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3,4-Diarylpiperidines as potent renin inhibitors

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ABSTRACT

The discovery and SAR of a series of potent renin inhibitors possessing a novel 3,4-diarylpiperidine scaffold are described herein. The resulting compound **38** exhibit low nanomolar plasma renin IC_{50}, had a clean CYP 3A4 profile and displayed micromolar affinity for the hERG channel. Furthermore, it was found to be efficacious in the double transgenic rat hypertension model and show good to moderate oral bioavailability in two animal species.

Treatment of hypertension, one of the major risk factors for cardiovascular diseases, has been an area of intense research for many years. Despite this, most patients still cannot achieve the targeted 140/90 mmHg as recommended by the American Heart Association (AHA) using the currently available therapies (i.e. diuretics, β-blockers, calcium channel blockers, ACE inhibitors, AT1R antagonists, etc.).[1] Disruption of the renin–angiotensin–aldosterone system (RAAS) at its various points (Fig. 1) has proven to be an effective strategy for the lowering of blood pressure.[2] Inhibition of renin, the enzyme responsible for the cleavage of angiotensinogen to angiotensin I, which is the first and rate limiting step of the RAAS pathway, should then afford the maximum blood pressure lowering effect.[3] Furthermore, it is believed that the inhibition of renin should have fewer mechanism-related side effects than the current therapies targeting downstream events of the RAAS pathway.[4] Indeed, significant research effort and resources from the pharmaceutical industry have been invested toward the discovery of a renin inhibitor suitable for the treatment of hypertension. Many compounds have entered clinical development,[5] but so far, only Aliskiren has received FDA approval (2007) for the treatment of hypertension.[6]

Previously, we described the discovery of a new diaryl scaffold generating potent renin inhibitors with compound **1** (Fig. 2) as a representative example.[7] Although **1** showed good potency against human renin and displayed interesting pharmacokinetic properties, two major liabilities were revealed when it was further profiled. Namely, **1** demonstrated a high affinity for the potassium channel encoded by the human ether-a-go-go gene (hERG) and inhibited P450 CYP3A4 both reversibly and in a time-dependent manner. Part of our efforts toward overcoming these two liabilities

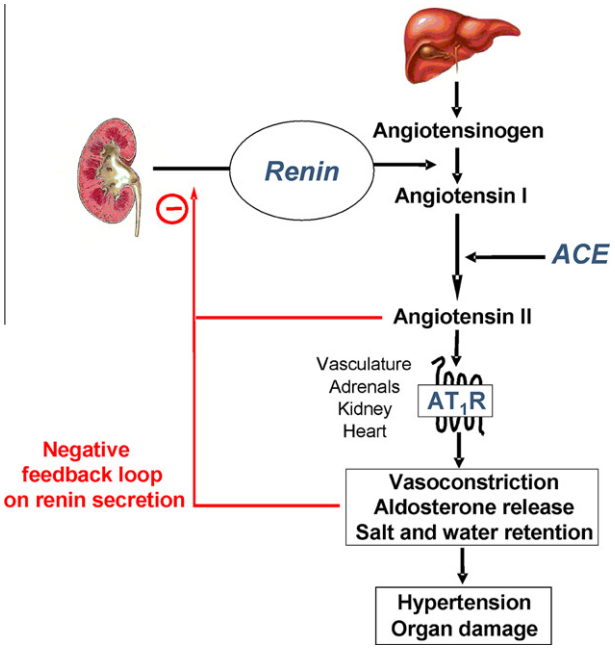

Figure 1. Renin–angiotensin–aldosterone system (RAAS).

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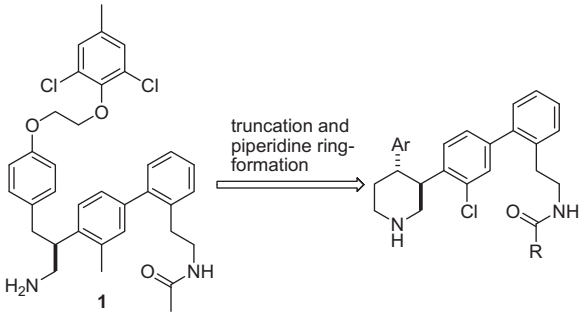


Figure 2. 3,4-Diarylpiperidine as primary amine replacement.

was recently published.[8] In an extension of this work, we report herein our discovery of a novel series of 3,4-diarylpiperidine as potent renin inhibitors.

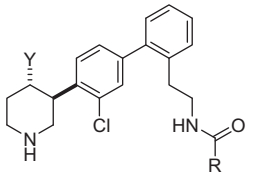
Our initial SAR efforts were focused on the removal of the northern lipophilic appendage of compound **1** (Fig. 1). However, in order to minimize the magnitude of the anticipated loss in potency associated with this truncation, the primary amine warhead of **1** was replaced by a piperidine moiety. Indeed, as was demonstrated in a previous publication,[9] decreasing the number of freely rotatable bonds has resulted in piperidine analogs with a tendency to be more potent in vitro towards renin and more efficacious in double transgenic rats than their acyclic counterparts. A series of 3,4-diarylpiperidines as represented in Tables 1 and 2 were then prepared following the synthetic route described in Scheme 1. Briefly, the Michael addition of 2-chloro-4-bromophenylacetonitrile **2** to a suitable ethyl aryl acrylate led to a diastereoisomeric mixture of the desired intermediate **3**. The latter was then cyclized under acidic conditions to afford the racemic *trans* diastereomer **4**. Subsequently, cyclic imide **4** was reduced with borane, protected as the *tert*-butyl carbamate, and resolved to give the the enantiomerically pure, *trans*-3,4-diarylpiperidine scaffold **5**. Finally, Suzuki cross-coupling of **5** with a suitable 2-substituted phenyl boronic acid **6** afforded compound **7**. Elaboration of derivative **7** could be carried out by first unmasking the terminal primary amine functionality via hydrogenation followed by acylation and Boc-deprotection with hydrochloric acid to give the desired amine **8**.

In addition, the requisite 4-substituted phenyl bromides for the preparation of the spirocycle derivative **27** (Scheme 3), as well as the tertiary alcohol derivatives **34** and **35** (Scheme 4), can be obtained using the synthetic routes described in Scheme 2. Starting from commercially available alcohol **9**, it can be converted to benzyl ether **10** by treatment with 4-bromobenzyl bromide under base promoted alkylation conditions. A two-step reduction–alkylation sequence then afforded the desired intermediate **12**. Starting from known alcohol **13**,[10] it can be converted to methyl ether **14** by treatment with MeI under base promoted alkylation conditions, followed by TBAF-mediated removal of the silyl protecting group. The resulting alcohol **14** was then converted to the desired phenyl bromide **15** as described previously. Furthermore, phenyl bromide derivative **18** could be accessed via a three-step sequence from commercially available carboxylic acid **16**. Indeed, borane reduction, Dess–Martin periodinane (DMP) oxidation and acetal formation under acidic conditions led to the desired aryl bromide **18**.

The spirocyclic derivatives **27** and **44** were prepared according to the synthetic route described in Scheme 3. Chemoselective Suzuki cross-coupling reaction of 2-bromo-5-iodotoluene **19** with commercially available 2-hydroxymethylbenzene boronic acid afforded, after alcohol protection, the requisite diaryl intermediate **20**. The latter was then converted to the desired keto-piperidine **21** using a Buchwald–Hartwig palladium catalyzed α-arylation protocol.[11] Further elaboration of **21** was then accomplished with Grignard reagents freshly prepared from either compound **16** to affords the hydroxy-carboxylic acid derivative or **18** to afford compound **22** which, following acidic treatment, gave the lactone (not shown) or lactol **23**, respectively. Reduction of lactol **23** with lithium borohydride and removal of the silyl protecting group afforded triol **24** which upon treatment with methanesulfonyl chloride could be readily converted to spirocyclic ether **25**. The benzylic mesylate group of **25** was then displaced with cyanide, reduced with cobalt borohydride[12] prepared in situ from cobalt(II) chloride hexahydrate and sodium borohydride, and acetylated. Finally, resolution using preparative chiral HPLC afforded, following Boc-deprotection with hydrochloric acid, the desired acetamide **27**.

In addition, the tertiary alcohols **34** and **35** were prepared via the route described in Scheme 4. Briefly, Buchwald–Hartwig α-arylation of Boc-piperid-4-one with 2-bromo-4-hydroxytoluene (**28**) readily delivered ketopiperidine **29**. Subsequent triflate formation and Suzuki cross-coupling reaction with aryl boronate ester **6** gave diaryl **30**. Hydrogenation in presence of acetic anhydride then afforded the requisite derivative **31**. Finally, addition of a suitable Grignard reagent prepared from either aryl bromide **12** or **15** affor-

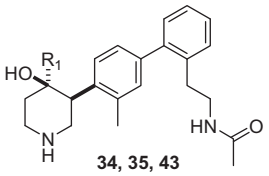
Table 1
In vitro data for compounds **36**–**42**



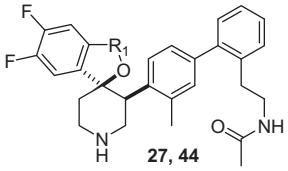
Compounds	Y	R	Renin buffer IC_{50}[a,b] (nM)	Renin plasma IC_{50}[a,b] (nM)	hERG IC_{50}[a] (μM)	CYP 3A4	
						Reversible inhibition[c] (% activity @ 10 μM)	Time-dependent inhibition[c] (% activity loss @ 10 μM)
36	3-4-Difluorophenyl	Me	0.5	7.5	1.1	98	49
37	Pyridin-3-yl	Me	1.4	3.6	4.8	98	55
38	1-Methyl-4-pyridinyl-2-one	Me	2.1	3.4	5.8	97	0
39	1-Methyl-4-pyridinyl-2-one	Et	4.8	8.0	2.8	92	21
40	1-Methyl-4-pyridinyl-2-one	OMe	3.52	6.1	10.3	92	46
41	1-Methyl-4-pyridinyl-2-one	OEt	11.0	28.2	3.2	91	58
42	1-Methyl-4-pyridinyl-2-one	CH_2OMe	73.8	97.4	—	—	—

[a] Values are means of at least two experiments.
[b] See Ref. 16 for assay protocol.
[c] See Ref. 17 for assay protocol.

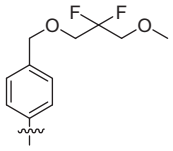
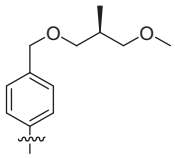
Table 2
In vitro data for tertiary alcohol and ether derivatives



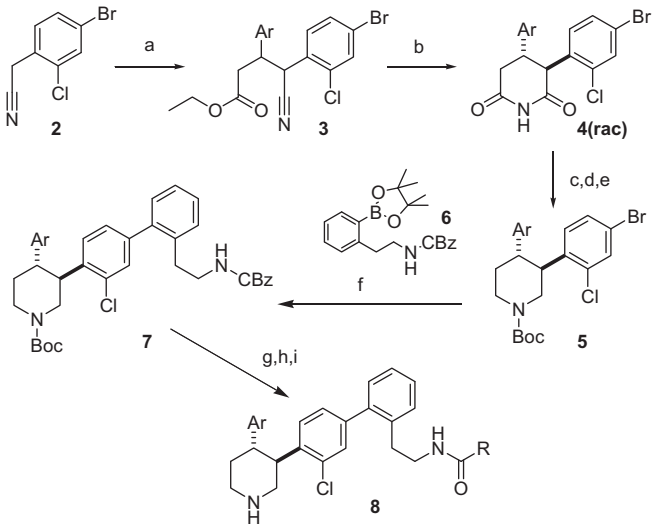
34, 35, 43



27, 44

Compounds	R^1	Renin buffer IC_{50}[a,b] (nM)	Renin plasma IC_{50}[a,b] (nM)	hERG IC_{50}[a] (μM)	CYP 3A4	
					Reversible inhibition[c] (% activity @ 10 μM)	Time-dependent inhibition[c] (% activity loss @ 10 μM)
43	3,4-Difluorophenyl	0.50	4.95	4.2	87	40
44	CO	0.14	1.6	12.9	80	88
27	CH_2	0.17	1.3	5.1	88	68
34		0.08	0.32	9.8	76	37
35		0.05	0.44	9.0	88	72

[a] Values are means of at least two experiments.
[b] See Ref. 10 for assay protocol.
[c] See Ref. 11 for assay protocol.

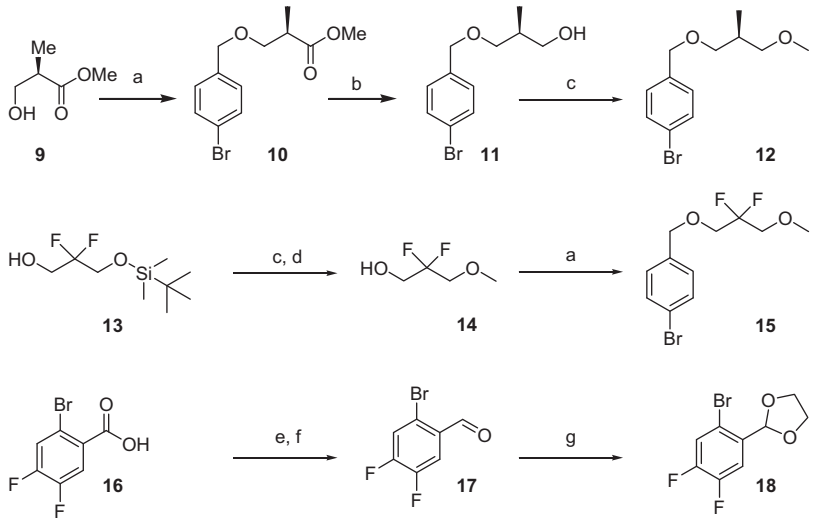


Scheme 1. Synthesis of **8**. Reagents and conditions: (a) NaOEt, EtOH, ethyl aryl acrylate; (b) H_2SO_4, AcOH; (c) BH_3·DMS, THF; (d) $(BOC)_2O$, Hünig's base, CH_2Cl_2; (e) Chiralpak AD separation; (f) $Pd(OAc)_2$, Ph_3P, Na_2CO_3, *n*-PrOH; (g) H_2, Pd/C, MeOH; (h) RCOCl, Hünig's base, CH_2Cl_2; (i) HCl, dioxane/CH_2Cl_2.

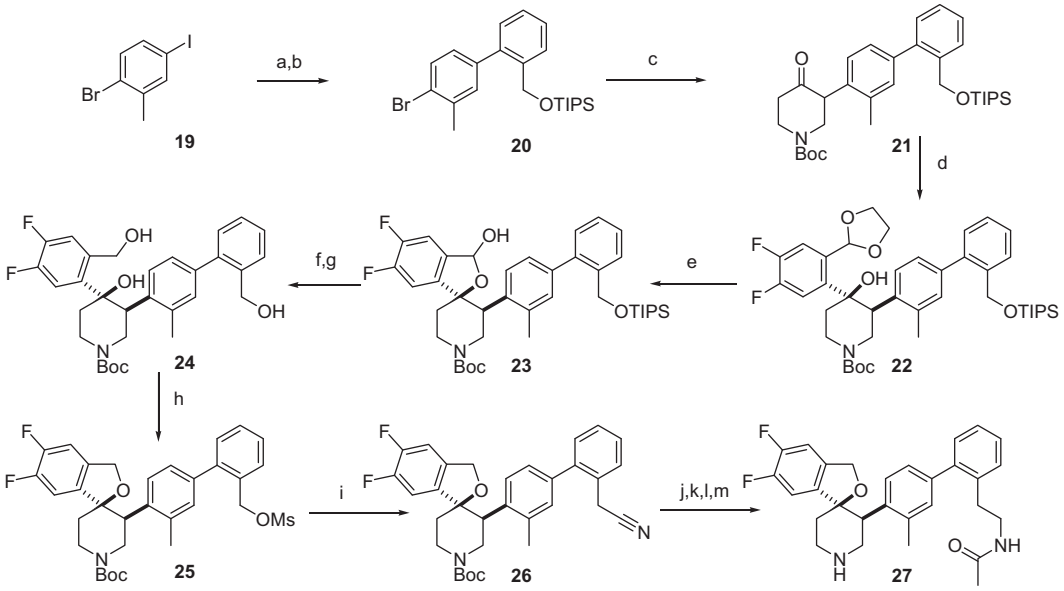
ded, after chiral resolution and acidic protecting group removal, the desired 3,4-diarylpiperidine **34** and **35**, respectively.

In agreement with what was previously observed, replacing the lipophilic 2-(2,6-dichloro-4-methylphenoxy)ethoxy residue present in **1** with either the 3,4-difluorophenyl (**36**),[13] pyrid-3-yl (**37**),[14] or the 1-methyl-4-pyridinyl-2-one moiety (**38**)[15] furnished potent, low nanomolar inhibitors of renin (Table 1). Indeed, these three privileged motifs are known to form both a stabilizing π-stack and a hydrogen bond with Tyr83 and Trp45 of the renin enzyme, respectively. Although all three compounds were intrinsically less potent against renin than **1** (for data, see Table 3), they were however less shifted in the presence of human plasma.[16] Consequently, the measured renin plasma IC_{50}s were found to be comparable across all four compounds. More importantly, these three truncated derivatives were found to demonstrate much lower affinity for the hERG channel as well as a decreased tendency to reversibly inhibit CYP3A4 activity versus their non-truncated counterpart **1** (Tables 1 and 2). Furthermore, the pyridone-bearing analog **38** did not suffer from time-dependent CYP3A4 inhibition and offered the optimum balance between renin potency and off-target profile. Consequently, further fine-tuning of compound **38** ethyl acetamide side chain was carried out. Unfortunately, replacement of the acetamide cap by either a propionamide (**39**), a methyl carbamate (**40**), an ethyl carbamate (**41**), or 2-methoxyacetamide (**42**) all failed to afford any tangible improvement. In fact, these modifications led to a loss in renin potency, as well as an increase in time-dependent CYP 3A4 inhibition. From the results in Table 1, it is also apparent that the compound **36** bearing the 3,4-difluorophenyl residue was the intrinsically most potent renin inhibitor in this series. Although a high level of protein binding blunted its potency in the presence of human plasma by 15-fold, we hypothesized that the judicious introduction of polarity into the scaffold of **36** would offer the best opportunity to decrease this plasma shift. Indeed, the incorporation of a tertiary alcohol at the 4-position of the piperidine ring led to a 33% drop in plasma shift without adversely impacting the intrinsic renin potency (i.e. **43**, Table 2). Furthermore, we were gratified to observe that a fourfold decrease in hERG affinity was also achieved with this addition.

Previously, we have demonstrated that the tertiary alcohol also offered a handle to freeze the adjacent 3,4-difluorophenyl moiety into its bioactive conformation via spirocyclization which led to a marked improvement in both renin potency and off-target profile.[15] Indeed, the same benefits were realized here as both spirocyclic ether **27** and spirocyclic lactone **44** were both more effective inhibitors of renin than their non-spirocyclic congener **43**. In addi-



Scheme 2. Synthesis of aryl bromide intermediates. Reagents and conditions: (a) 4-Bromobenzyl bromide, NaH, DMF; (b) DIBAL-H, CH_2Cl_2; (c) MeI, NaH, THF; (d) TBAF, THF; (e) BH_3·DMS, THF; (f) DMP, CH_2Cl_2; (g) ethylene glycol, $TsOH·H_2O$, benzene.

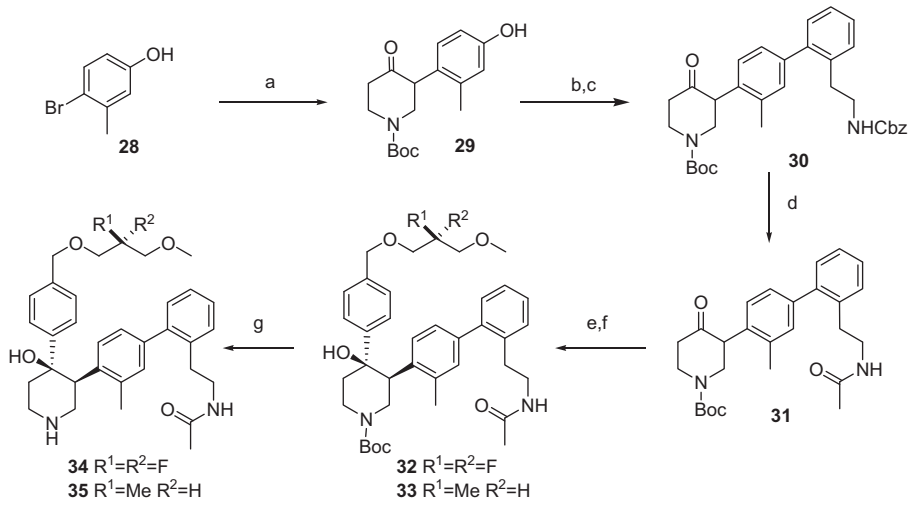


Scheme 3. Synthesis of spirocyclic compound **27**. Reagents and conditions: (a) 2-Hydroxymethylbenzene boronic acid, $Pd(PPh_3)_2Br_2$, Na_2CO_3, toluene/ethanol; (b) TIPSiCl, imidazole, DMF; (c) Boc-piperid-4-one, $Pd_2(dba)_3$, DTBPF, *t*-BuONa, THF; (d) aryl bromide **18**, Mg, LiCl, THF; (e) PPTS, acetone/H_2O; (f) $LiBH_4$, THF; (g) TBAF, THF; (h) MsCl, Et_3N, CH_2Cl_2; (i) KCN, TBAI, DMF; (j) $CoCl_2·6H_2O$, $NaBH_4$, MeOH; (k) AcCl, Hunig's base, CH_2Cl_2; (l) Chiralpak AD separation; (m) HCl, dioxane/CH_2Cl_2.

tion, lactone **44** exhibited the lowest affinity for the hERG channel of all the diaryl compounds presented here. Although all three compounds showed little affinity for CYP3A4, they unfortunately did inhibit CYP3A4 in a time-dependent manner. To address this, it was felt that further introduction of polarity may serve to decrease the oxidative stress on the molecule. Indeed, we hypothesized that this was the main reason why pyridone **38** possessed such a clean CYP profile. In this regard, the 3,4-difluorobenzene of **43** was replaced by two representative phenyl derivatives bearing polyether side-chain (i.e. **34**, **35**). While these modifications did deliver sub-nanomolar renin inhibitors even in the presence of plasma, they unfortunately failed to completely dial out the CYP3A4 time-dependent inhibition liability.

Having established that 3,4-diarylpiperidine **38** was still the best representative of this novel series of renin inhibitors, the compound was profiled further (Table 3). As previously mentioned, even though compound **38** was found to be intrinsically ~50× less potent against renin than **1**, it was less protein shifted and found to be 3× more potent in the presence of plasma. Furthermore, analog **38** showed a 1000-fold improvement over **1** with regards to its hERG binding affinity and, unlike **1**, possessed a clean CYP3A4 profile. When dosed orally as the hydrochloride salt to Sprague–Dawly rats at 30 mpk, or to dogs at 3 mpk (0.5% methocel solution, 5 mL/kg), compound **38** was found to have good to moderate bioavailability. Finally, it was found to be efficacious in a hypertensive double transgenic rat model (dTGR) harboring both human renin and human angiotensinogen genes. At a dose of 10 mpk, robust blood pressure lowering over a period of 24 h was achieved with compound **38** (Table 3).

In conclusion, we were able to design a novel series of 3,4-diarylpiperidine renin inhibitors with good in vitro potency and lower affinity for the hERG channel. Furthermore within this series, it was demonstrated that potent compounds devoid of CYP3A4-driven drug–drug interaction liabilities can be identified. One such



Scheme 4. Synthesis of tertiary alcohol derivatives **34** and **35**. Reagents and conditions: (a) Boc-piperid-4-one, $Pd_2(dba)_3$, Q-Phos, *t*-BuONa, THF; (b) Tf_2O, pyridine, CH_2Cl_2; (c) boronate ester, $Pd(OAc)_2$, S-Phos, K_3PO_4, THF/H_2O; (d) H_2, Pd/C, Ac_2O, EtOH; (e) aryl bromide **12** or **15**, Mg, LiCl, THF; (f) Chiralpak AD separation; (g) HCl, dioxane/CH_2Cl_2.

Table 3
Comparative profiles for compounds **1** and **38**

Compounds		**1**	**38**
Renin	Buffer IC_{50}, nM[a,b]	0.04	2.1
Renin	Plasma IC_{50}, nM[a,b]	9.6	3.4
hERG	IC_{50} (μM)[a]	0.006	5.8
CYP3A4	Reversible inhibition[c]	53	97
	Time-dependent inhibition[c]	74	0
	F (%)	23	12
SD Rat	C_{max} (μM)	0.44	0.45
(30 mpk po)	Cl (mL/min/kg)	34	115
(5 mpk iv)	$T_{½}$ (h)	7	4
	V_{dss} (L/kg)	9	25
	F (%)	—	30
BeagleDog	C_{max} (μM)	—	0.35
(3 mpk po)	Cl (mL/min/kg)	—	24
(1 mpk iv)	$T_{½}$ (h)	—	6.2
	V_{dss} (L/kg)	—	4.6
Efficacy in dTGR (10 mpk po)	Max. BP↓ (mmHg)	—	32
	Duration (h)	—	24

[a] Values are means of at least two experiments.
[b] See Ref. 16 for assay protocol.
[c] See Ref. 17 for assay protocol.

compound, renin inhibitor **38**, was found to exhibit good to moderate bioavailability in both rodents and dogs and to be efficacious in dTGR. Other biaryl-based renin inhibitors, as well as their associated properties, will be disclosed in due course.

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16. *Buffer assay*: Human recombinant renin (Proteos) at 100 pM was incubated in the presence or absence of renin inhibitors and 6 μM of Q-FRET substrate **9** *DNP-Lys-His-Pro-Phe-His-Leu-Val-Ile-His-D,L-Amp* in 50 mM MOPS, 100 mM NaCl, pH 7.4, 0.002% Tween. The reactions were performed in Costar 384 well black plates at 37 °C for 3 h. Fluorescence was measured at times 0 and 3 h in a SpectraMax Gemini EM reader with excitation and emission filters at 328 nm and 388 nm, respectively. *Plasma assay*: Frozen human EDTA-plasma was rapidly thawed in warm water and centrifuged at 2900 g for 15 min at 40 °C. The supernatant was collected and recombinant human renin (Proteos) were added at 1 nM nominal concentration. The plasma was transferred to Costar black 384 well plates, renin inhibitors added and the mixture was pre-incubated at 37 °C for 10 min. The renin Q-FRET substrate *QXL520-Lys-His-Pro-Phe-His-Leu-Val-Ile-His-Lys-(5-FAM)* (Proteos), diluted in 3 M Tris/200 mM EDTA, pH 7.2 was added to the plasma with final concentrations of 342 mM Tris, 23 mM EDTA and 6.8 μM substrate. The plate was incubated at 37 °C for 1 h and the plates were analyzed in a SpectraMax Gemini EM reader with excitation and emission filters at 490 nm and 520 nm, respectively, at time 0 and 1 h.
17. (a) Calculated as percentage of the difference in the rate of CYP3A4-mediated conversion of testosterone (250 μM) to 6-β-hydroxytestosterone in the presence of compound (10 μM in DMSO) versus blank DMSO. A 50% change corresponds to a reversible CYP3A4 inhibition IC_{50} of 10 μM.; (b) Calculated as percentage of the difference in the rate of CYP3A4-mediated conversion of testosterone (250 μM) to 6-β-hydroxytestosterone before and after 30 min incubation period with the compound (10 μM in DMSO). A 0% change corresponds to no measurable time-dependent CYP3A4 inhibition.