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Renin inhibitors for the treatment of hypertension: Design and optimization of a novel series of pyridone-substituted piperidines

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Keywords: Renin Inhibitor Anti-hypertensive ABSTRACT

An SAR campaign aimed at decreasing the overall lipophilicity of renin inhibitors such as 1 is described herein. It was found that replacement of the northern appendage in **1** with an *N*-methyl pyridone and subsequent re-optimization of the benzyl amide handle afforded compounds with in vitro and in vivo profiles suitable for further profiling. An unexpected CV toxicity in dogs observed with compound 20 led to the employment of a time and resource sparing rodent model for in vivo screening of key compounds. This culminated in the identification of compound 31 as an optimized renin inhibitor.

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Essential hypertension is known to affect over a billion people worldwide. If left untreated, hypertension can lead to multiple organ failure. Compounds capable of perturbing the renin-angiotensin-aldosterone system (RAAS, Fig. 1), a tightly regulated biological cascade known to play a pivotal role in the control of blood pressure,¹ have the potential to address this medical need.² Effective anti-hypertensive therapies involving either the inhibition of angiotensin I converting enzyme (ACE)³ or the antagonism of angiotensin II type I receptor⁴ have already been developed. However, it is believed that the inhibition of renin, an enzyme that is involved in the first and rate-limiting step of RAAS,⁵ would offer the best potential for blood pressure control and end organ protection. Furthermore unlike ACE inhibition,⁶ renin blockade should not be plagued by mechanism-based adverse events as the only known substrate for the renin enzyme is angiotensinogen, which is a protein with no known biological function.⁷

It has been previously reported that compounds bearing a piperidine warhead flanked at the 3,4-position by two optimized but lipophilic appendages (e.g., **1**, Fig. 2) can serve as highly potent and orally-bioavailable inhibitors of renin.^{8,9} Unfortunately the same structural features responsible for both renin potency and selectivity against other aspartic proteases also rendered the development of these compounds challenging. Specifically, the majority of these lipophilic amines suffered from CYP 3A4 inhibi-

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tion, high affinity for the hERG channel, poor physicochemical properties and potential to induce phospholipidosis. We attempted to address these four issues through the judicious incorporation of polar functional groups onto these privileged scaffolds.⁹ Although the resulting compounds (e.g., 2, Fig. 2) did show the desired improvements across these key parameters while keeping their intrinsic potency against renin, they were, however, not orally bioavailable in either rodents or dogs.

Having demonstrated with 2 that most of the issues associated with 1 could be addressed by decreasing its overall 'greasiness', an alternate approach to achieving this goal could involve truncating one of the compound's two lipophilic appendages. Since we were also cognizant of the highly mobile nature of the renin flap under which the 2-(2,6-dichloro-4-methylphenoxy)-ethoxy residue sits, we decided to initiate a program targeting the synthesis of truncated renin inhibitors.

Although a complete replacement of the 2-(2,6-dichloro-4methylphenoxy)ethoxy group in compound 1 by hydrogen (i.e., 3) led to a precipitous drop in renin potency, we were however encouraged by the attendant improvement made on both the hERG and CYP 3A4 profiles (Table 1). When compound **3** was docked into the renin active site, computer modeling suggested that this truncation would now force the renin flap to adopt a half-closed conformation thereby leading to a small clash between Trp45 and the phenyl stub. This unfavorable interaction could however be alleviated by replacing the offending benzene with a pyridine. Indeed, on going from compound **3** to **4**, we were able to improve

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Figure 1. The renin-angiotensin-aldosterone system.

the renin potency by 4-fold without sacrificing the gains made in the off-target profile. Furthermore, compound **4** was also found to be orally bioavailable in rodents. The proximity of Trp45 to the pyridine nitrogen of compound **4** offered us another opportunity to improve the renin potency further. While the compound **4** pyridine did not possess the necessary reach to engage in an effective H-bond with the indole NH of Trp45, its replacement by a pyridone delivered a compound (i.e., **5**) with another 4-fold increase in renin potency (Fig. 3). Unfortunately, compound **5** suffered from poor rodent PK. Our attempts to address this and improve the renin potency via further fine-tuning of the pyridone scaffold afforded only limited success. Indeed, while compound **9** did demonstrate an improvement in both renin potency and rodent PK over **5**, its associated off-target profile was not optimal.

Keeping the simple *N*-methyl pyridone plate in **5**, an optimization campaign of the benzyl amide handle led to our next breakthrough. The requisite pyridone acid **10** could be accessed using the chemistry shown in Scheme 1. Briefly, the N-benzyl protecting group in β -ketoester **11** was cleaved and the resulting free amine re-protected in situ as the corresponding BOC carbamate 12. High conversion was achieved with $Pd(OH)_2$ as the catalyst, ethanol as the solvent and triethylamine as the base under pressurized H₂ atmosphere (50 psi). Subsequent reaction with sodium hydride and triflic anhydride resulted in the clean formation of triflate 13, which could in turn be converted to the corresponding pinacol boronate **14** using standard Miyaura conditions.¹¹ Suzuki coupling of boronate 14 with 4-bromo-2-methoxypyridine was best accomplished with $Pd(dppf)Cl_2$ and 2 N aq Na_2CO_3 in *n*-propanol at 80 °C.12 Mg-mediated single electron reduction of the tetra-substituted olefin in ester 15 in methanol afforded the reduced ester 16 as a mixture of diastereomers, with the *cis*-isomer constituting the bulk of the mass balance. Although the two diastereomers could be readily separated, it was more convenient to heat an ethanol solution of the crude product mixture from the reduction step in the presence of excess sodium ethoxide. After 18 h. only the desired trans-isomer 17 remained. It was discovered that prolonged heating of methoxypyridine 17 with an excess of iodomethane and sodium iodide in acetonitrile would trigger a formal N-to-O methyl migration to furnish pyridone 18 in nearly quantitative yield. Racemic 18 could then be resolved on a chiral AD column with 72:14:14 (v/v/v) hexanes/ethanol/*i*-propanol as the eluent. Finally, saponification of the slower eluting isomer with lithium hydroxide in a 2:1 (v/v) mixture of THF and methanol afforded the desired pyridone acid 10.

All the amides shown in Table 2 could be rapidly synthesized from **10** via a two-step sequence: HATU-mediated amide coupling with an appropriate amine in DMF, followed by removal of the BOC protecting group. Although the latter step could often be performed in the presence of a large excess of 4 M HCl in dioxane, BOC-deprotection from more acid-sensitive substrates was best accomplished with zinc(II) bromide in CH₂Cl₂.¹³

It became apparent that the best amine identified for our earlier generations of non-truncated renin inhibitors was no longer optimal.¹⁴ We hypothesized that the downward movement of the renin flap observed with truncated inhibitors such as **5** would also lead to some re-organization of the renin s3 pocket. Consequently when the benzyl amine used for the synthesis of **5** was switched for *N*-(2,3-dichlorobenzyl)cyclopropanamine (i.e., **19**), an equipotent but orally bioavailable compound was obtained. Re-introduction of a 3-methoxypropyl tail designed to reach into the renin s3 sub-pocket afforded compound **20**, an inhibitor with a renin plasma IC₅₀ value of 1.3 nM. Both chlorines in **20** proved to be important for renin potency as the removal of either led to less potent



good potency against renin high selectivity against other aspartic protease high oral bioavailabiliy in preclinical species

moderate time-dependent CYP3A4 inhibitor high affinity for hERG channel poor physicochemical properties causes phospholipidosis



good potency against renin high selectivity against other aspartic protease acceptable CYP3A4 profile lower affinity for hERG improved physicochemical properties low potential to cause phospholipidosis

not orally bioavailable in preclinical species



corresponds to a reversible CYP 3A4 inhibition IC₅₀ of 10 μM. 10% activity corresponds to a reversible CYP 3A4 inhibition IC₅₀ of 2 μM.

3% loss corresponds to no measurable time-dependent CYP 3A4 inhibition. ND = not determined.

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 Figure 3. Modeling of compound 5 in the renin active site.

compounds (i.e., **21**, **22**). Further alterations of the p3 sub-pocket tail of compound **20**; specifically, shortening the propyl chain by one methylene unit (i.e., **23**), replacing the methoxy capping group with either cyanide (i.e., **24**), *N*-acetamide (i.e., **26**) or methyl carbamate (i.e., **27**), and changing the linking atom from carbon to nitrogen (i.e., **28**), all led to a drop in renin potency, oral bioavailability or both. On the other hand, the two chlorines in **20** could be replaced with methyl groups (i.e., **29**) without sacrificing renin potency. Other preferred p3 aromatic plates include naphthalene (**33**), quinoline (**34**, **35**) and indole (**36** to **45**). Although some of these derivatives were found to be sub-nanomolar renin inhibitors in our plasma assay (i.e., **37–39**, **41–43**, and **45**), compound **20** still stood out by virtue of its renin potency, clean off-target profile (i.e., hERG, CYP 3A4 inhibition) and good pharmacokinetics in rodents.

When compound **20** was infused intravenously at a dose of 10 mpk over 1.5 h into anesthetized, vagotomized and ventilated mongrel dogs, we observed significant prolongation of QTc, PR and QRS intervals (Table 3). The same CV signals were also observed in vivo with **36**, a compound that was found to be inactive (IC₅₀ >30 µM) in the IKr, Ca L-type, hCaV1.2, hNaV2, and hNaV1.5 in vitro channel assays. Faced with the scenario of having to screen all the key compounds in an in vivo setting, a more time and resource sparing rodent alternative to the CV dog model was established.¹⁵ Although the cardiac electrophysiology (EP) rat model proved to be unsuitable for assessing a given compound's potential to induce QTc prolongation in either dog or human, there was however good agreement in the magnitude of both QRS and PR prolongations between what was observed in the EP rat and the CV dog model for compounds 20 and 36. With this tool in hand, all key compounds were screened leading to the identification of compound **31** as a renin inhibitor suitable for further development.

The key characteristics of compound **31** are summarized in Table 4. It is a potent renin inhibitor with moderate oral bioavailability in rats, dogs, and cyno monkeys. The corresponding HCl salt was found to be crystalline, non-hygroscopic and highly soluble in water. Compound **31** was also found to be efficacious in the double transgenic rat model (dTGR),¹⁶ imparting a robust blood pressure decrease when given orally at 10 mpk. Compound **31** inhibited CYP 3A4 reversibly with an IC₅₀ of 6.5 μ M and demonstrated little potential to inactivate CYP 3A4 in a time-dependent manner. This compound also exhibited minimal potential to covalently label proteins in the presence of liver microsomes. Most importantly, compound **31** did not cause significant prolongation of QTc, PR nor QRS intervals in vivo in both rats and dogs.

Table



Scheme 1. Synthesis of **10**: Reagents and conditions: (a) Pd(OH)₂, BOC₂O, TEA, ethanol, 50 psi H₂, 4 h, 82%; (b) NaH (60% dispersion in oil), Tf₂O, THF, 0 °C to rt, 2 h, 79%; (c) Pd(dppf)Cl₂, KOAc, B₂Pin₂, dioxane, 80 °C, 14 h; (d) Pd(dppf)Cl₂, 2 N aq Na₂CO₃, 4-bromo-2-methoxypyridine, *n*-PrOH, 80 °C, 14 h, 84% over two steps; (e) Mg powder, MeOH, sonication, 2 h; (f) NaOEt, EtOH, 80 °C, 16 h, 70% over two steps; (g) iodomethane, NaI, CH₃CN, sealed tube, 45 °C, 72 h, 97%; (h) chiral resolution, AD column, 72:14:14 hexanes/EtOH/*i*PrOH; (i) 1 N aq LiOH, MeOH, THF, 16 h, 48% over two steps.

Table 2

SAR of select renin inhibitors: Amide modifications



Compound	\mathbb{R}^1	\mathbb{R}^2	R ³	R ⁴	Renin I	Potency ^a	hERG K_i (nM)	(nM) CYP 3A4 inhibition		F in SD rats at 20 mpk po (%)
					Buffer (nM)	Plasma (nM)		Reversible (% activity) ^b	Time dependent (% loss) ^c	
19	Cl	Cl	Н	Н	20	42	13,000	61	37	23
20	Cl	Cl	Н	(CH ₂) ₃ OMe	0.3	1.3	4,800	53	16	24
21	Cl	Н	Н	(CH ₂) ₃ OMe	8	25	28,000	75	29	14
22	Н	Cl	Н	(CH ₂) ₃ OMe	3	6.5	7,600	23	1	28 at 30 mpk
23	Cl	Cl	Н	(CH ₂) ₂ OMe	0.6	2.2	11,000	68	23	18
24	Cl	Cl	Н	(CH ₂) ₃ CN	4.9	10	8,400	55	20	7
25	Cl	Cl	Н	(CH ₂) ₂ CN	8.5	17	17,000	52	15	8
26	Cl	Cl	Н	(CH ₂) ₂ NHAc	4.5	9	>29,000	97	0	0
27	Cl	Cl	Н	(CH ₂) ₂ NH(C=O)OMe	61	210	15,000	45	56	1
28	Cl	Cl	Н	NH(CH ₂) ₂ OMe	7.1	26	18,000	71	0	1
29	Me	Me	Н	(CH ₂) ₃ OMe	0.6	1.0	>29,000	74	15	7 at 30 mpk
30	Н	Br	Н	(CH ₂) ₃ OMe	1.7	3.5	5,000	15	45	15
31	Н	Br	Me	(CH ₂) ₃ OMe	1.0	2.4	18,000	22	0	30
32	Н	Ph	Н	(CH ₂) ₃ OMe	18	81	1,700	23	0	8
33	-	-	-	-	0.4	1.4	17,000	54	34	4
34	—	—	-	-	1.4	3.0	>29,000	95	13	1
35	—	—	-	-	0.8	2.2	27,000	85	16	2 at 30 mpk
36	Н	—	-	(CH ₂) ₃ OMe	2.3	5.2	>29,000	59	45	8 at 30 mpk
37	F	-	-	(CH ₂) ₃ OMe	0.32	0.87	>29,000	41	0	8 at 30 mpk
38	Cl	-	-	(CH ₂) ₃ OMe	0.12	0.64	23,000	48	19	22 at 30 mpk
39	Br	-	-	(CH ₂) ₃ OMe	0.07	0.57	11,000	63	48	3 at 3 mpk
40	F	-	-	Н	12	8.4	>29,000	74	0	0 at 3 mpk
41	F	-	-	CH ₂ Ph	0.02	0.62	950	19	43	16 at 3 mpk

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(continued on next page)

Table 2 (continued)

Compound	\mathbb{R}^1	R ²	R ³	R ⁴	Renin Potency ^a		hERG K_i (nM)	CYP 3A4 inhibition		F in SD rats at 20 mpk po (%)
					Buffer (nM)	Plasma (nM)		Reversible (% activity) ^b	Time dependent (% loss) ^c	
42	F	_	_	CH ₂ (3-FPh)	0.06	0.63	1,200	18	39	30 at 30 mpk
43	F	-	_	CH ₂ (4-FPh)	0.05	0.48	750	16	70	17 at 30 mpk
44	F	-	-	CH ₂ (3-Pyr)	0.88	2.0	12,000	61	44	0 at 3 mpk
45	F	-	—	CH ₂ (4-Pyr)	0.24	0.53	15,000	44	29	0 at 3 mpk

^a Average of at least two replicates. See Ref. 10 for assay protocols.

^b Calculated as a 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. 50% activity corresponds to a reversible CYP 3A4 inhibition IC₅₀ of 10 μM. 10% activity corresponds to a reversible CYP 3A4 inhibition IC₅₀ of 2 μM.

 c Calculated as a percentage of the difference in the rate of CYP3A4-mediated conversion of testosterone (250 μ M) to 6- β -hydroxytestosterone before and after 30 min incubation period with the compound (10 μ M in DMSO). A 0% loss corresponds to no measurable time-dependent CYP 3A4 inhibition.

Table 3

CV profiles of representative compounds

		20	36	31
CV Dog (10 mpk/1.5 h)	QTc (%) ^a	10	6	0
	PR (%) ^a	50	14	5
	QRS (%) ^a	33	12	4
	[Plasma] (µM)	15	25	9.3
EP Rat (10 mpk/0.5 h)	PR (%) ^a	35	23	16
	QRS (%) ^a	54	30	14
	[Plasma] (µM)	7.4	12.7	7.9

^a Prolongation expressed as a percentage change from baseline.

Table 4

Key profile of compound **31**

Renin $IC_{50}^{a,b}$ (nM)	Buffer	1.0
	Plasma	2.4
SD rat (20 mpk po, 5 mpk iv)	F (%)	30
	po AUC (μM h)	4.2
	Cl (mL/min/kg)	67
	$T_{1/2}$ (h)	5.2
	V _{dss} (L/kg)	17
Beagle dog (3 mpk po, 1 mpk iv)	F (%)	36
	po AUC (µM h)	2.1
	Cl (mL/min/kg)	16
	$T_{1/2}$ (h)	13.7
	V _{dss} (L/kg)	4.5
Cyno monkey (3 mpk po, 1 mpk iv)	F (%)	14
	po AUC (µM h)	0.9
	Cl (mL/min/kg)	14
	$T_{1/2}$ (h)	7.8
	$V_{\rm dss}$ (L/kg)	4.8
COBILM ^c , human	With NADPH pmole eq/(mg at 1 h)	34.5
	Without NADPH pmole eq/(mg at 1 h)	8.3
Efficacy in dTGR (10 mpk po)	Max BP \downarrow (mm Hg)	35
	Duration (h)	24
CYP 3A4 IC ₅₀ (nM)	Reversible	6,500
	30 min Pre-Inc/0 min Pre-Inc ^d	16,000/20,000

^a See Ref. 10 for assay protocols.

^b Average of at least two replicates.

^c COBILM assay (covalent binding in liver microsomes): incubations (n = 3) were done with human liver microsomes at 10 μ M, with 1 mg protein/mL, for 60 min with or without NADPH (1 mM) as additive. For complete protocol, see Ref. 17.

^d TDI assay (time dependent inhibition): the CYP 3A4 IC₅₀ observed with or without a 30 min pre-incubation period with the compound in question.

In summary, we have described a novel series of pyridonesubstituted piperidines with a profile suitable for their further clinical development as renin inhibitors.

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- 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 take place in a Costar 384 well black

plate 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 and 388 nm, respectively. *Plasma assay*: Frozen human EDTA-plasma was rapidly thawed in warm water and centrifuged at 2900g for 15 min at 40 °C. The supernatant was collected and recombinant human renin (Proteos) added at 1 nM nominal concentration. The plasma was transferred to Costar black 384 well plates, renin inhibitors added and the mixture pre-incubated at 37 °C for 0 min. The renin Q-FRET substrate QXL520-Lys-His-Pro-Phe-His-Leu-Val-Ile-His-Ly-(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 plate read in a SpectraMax Gemini EM reader with excitation and emission filters at 490 and 520 nm, respectively, at time 0 and 1 h.

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