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Design and optimization of a substituted amino propanamide series of renin inhibitors for the treatment of hypertension

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ABSTRACT

The discovery and SAR of a new series of substituted amino propanamide renin inhibitors are herein described. This work has led to the preparation of compounds with in vitro and in vivo profiles suitable for further development. Specifically, challenges pertaining to oral bioavailability, covalent binding and time-dependent CYP 3A4 inhibition were overcome thereby culminating in the identification of compound **50** as an optimized renin inhibitor with good efficacy in the hypertensive double-transgenic rat model.

The renin–angiotensin–aldosterone system (RAAS, Fig. 1) plays a key role in the regulation of extracellular fluid volume and blood pressure.[1] Over the past decades, agents capable of antagonizing the RAAS pathway at its various steps (i.e., angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers) have emerged as effective treatments for hypertension.[2] Despite these significant advances and the availability of therapies that operate distinctly from the RAAS pathway (i.e., diuretics, β-blockers and calcium channel blockers)[3], hypertension remains poorly controlled in many patients and often requires combination therapy to achieve the targeted blood pressure lowering.[4] Since the cleavage of angiotensinogen by renin represents the first and rate-limiting step of the RAAS cascade, the maximum benefit that can be gleaned from the modulation of this pathway could come in the form of a direct inhibitor of renin.[5,6]

Despite significant research investments from the pharmaceutical industry directed towards the discovery of renin inhibitors suitable for clinical development,[7,8] only Aliskiren (**1**, Fig. 2) has gained FDA approval for the treatment of hypertension.[9]

All of the first generation renin inhibitors synthesized during the 1980s such as remikiren (RO 42-5892, **2**, Fig. 2), were pepti-

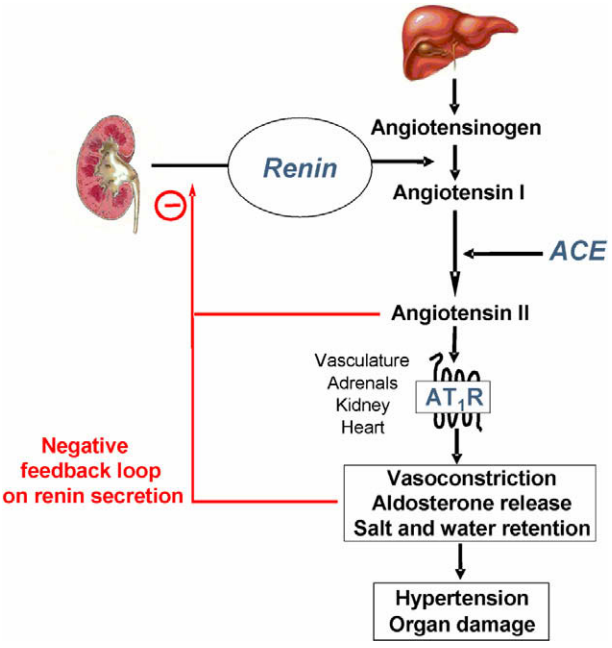

Figure 1. The renin–angiotensin–aldosterone system.

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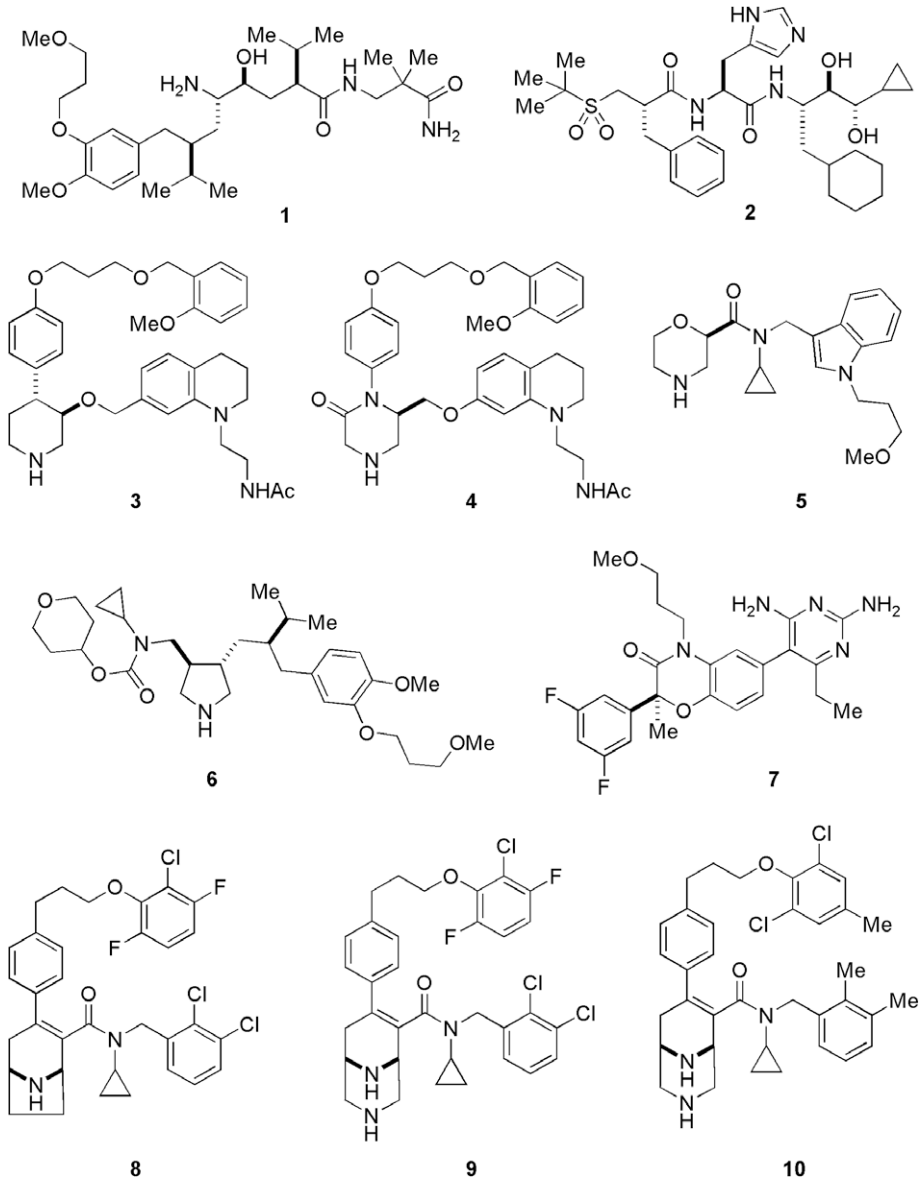


Figure 2. Representative renin inhibitors.

domimetics and possessed identical peptide hydrolysis transition state isostere.[10] Consequently, these compounds often suffered from high molecular weight, limited metabolic stability and low oral bioavailability. While efforts to decrease the peptidic character eventually culminated in the discovery of second generation renin inhibitors such as Aliskiren,[9] vestiges of the peptide backbone and side chains present in the first generation renin inhibitors remained as key structural features. Consequently, these second generation compounds were also plagued by low oral exposure in preclinical species and man. The first significant structural departure from these original scaffolds came from the recognition that the nitrogen of 3,4-disubstituted piperidines (**3**),[11] when protonated, would bind strongly to the aspartic acid dyad at the renin active site. Further structural diversifications have since revealed that morpholine (**4**),[12] piperazine (**5**)[13] and pyrrolidine (**6**),[14] as well as their bicyclic analogues (**8, 9, 10**),[15,16] along with diamino-pyrimidine (**7**),[17] could also serve as suitable warheads for renin inhibition.

Motivated by the need to simplify the often laborious synthesis of typical renin inhibitors; many of which display multiple stereocenters, we were intrigued by the idea that compounds bearing a simple methylene amine warhead could serve as a suitable starting scaffold. For the initial proof-of-concept experiment, direct mapping of this functionality onto the known diaza-bicyclononene renin inhibitor **9** was envisioned (Fig. 3). Indeed, using well-known, high-yielding reactions, compound **11** could be rapidly accessed from the known bromoarene **13** in 7 steps (Scheme 1). Pd-catalyzed formylation of **13** under standard conditions with sodium formate as the reducing agent afforded aldehyde **14** in 81% yield.[18] Subsequent Knoevenagel condensation of **14** with methyl cyanoacetate in the presence of catalytic amount of piperidine was carried out quantitatively. α,β-Unsaturated cyanoester **15** thus obtained could then be hydrogenated in $CHCl_3$-spiked MeOH with Pd black as the catalyst before the resulting amine was protected as the Boc-carbamate **16** in 42% overall yield. Hydrolysis to carboxylic acid **17** with aq lithium hydroxide followed by coupling with amine **18** both proceeded without incidents. Finally, removal of the Boc-protecting group from amide **19** was accomplished with a large excess of HCl and afforded **11** in 85% yield over three steps.[15] Synthesis of compound **12** on the other hand, commenced

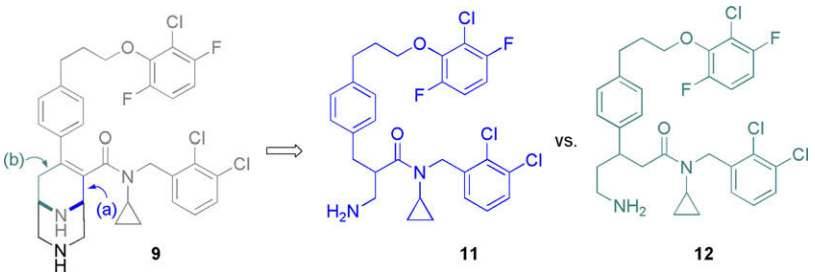
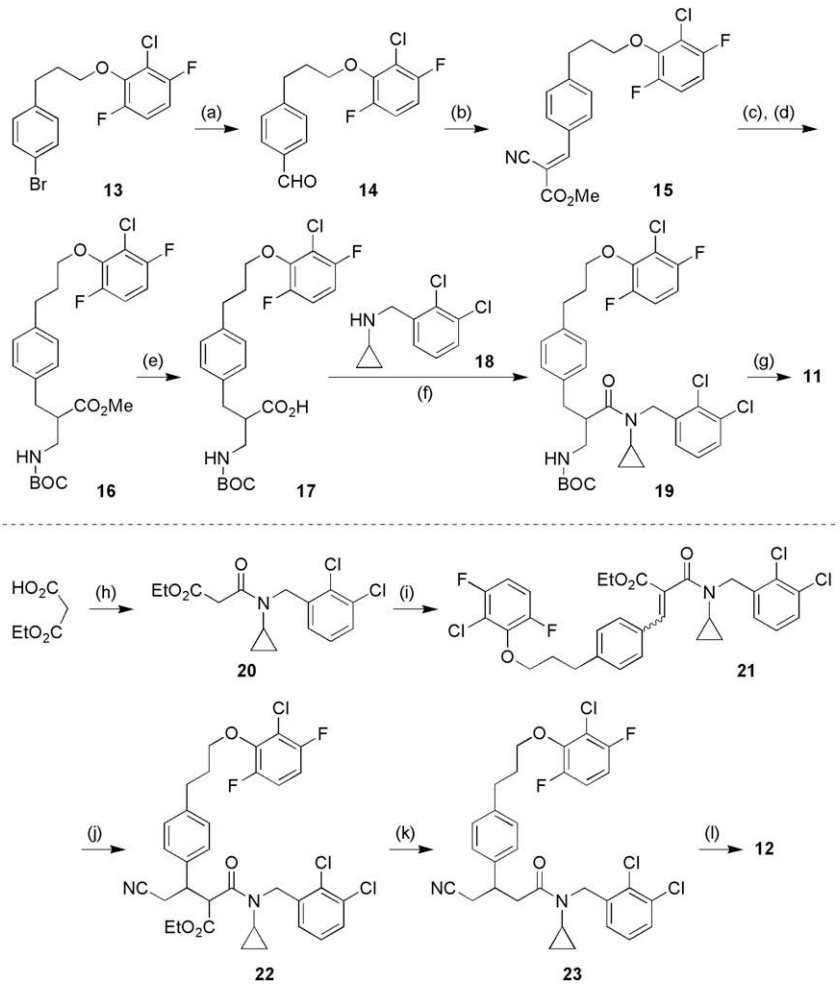


Figure 3. Design of amino methylene warhead scaffold.

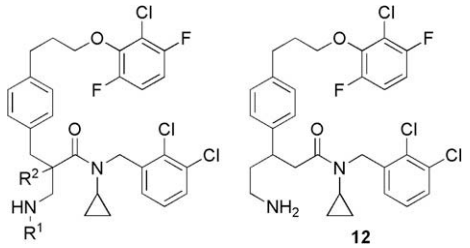


Scheme 1. Synthesis of **11** and **12**. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CO, $NaCO_2H$, DMF, 110 °C, 8 h, 81%; (b) methyl cyano-acetate, piperidine, PhH, 80 °C, 5 h, >99%; (c) Pd/C, H_2, MeOH, $CHCl_3$, 14 h; (d) BOC_2O, 1 N aq NaOH, CH_2Cl_2, 14 h, 42% over two steps; (e) 2 N aq LiOH, MeOH, THF, 16 h, >99%; (f) **18**, HATU, Hunig's base, DMF, 5 h, 75%; (g) 4 M HCl in dioxane, CH_2Cl_2, 4 h, 85%; (h) **18**, HATU, Hunig's base, DMF, 15 h, 95%; (i) piperidine, PhH, 80 °C, 18 h, >99%; (j) MeCN, *n*BuLi, −78 °C, 45 min, then CuI, −30 °C, 30 min, then **21**, −30 °C to rt, 2 h, 66%; (k) 1 N aq LiOH, MeOH, reflux, 24 h, 90%; (l) Pt/C, H_2, AcOH, 23%.

with the coupling of ethyl malonate with amine **18**. Knoevenagel condensation between amide **20** and aldehyde **14** then afforded α,β-unsaturated amide **21** as a ~1.2:1 mixture of geometric isomers. Amide **21** readily underwent conjugate addition when treated with the cuprate prepared in situ from lithiated acetonitrile and copper(I) iodide.[19] When the resulting amide **22**, a ~2.2:1 mixture of diastereomers, was heated to reflux in the presence of aqueous lithium hydroxide in methanol, ester hydrolysis followed by decarboxylation took place and afforded amide **23** in 90% yield. Finally, reduction of the cyano group could be accomplished in acetic acid with Pt/C as the hydrogenation catalyst.

We were pleased to observe that compound **11**, synthesized as a racemate, was a potent inhibitor of renin in both the absence or presence of human plasma, with an IC_{50} of 0.79 nM and 21 nM, respectively (Table 1). On the other hand, its constitutional isomer **12** was >800-fold less potent in the renin buffer assay. While tolerated, methylation of the primary amine in **11** using standard reductive amination conditions did not further improve the intrinsic potency against renin and the resulting compound was found to be more plasma-shifted. On the other hand, methylation of the amide-bearing carbon in **11** to furnish **26** led to a precipitous drop in intrinsic potency.

Table 1
SAR of select renin compounds: core modifications



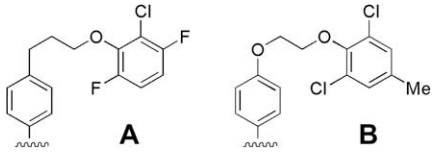
Compound	R^1	R^2	Renin IC_{50}[a,b] (nM)	
			Buffer	Plasma
11	H	H	0.79	21
12	—	—	650	ND[c]
24	Me	H	1.9	350
25	CH_2Ph	H	260	ND[c]
26	H	Me	210	ND[c]

[a] See Ref. 20 for assay protocols.
[b] Average of at least two replicates.
[c] ND = not determined.

The discovery that related compounds possessing the 2-chloro-3,6-difluorophenol residue can bind covalently to protein in the presence of NADPH and liver microsomes[15] necessitated a re-optimization of the northern chain. Gratifyingly, it was found that a switch to the 2-(2,6-dichloro-4-methylphenoxy)ethoxy northern terminus led to a decrease in the compound's covalent binding potential (Table 2) but without jeopardizing its potency against renin (vide infra).

Computer modeling suggested that re-optimization of the benzyl amine aromatic plate would offer the best opportunity for improving renin potency. Namely, we hypothesized that the introduction of an aliphatic chain of an appropriate length and functionality at the *meta* position of the benzyl amide residue would better anchor the substrate to the renin enzyme by filling the still vacant S3 sub-pocket. In this task, a 3-methoxypropyl tail proved to be optimal (Table 3). Furthermore, since the renin inhibition observed for racemate **28** should be ascribed specifically to one of its two antipodes, a stereoselective synthesis of the (*S*)-enantiomer was carried out via an adaptation of a sequence previously developed by Hintermann and Seebach for the synthesis of unnatural, homologated amino acids.[22] Indeed, the ~2-fold increase in potency observed on going from **28** to **29** confirmed that the active enantiomeric form to be of the (*S*)-configuration.

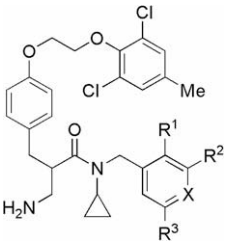
Table 2
Covalent binding potential of different northern termini[a]



Terminus	Species	Additive	pmol equiv/(mg @ 1 h)
A	Rat	None	82
		NADPH	415
	Human	None	71
		NADPH	508
B	Rat	None	25
		NADPH	111
	Human	None	22
		NADPH	159

[a] COBILM assay (**co**valent **b**inding **i**n **l**iver **m**icrosomes): incubations ($n = 3$) were done with rat or human liver microsomes at 10 μM, with 1 mg protein/mL, for 60 min with or without NADPH (1 mM) as additive. For complete protocol, see Ref. 21.

Table 3
SAR of select renin compounds: P_3 arene modifications

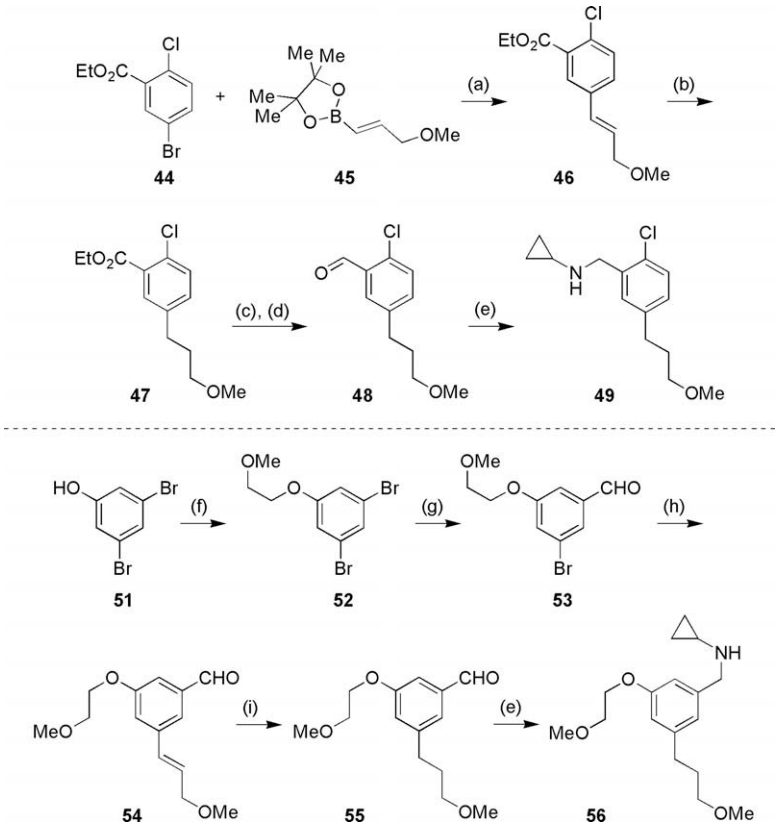


Compd	R^1	R^2	R^3	X	Renin IC_{50}[a,b] (nM)	
					Buffer	Plasma
27	Cl	Cl	H	CH	0.40	29
28	Cl	H	$(CH_2)_3OMe$	CH	0.26	5.8
29[c]	Cl	H	$(CH_2)_3OMe$	CH	0.09	2.4
30	Cl	H	$O(CH_2)_2OMe$	CH	0.21	18
31	Cl	H	$(CH_2)_2OMe$	CH	0.19	7.0
32	Cl	H	$CH_2N(Me)Ac$	CH	2.9	250
33	Cl	H	$(CH_2)_3N(Me)_2$	CH	1.5	103
34	Cl	Cl	$(CH_2)_3OMe$	CH	0.26	31
35	Cl	H	$(CH_2)_2CN$	CH	0.27	10
36[c]	Cl	H	$(CH_2)_3CN$	CH	0.11	4.6
37	Cl	H	$(CH_2)_3OH$	CH	0.09	6.4
38	Cl	H	$(CH_2)_3OMe$	N	0.19	2.2
39	Cl	H	$(CH_2)_3OMe$	$N^+–O^-$	0.3	1.8

[a] See Ref. 20 for assay protocols.
[b] Average of at least two replicates.
[c] (*S*)-Enantiomer only.

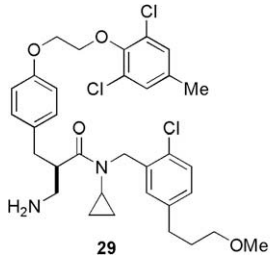
Compound **29** was chosen for further profiling. The requisite amine **49** could be accessed from ester **44** using the chemistry disclosed in Scheme 2. Specifically, after an initial Suzuki-coupling of **44** with commercially available pinacolboronate **45**, subsequent hydrogenation of the double bond in **46** was best accomplished with Wilkinson's catalyst on a Parr shaker (50 psi H_2). Ester **47** thus obtained was then converted into aldehyde **48** via a two-step sequence: reduction with DIBAl-H followed by Swern oxidation.[23] Finally, reductive amination with cyclopropylamine afforded amine **49**. When compound **29** was formulated either as a hemifumarate or HCl salt, moderate to good oral exposure was observed in SD rats, and beagle dogs (Table 4). When tested in a telemetered double-transgenic rat (dTGR) cohort ($n = 8$)[24], maximum blood pressure lowering of 27 mm Hg could be achieved at a P.O. dose of 3 mpk. Duration of action was found to exceed 48 h. Although compound **29** did exhibit worrisome affinity for the hERG channel (220 nM), none of the key CV intervals (i.e., QTc, PR, QRS) were significantly prolonged (<5%) when **29** was given intravenously to beagle dogs at up to 12 μM plasma exposure. Unfortunately, compound **29** was found to inhibit CYP 3A4 activity in a time-dependent manner and thus possesses the potential to perpetrate drug–drug interaction when co-dosed with drugs known to be cleared mainly by CYP 3A4 metabolism (e.g., simvastatin).

Given the promiscuous nature of the CYP 3A4 pocket, it proved highly challenging to completely dial out the time-dependent inhibition associated with this structural series while at the same time maintaining potency against renin. After much optimization, it was observed that the installation of a second appendage onto the eastern aromatic plate (e.g., **50**, Table 4) did lead to a significant improvement in CYP profile but without jeopardizing the

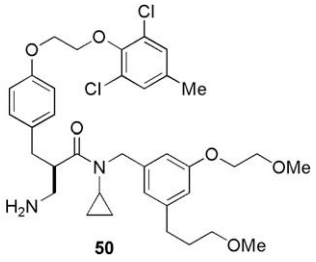


Scheme 2. Synthesis of **49** and **56**. Reagents and conditions: (a) $Pd(PPh_3)_4$, 2 N aq Na_2CO_3, *n*PrOH, 80 °C, 1 h, 90%; (b) $Rh(PPh_3)_3Cl$, 50 psi H_2, EtOH, 24 h, 85%; (c) DIBAl-H, CH_2Cl_2, −78 °C to rt, 3 h; (d) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, 0 °C to rt, 1 h, 82% over two steps; (e) cyclopropylamine, $MgSO_4$, CH_2Cl_2, 18 h, then $NaBH_4$, MeOH, 1.5 h, 95% for **49**, 88% for **56**; (f) $Br(CH_2)_2OMe$, Cs_2CO_3, DMF, 80 °C, 16 h, 90%; (g) *n*BuLi, DMF, THF, −78 °C, 30 min, 45%; (h) $Pd(PPh_3)_2Br_2$, **45**, 2 N aq Na_2CO_3, DMF, 80 °C, 3 h, 77%; (i) Pd/C, H_2, EtOAc, 75%.

Table 4
Key profiles of compounds **29** and **50**



29



50

		29	**50**
Renin IC_{50}[a,b] (nM)	Buffer	0.09	0.02
	Plasma	2.4	3.5
SD Rat (30 mpk P.O., 5 mpk I.V.)	*F* (%)	41	48
	P.O. AUC (μM h)	8.3	27.5
	Cl (mL/min/kg)	35	13
	$T_{1/2}$ (h)	6	4
	V_{dss} (L/kg)	12	3.6
Beagle Dog (3 mpk P.O., 1 mpk I.V.)	*F* (%)	32	36
	P.O. AUC (μM h)	1.3	0.7
	Cl (mL/min/kg)	16	35
	$T_{1/2}$ (h)	17	7.5
	V_{dss} (L/kg)	8.4	15
COBILM[c], human	With NADPH pmol equiv/(mg @ 1 h)	115	90
	Without NADPH pmol equiv/(mg @ 1 h)	44	32
Efficacy in dTGR (3 mpk P.O.)	Max. BP ↓ (mm Hg)	27	35
	Duration (h)	~2 days	~2 days
CYP 3A4 IC_{50} (nM)	Reversible	14000	2000
	30 min pre-incubation	130	1300

[a] See Ref. 23 for assay protocols.
[b] Average of at least two replicates.
[c] COBILM assay (**co**valent **b**inding **i**n **l**iver **m**icrosomes): incubations ($n = 3$) were done with human liver microsomes at 10 μM, with 1 mg protein/mL, for 60 min with or without NADPH (1 mM) as additive. For complete protocol, see Ref. 21.

compound's potency against renin. An X-ray of **50** bound (Fig. 4) to renin revealed that the second appendage was threaded through a small channel in the renin S3 pocket into the solvent. Compound **50** was then selected for further profiling and the synthesis of the requisite amine **56** used for its preparation is shown in Scheme 2. Starting with 3,5-tribromophenol (**51**), O-alkylation with 2-bromoethyl methyl ether with cesium carbonate as base furnished ether **52** in 90% yield. Initial treatment of **52** with 0.8 equiv of *n*-butyl lithium, followed by quenching with DMF afforded aldehyde **53** in 45% yield. Using a similar end game (e.g., Suzuki-coupling with **45**, hydrogenation and reductive amination with cyclopropylamine), aldehyde **53** was converted to amine **56** in 51% yield over three steps. Compound **50** also exhibited good oral bioavailability in both SD rats (48%) and beagle dogs (36%). Robust blood pressure lowering at 3 mpk was achieved with **50** when the compound was tested in the dTGR model ($n = 8$) with a duration of action also exceeding 48 h.

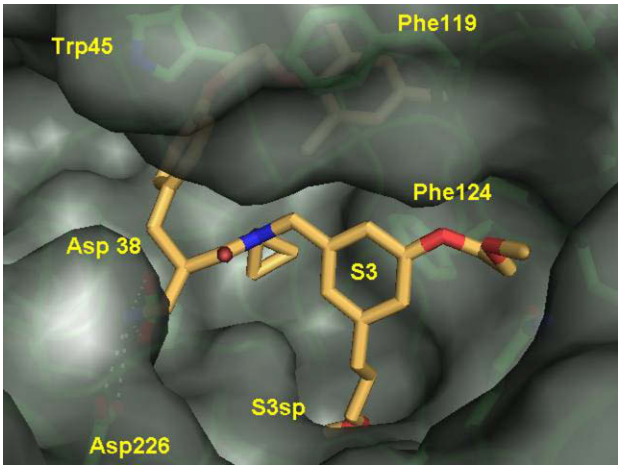


Figure 4. X-ray structure of **50** bound to renin.

In conclusion, we have described a novel series of potent and orally-bioavailable renin inhibitors. Modification of the northern terminus led to compounds with lower potential to bind covalently to proteins when incubated with liver microsomes. To address the pre-incubation dependent CYP 3A4 inhibition observed with compound **29**, further optimization of the eastern aromatic plate afforded compound **50**, a renin inhibitor with a profile suitable for further clinical development.

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20. *Buffer assay*: recombinant human renin (3 pg/μL) in assay buffer (PBS1X, 1 mM EDTA, 0.1% BSA, pH = 7.4), human tetradecapeptide (1–14) substrate (5 μM in 10 mM HCl), hydroxyquinoline sulfate (30 mM in H_2O) and assay buffer were pre-mixed at 4 °C at a ratio of 100:30:10:145. 47.5 μL per well of this pre-mix was transferred into polypropylene plates. Test compounds were dissolved and diluted in DMSO and 25 μL added to the pre-mix, then incubated at 37 °C for 3 h. At the end of the incubation period, 5 μL of the renin reaction (or standards in the assay buffer) were measured for AngI accumulation. The percentage of renin inhibition (AngI decrease) was calculated for each concentration of compound and an IC_{50} was determined by curve fitting software. *Plasma assay*: citrate-plasma from human volunteers was pooled, aliquoted and stored frozen at −20 °C. Renin activity in pooled plasma was supplemented with recombinant human renin (150 pg/mL final concentration) in order to increase the readout of the assay. Five microlitre of renin inhibitors, at various concentrations in DMSO, was added to 80 μL of a mixture (7:1) of human plasma and a fast trapping primary AngI antibody (anti-AngI: AS1, bleed 6, pre-diluted 1:10 in horse serum) diluted initially 1:26.5 in assay buffer (PBS1X, 1 mM EDTA, 0.1% BSA, pH 7.4) and then diluted 1:3.3 in 3 M Tris, 200 mM EDTA, pH 7.2 (final anti-serum dilution 1:50,000) and was incubated at 37 °C for 2 h. As was done in the buffer assay, 12 μL of the renin reaction (or standards in the assay buffer) were measured for AngI accumulation by immunoassay.
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