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Design and synthesis of potent, isoxazole-containing renin inhibitors

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ABSTRACT

The design and optimization of a novel isoxazole S_1 linker for renin inhibitor is described herein. This effort culminated in the identification of compound **18**, an orally bioavailable, sub-nanomolar renin inhibitor even in the presence of human plasma. When compound **18** was found to inhibit CYP3A4 in a time dependent manner, two strategies were pursued that successfully delivered equipotent compounds with minimal TDI potential.

Hypertension is a major risk factor for a variety of cardiovascular diseases.[1] Despite the fact that a wide variety of anti-hypertensive medications are now available in the developed world, more than 60% of hypertensive patients are still unable to reach the blood pressure goal of 140/90 mm Hg as recommended by the American Heart Association. As a result, hypertension is still an area of active research in several pharmaceutical companies. In this regard, the perturbation of the renin-angiotensin-aldosterone system (RAAS) at its various stages continues to be an attractive strategy for the development of novel anti-hypertensives (Fig. 1).[2] Since renin is involved in the first and rate-limiting step of this cascade, the development of a suitable renin inhibitor should offer the best potential for optimal blood pressure control and superior end organ protection than therapies targeting other steps of the RAAS pathway.[3,4] However to date, only Aliskiren, marketed as Tekturna in the U.S. and Rasilez in the EU and Japan, has received regulatory approval for the treatment of moderate essential hypertension.[5]

We have previously reported that suitably functionalized 3,4-biarylpiperidines can serve as highly potent renin inhibitors (Fig. 2).[6,7] In this scaffold, a 2-chlorophenyl ring at the S_1 position was employed as an equipotent replacement for the classic cyclopropyl amide linker.[8] In the course of our investigations, we also determined that piperidines substituted at the 4-position with a pyridone ring were necessary to obtain compounds with a sufficiently clean off-target profile (hERG and CYP 3A4 inhibition).[6,9] However, the main issue associated with the use of such a functionality is that pyridone's inherent polarity renders the resulting renin inhibitors poorly permeable to cellular membranes. As a result, most of these compounds suffered from low and dose-dependant oral exposure.[10] For this reason, we hypothesized that replacement of the lipophilic phenyl S_1 linker in **1** with a more polar heterocycle may enlarge the off-target/potency window sufficiently such that the pyridone 'band-aid' would no longer be necessary. In this regard, the suitability of several metabolically stable heterocycles bearing the required substitution pattern were evaluated and isoxazoles were rapidly identified as being optimal. It is also worth highlighting that the isoxazole linker can be assembled late in the synthesis to allow the convergent union of two pieces of comparable synthetic complexity. This retrosynthetic dissection would in turn facilitate parallel SAR explorations at both the northern and eastern aromatic rings as well as increase the compound output.

Both isoxazole isomers shown in Figure 2 can be readily accessed via a 1,3-dipolar cycloaddition between an alkyne and a nitrile oxide generated in situ from its corresponding oxime. In this regard, we prepared oxime **4** and alkynes **5** and **6** from the known carboxylic acid **2** (Scheme 1).[7] Borane reduction of **2** and subsequent oxidation of the resulting alcohol with Dess-Martin's periodinane (DMP) yielded aldehyde **3** in excellent yield. Its condensation with hydroxylamine then gave oxime **4** in quantitative yield. Alternatively, treatment of aldehyde **3** with Ohira-Bestmann's reagent delivered terminal alkyne **5**.[11] Finally, 2-bromoalkyne **6** was prepared from aldehyde **3** via an initial Ramirez condensation with CBr_4 followed by potassium *tert*-butoxide promoted HBr elimination.[12]

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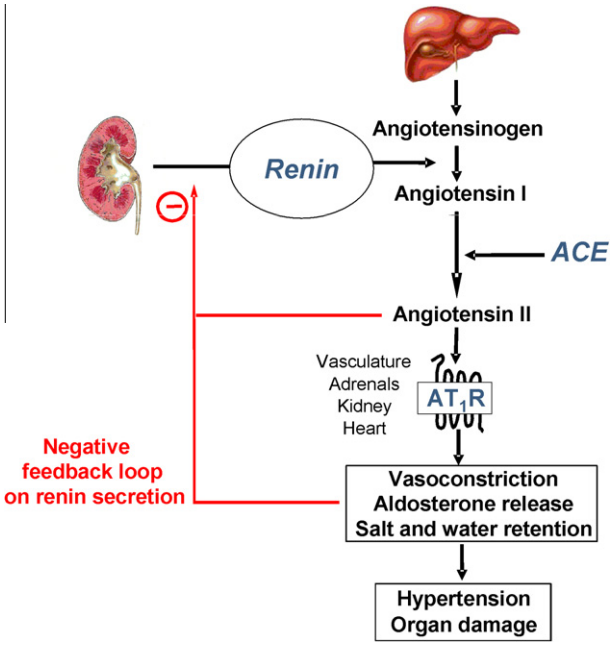


Figure 1. The renin-angiotensin-aldosterone system.

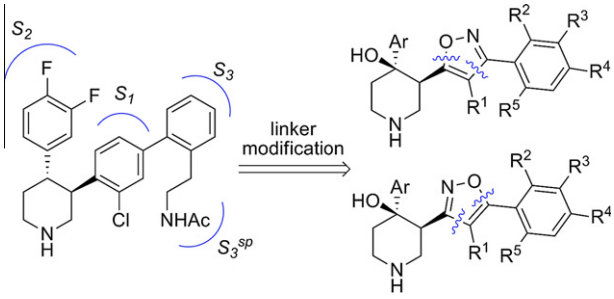
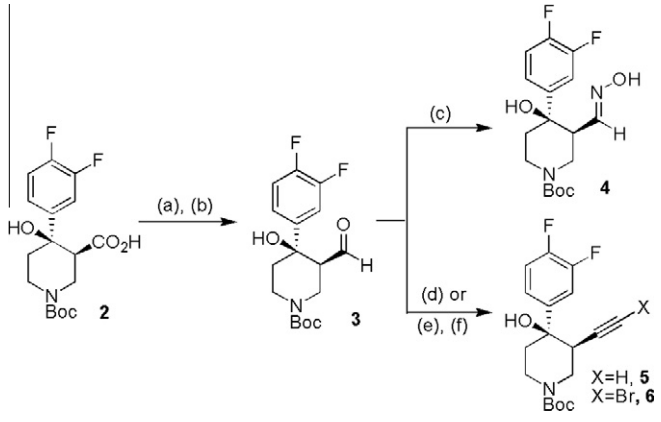
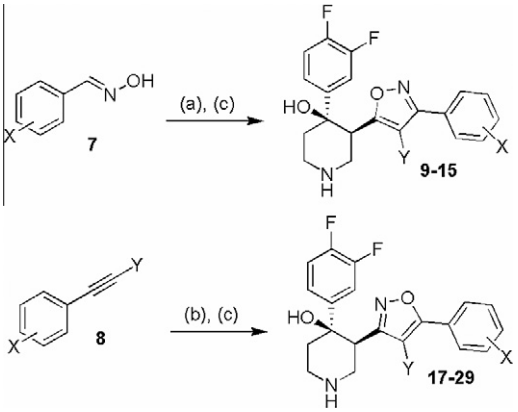


Figure 2. General topology of the renin active site and isoxazole as replacement for phenyl linker.

With the requisite alkynes and oximes in hand, we performed the key 1,3-dipolar cycloaddition (Scheme 2).[13] Treating the appropriate oxime **7** with Chloramine-T resulted in the in situ generation of the corresponding nitrile oxide. Addition of alkyne **5** or **6** then gave the desired isoxazole core. Deprotection of the Boc protecting group using HCl gave renin inhibitors **9**–**17**. Analogously, the same steps were repeated using oxime **4** and alkynes **8** to deliver inhibitors **18**–**27**.



Scheme 1. Reagents and conditions: Synthesis of **6**: (a) 3 equiv BH_3DMS, THF, RT, 6 h, >99%; (b) 3 equiv DMP, 30 equiv pyridine DCM:*t*-BuOH (4:1), 18 h, 88% (c) 1.5 equiv hydroxylamine hydrochloride, 1.55 equiv Na_2CO_3, EtOH:H_2O (9:1), >99%; (d) 1.2 equiv Ohira-Bestmann's reagent, 2 equiv K_2CO_3, MeOH, RT, 8 h, 72%; (e) 1 equiv Et_3N, 2 equiv PPh_3, 3.8 equiv CBr_4, DCM, −78 °C to RT, 3 h, 88%; (f) 1.2 equiv KO*t*Bu, THF, RT, 2 h, 78%.



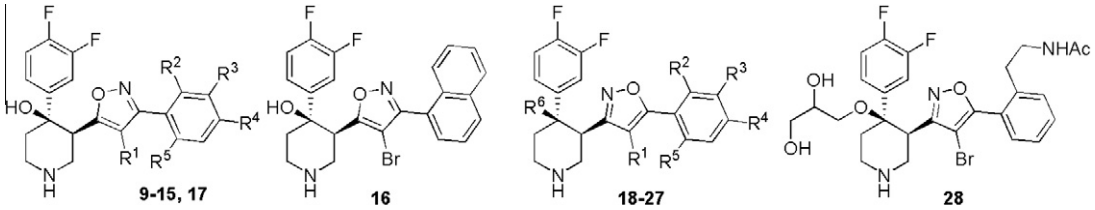
Scheme 2. Reagents and conditions: Synthesis of **9**–**27**: (a) 2 equiv **7**, 2 equiv Chloramine-T, MeOH, 20 min., then 1 equiv **5** or **6**, 60 °C, 3 h; (b) 1 equiv **4**, 1.15 equiv Chloramine-T, MeOH, 20 min., then 3 equiv **8**, 60 °C, 3 h; (c) 30 equiv 4 M HCl in dioxane, RT, 1 h.

The key data are summarized in Table 1. Firstly, compounds lacking a 4-substituent at the isoxazole bridge (i.e., **9**–**11**, **19**, **21**), regardless of the substitution patterns of the terminal aromatic group and which isoxazole isomer it contains, were all found to be weaker inhibitors of renin. However, greater than 200× boost in renin potency could be realized following the installation of a bromine at the 4-position of the isoxazole tether (i.e., **11** vs **12**). Indeed, the bromine substituent was found to occupy the previously vacant renin S_1 subpocket and effectively brought the potency of the isoxazole series to the same range as that of the reference compound **1**.[14]

With the 4-bromoisoxazole core in hand, we then set out to optimize the terminal aromatic ring that sits in the highly lipophilic renin S_3 pocket. On going from 2-chlorophenyl (i.e., **12**) to 2,3-dichlorophenyl (i.e., **13**), we were able to increase the intrinsic renin potency further, although this gain was partially negated by the concomitant increase in plasma shift. Replacement of the two chlorine atoms by methyl groups (i.e., **14**) unfortunately proved to be detrimental for both renin potency and plasma shift. Similarly, shuffling the *meta*-chlorine in compound **13** to the other *ortho*-position of the phenyl ring (i.e., **15**) was also not beneficial. Although replacement of the 2,3-dichlorobenzene in compound **13** with its well known 2-naphthalene isostere (i.e., **16**) did improve the intrinsic renin potency further, this again came at the expense of a concomitant increase in plasma protein binding. To get around this, we decided instead to focus our attention on improving the interactions between our inhibitor and the less lipophilic renin S_3 subpocket. Indeed, following the replacement of the chlorine in **12** with the same polar ethyl acetamide tail present in compound **1** (i.e., **17**), both the intrinsic renin potency and the plasma shift were significantly improved. Furthermore on going from **12** to **17**, there was a noticeable decrease in the compound's affinity for the hERG channel as well as its ability to reversibly inhibit CYP3A4, although the extent of CYP3A4 time-dependent inhibition (TDI) remained an issue.[15]

We then investigated which isoxazole isomer possessed the best in vitro profile. Gratifyingly compound **18**, which bears the regioisomer of the isoxazole present in **17**, exhibited superior renin potency, reaching sub-nanomolar activity even in the presence of

Table 1
SAR of select renin inhibitors



#	R^1	R^2	R^3	R^4	R^5	R^6	Renin potency[a]		hERG K_i (μM)[b]	CYP 3A4 inhibition	
							Buffer (nM)	Plasma (nM)		Reversible (μM)[c]	Time dependent (% loss)[d]
1	—	—	—	—	—	—	0.5	7.5	1.1	20	49
9	H	Br	H	F	H	—	402	8654	1.7	3	22
10	H	Cl	H	H	Cl	—	1213	>9500	2.8	8	59
11	H	Cl	H	F	H	—	2500	>9500	3.4	7	36
12	Br	Cl	H	H	H	—	1.14	27	6.2	18	76
13	Br	Cl	Cl	H	H	—	0.21	7.8	—	19	60
14	Br	Me	Me	H	H	—	0.47	21	6.0	20	77
15	Br	Cl	H	H	Cl	—	3.7	131	3.9	15	82
16	—	—	—	—	—	—	0.06	4.7	4.2	>50	67
17	Br	C_2H_4NHAc	H	H	H	—	0.36	1.6	10.5	45	74
18	Br	C_2H_4NHAc	H	H	H	OH	0.27	0.6	10.7	33	68
19	H	Cl	H	H	Cl	OH	1333	>9500	9.8	17	60
20	Br	C_2H_4NHAc	H	F	H	OH	0.14	0.7	23	45	78
21	H	C_2H_4NHAc	H	H	H	OH	166	331	3.3	32	21
22	Cl	C_2H_4NHAc	H	H	H	OH	0.73	1.9	15.3	>50	74
23	C(O)Me	C_2H_4NHAc	H	H	H	OH	2.82	4.2	13.7	8	32
24	CH(OH)Me	C_2H_4NHAc	H	H	H	OH	4.30	4.5	33.6	>50	48
25	Br	C_2H_4NHAc	H	H	H	H	0.26	1.2	19.5	13	75
26	Br	C_2H_4NHAc	H	H	H	OMe	0.17	1.2	21.2	28	60
27	Br	C_2H_4NHAc	H	H	H	$C(O)NH_2$	0.22	0.6	30.0	36	86
28	—	—	—	—	—	—	0.37	1.4	>60	>50	18

[a] Average of at least two replicates. See Ref. 16 for assay protocols.
[b] Average of at least two replicates. Binding affinity.
[c] Calculated as an IC_{50} for the inhibition of CYP3A4-mediated conversion of testosterone (50 μM) to 6-β-hydroxytestosterone in the presence of compound.
[d] Calculated as a 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% loss corresponds to no measurable time-dependent CYP 3A4 inhibition.

human plasma. Unfortunately, the extent of TDI observed was not improved with **18**. To address this, we sought to decrease the lipophilicity of the molecule further by changing the bromine at the 4 position of the isoxazole core. To evaluate the viability of this approach, we first prepared compound **21** where the bromine was replaced by a hydrogen atom. Although this led to a major loss in renin potency, the CYP3A4 TDI was indeed improved, going from a 68% loss in CYP3A4 activity after a 30 min pre-incubation period with **18** to a 21% loss with **21**. Encouraged by this result, we then prepared a variety of compounds with different R_1 groups (i.e., compounds **22**–**24**). Although improvements in their respective off-target profile versus compound **20** were observed, the attendant loss of potency against renin was unfortunately too large to warrant further profiling of these compounds since the safety margin was not improved.

As an alternative approach to reduce the CYP3A4 TDI potential of **18**, we also tried to increase the polarity of the molecule. Previous in silico docking experiments involving scaffolds similar to that found in compound **18** suggested that the tertiary alcohol residue does not engage in any stabilizing interactions with the renin enzyme. Indeed, this was independently confirmed by the observation that both compounds **25** and **26**, where the tertiary alcohol in question was replaced by either a hydrogen or a methoxy group, respectively, were equipotent to **18** in the absence of human plasma. Furthermore, since the tertiary alcohol was suspected to be solvent-exposed, we felt that this would be an ideal position to append a polar residue. While carbamate **27** exhibited comparable renin potency to that of **18**, its CYP3A4 profile was not improved. However, the introduction of an even more polar 2,3-dihydroxypropyl chain (i.e., **28**) had the desired impact on both reversible and time-dependent CYP3A4 inhibition, with only a small loss in renin potency versus compound **18**. Unfortunately, this compound was not orally bioavailable in rats ($F = 0\%$, Table 2), most likely a consequence of its poor cellular permeability. This further examplifies the delicate balance between lipophilicity, CYP3A3 time-dependant inhibition and permeability present in multiple renin inhibitor series.

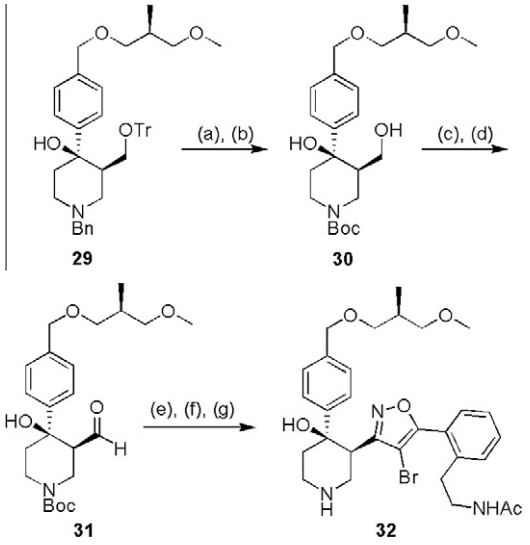
Since it was suspected that the TDI observed with compound **18** was caused by the formation of an unknown reactive metabolite, concurrent with the above effort, we were intrigued by whether this liability could also be addressed through the introduction of a distal metabolic soft spot that would shunt the metabolism to a more innocuous position of the molecule. With this idea in mind, we prepared compound **32** from the known piperidine **29** (Scheme 3). Removal of the trityl group with *p*-toluenesulfonic acid and a subsequent protecting group switch under hydrogenolysis condition yielded compound **30**. Following chiral resolution by HPLC, the desired diastereomer was oxidized to aldehyde **31** using DMP. The synthesis of **32** was then completed using the same synthetic manipulations as described above for **18**.

The introduction of a metabolic soft spot turned out to be a highly promising approach. Compound **32** was found to be equipotent to **18** both in the absence and presence of human plasma. Furthermore, the off-target profile of **32** was significantly improved over that of **18**. Most importantly, compound **32** does not irreversibly inactivate CYP3A4 at a measurable rate and hence should exhibit minimal potential to perpetrate drug-drug interactions (DDI) if co-dosed with drugs cleared mainly by CYP3A4 (e.g., simvasta-

Table 2
Key profiles of compounds **18**, **28** and **32**

Compound		**18**	**28**	**32**
Renin IC_{50}[a,b] (nM)	Buffer	0.27	0.37	0.26
	Plasma	0.63	1.4	0.67
SD Rat (3 mpk PO, 0.5 mpk IV)	*F* (%)	16	0	18
	PO AUC (μM h)	0.14	0	0.13
	Cl (mL/min/kg)	108	70	124
	$T_{1/2}$ (h)	5.7	1.9	1.4
	V_{dss} (L/kg)	36	12	12
Papp ($\times 10^{-6}$ cm s^{-1})[c]		—	<1	—
Efficacy in dTGR (3 mpk PO)	max. BP ↓ (mm Hg)	44	—	27
	duration (h)	18	—	8
hERG K_i (μM)		11.7	>60	31.2
CYP 3A4 IC_{50} (nM)	Reversible (μM)[d]	33	>50	29
	Time dependent (% loss)[d]	68	18	10
CYP 3A4 k_{obs} (min^{-1})		0.093	—	<0.004

[a] See Ref. 13 for assay protocols.
[b] Average of at least two replicates.
[c] See Ref. 9 for assay protocol.
[d] Calculated as a percentage of the difference in the rate of CYP3A4-mediated conversion of testosterone (250 μM) to 6-β-hydroxy-testosterone before and after 30 min incubation period with the compound (10 μM in DMSO). A 0% loss corresponds to no measurable time-dependent CYP 3A4 inhibition.



Scheme 3. Reagents and conditions: Synthesis of **32**: (a) 1.2 equiv TsOH, THF:MeOH (1:4), 4 h, >99%; (b) 1.05 equiv BOC_2O, 0.1 equiv Pd/C, MeOH, 18 h, 73%; (c) chiral resolution, AD column, 90:5:5 hexanes: *i*-PrOH: MeOH; (d) 3 equiv DMP, 30 equiv pyridine DCM:*t*-BuOH (4:1), 18 h, 69%; (e) 1.5 equiv hydroxylamine hydrochloride, 1.55 equiv Na_2CO_3, EtOH:H_2O (9:1), >99%; (f) 1.15 equiv Chloramine-T, MeOH, 20 min, then 3 equiv **8**, 60 °C, 3 h; (g) 30 equiv 4 M HCl in dioxane, RT, 1 h, 32% over 2 steps.

tin). Unlike compound **28**, compound **32** is bioavailable (*F* = 18%) in rats when dosed orally as a 3 mpk solution in 0.5% methyl cellulose. As expected, the presence of a metabolic softspot in **32** was manifested in a higher plasma clearance (Cl = 124 mL/min/kg) and a shorter half life ($T_{1/2}$ = 1.4 h) than compound **18**.

When compounds **18** and **32** were given to hypertensive double transgenic rats (dTGR) harboring both human renin and angiotensinogen, robust blood pressure lowering was observed with both compounds.[17] As is consistent with the higher volume of distribution and lower rate of plasma clearance in rat of compound **18**, its blood pressure lowering effect was both more extensive and prolonged than compound **32** in this hypertension efficacy model. Unfortunately when compound **32** was profiled in higher order species, namely beagle dogs and cynomolgus monkeys, the observed systemic exposure following oral dosing was not sufficient to warrant further characterization (data not shown).

In conclusion, an isoxazole S_1 linker was found to be a superior replacement for the classic amide linker as well as the more recent phenyl linker. Indeed, changing the 2-chlorophenyl linker in **1** for a 4-bromoisoxazole linker in **18** increased the plasma renin potency by more than 10-fold and improved the attendant off-target profile. Although compound **18** was found to inactivate CYP3A4 in a time dependent manner, the judicious incorporation of either polarity or a metabolic soft spot both proved to be viable strategies for addressing this liability. Further optimization of the pharmacokinetic properties of this series of inhibitors is currently underway and will be reported 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 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 2900 g 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 10 min. The renin Q-FRET substrate *QXL520-Lys-His-Pro-Phe-His-Leu-Val-Ile-His-Lys-(5-FAM)* (Anaspec), 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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