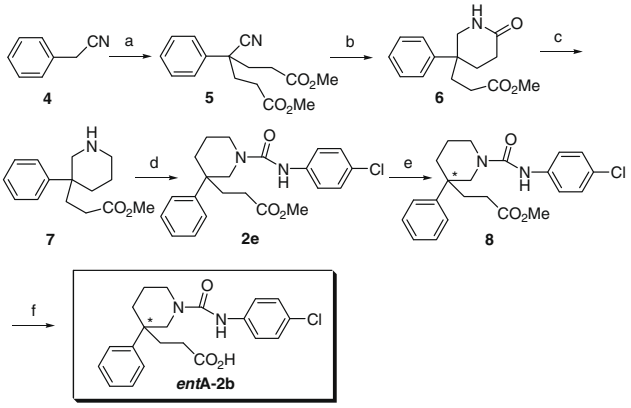


Figure 3. Panel A systolic and panel B diastolic blood pressure change from baseline (mmHg) of ***ent*A-2b** (50 mpk, po) in SHRs over 8 h (pink curve: ***ent*A-2b**; green curve: vehicle (Imwitor/Tween)).

different structures, good ex vivo target engagement and clean off-target profiles also failed to lower the blood pressure in SHR.[21] These results contradict with a previous report in which an sEH inhibitor acutely lowered blood pressure in SHRs.[22] It is conceivable that potential off-target activities of the previously communicated compound might have contributed to the observed anti-hypertensive effect.

The synthesis of the trisubstituted urea ***ent*A-2b** is shown in Scheme 1. Intermediate **7** was prepared following a literature procedure.[23] The subsequent urea formation, chiral separation of two enantiomers, and hydrolysis sequence provided ***ent*A-2b**.[24]



Scheme 1. Reagents and conditions: (a) Methyl acrylate, triton B, *t*-butanol, reflux, 4 h; (b) H_2 (50 psi), PtO_2, HOAc, rt, 5 h; (c) BH_3 dimethyl sulfide complex, THF, rt, 3 h, 48% over 3 steps; (d) parachlorophenyl isocyanate, DCM, rt, 14 h, rt, 72%; (e) SFC, 40%; (f) LiOH (1 N), THF/MeOH/water (3:1:1), rt, 2 h, 95%.

In conclusion, we have identified ***ent*A-2b**, a trisubstituted urea containing a 3,3-disubstituted piperidine motif, as a potent, selective sEH inhibitor with good target engagement and PK. However, this compound failed to demonstrate any significant blood pressure lowering activity in the SHR model. This is the third distinct sEH inhibitor we have reported on thus far that failed to exhibit an antihypertensive effect.[21] This most recent observation reinforces our conclusion that sEH may not be a validated antihypertensive target in the SHRs, a *bona fide* predictive model of the human activity of numerous anti-hypertensive therapeutics. Despite its lack of blood pressure lowering activity in SHRs, ***ent*A-2b** may still find utility in other indications.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.138.

References and notes

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24. *The procedures of the preparation of* **entA**-**2b**: to the boiling solution **4** (12 g, 102 mmol) and methyl acrylate (29 mL) in *tert*-butanol (30 mL) was added a solution of triton (40% in methanol, 9.5 mL) in *tert*-butanol (14 mL) through condenser dropwise. The solution was heated at reflux for 4 h. After distillation of solvents, the residue was dissolved in $CHCl_3$ (200 mL), washed with 1 N HCl (100 mL), water (200 mL), dried over Na_2SO_4 to give 30.6 g of **5** as a crude oil. The solution of **5** (4.7 g, 16.2 mmol) and PtO_2 (0.61 g) in acetic acid (50 mL) was subjected to hydrogenation at 50 psi for 5 h. After filtration, the filtrate was concentrated and the residue was dissolve in EtOAc (200 mL), washed with satd $NaHCO_3$ (200 mL), dried over Na_2SO_4 to give 4.5 g of **6** as an oil. To the solution of 6 (4.5 g, 17.2 mmol) in THF (100 mL) was added BH_3 (20 mL, 40 mmol, 2 M in THF) at 0 °C, The resulting solution was stirred at rt for 3 h before quenched with 3 N HCl (30 mL) at 0 °C. After removing the solvent, the residue was partitioned between water (200 mL) and EtOAc (200 mL), the aqueous phase was then neutralized by 1 N NaOH, extracted with 30% isopropanol/chloroform (2 × 100 mL). The combined organic phase was dried over Na_2SO_4, and concentrated to give 1.89 g of **7** as a crude oil with an overall yield of 48% over three steps. To the solution of **7** (1.57 g, 6.3 mmol) in DCM (100 mL) was added 4-chlorophenyl isocyanide (1.024 g, 6.7 mmol) and the resulting solution was stirred at rt for 16 h. After concentration, the residue was purified on silica gel using 25% EtOAc/hexane to get 1.83 g of **2e** as a colorless oil (72% yield). The racemic **2e** (4.2 g, 10.9 mmol) was subjected to chiral separation (SFC) to give a single enantiomer **8** (1.7 g, >99% ee, 40% yield). To the solution of **8** (1.7 g, 4.4 mmol) in THF/MeOH/water (3:1:1, 350 mL) was added LiOH (1 N, 50 mL) dropwise and the resulting solution was stirred for 1 h. After removing the volatile solvents, the aqueous phase was acidified to pH 4 using 1 N HCl, and then extracted with EtOAc (2 × 200 mL), the combined EtOAc phase was washed with brine (2 × 200 mL), dried over Na_2SO_4, and concentrated to give **entA**-**2b** (1.56 g, 95% yield) as a colorless oil. ^1H NMR (acetone-d_6, 500 MHz): δ 8.11 (1H, s), 7.53 (2H, d), 7.46 (2H, d), 7.35 (2H, t), 7.23 (3H, m), 4.01 (1H, d), 3.64 (1H, d), 3.56 (2H, m), 2.16–1.87 (6H, m), 1.71 (1H, m), 1.52 (1H, m); LC–MS *m*/*z*: 387 (M^++1).