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Benzyl ether structure–activity relationships in a series of ketopiperazine-based renin inhibitors

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Abstract—Inhibition of renin enzymatic activity by a series of ketopiperazine-based compounds containing a C6 benzyloxymethyl substituent correlated with a $+(\pi + \sigma)$ effect. A 3-pyridinyloxymethyl substituent was also found to be equipotent as higher molecular weight analogs, and exhibited decreased CYP3A4 inhibition levels and improved pharmacokinetic properties.

Hypertension is a leading risk factor for cardiovascular disease, such as congestive heart failure, stroke, myocardial infarction, and is a major cause of death in the Western world.[1] The renin angiotensin system (RAS) is well-established as an endocrine system that is involved in blood pressure regulation and fluid electrolyte balance (Fig. 1).[2] Activation of the RAS is stimulated by several signals, including a drop in blood pressure, a decrease in the circulating volume, or a reduction in plasma sodium concentration. These signals stimulate the release of renin, which cleaves angiotensinogen to angiotensin I (AngI). Angiotensin converting enzyme (ACE) then converts AngI into the vasopressor peptide angiotensin II (AngII). The binding of AngII to the AT_1 receptor triggers a number of physiological effects, such as sodium and water retention and vasoconstriction, ultimately leading to an increase in blood pressure. Since renin is the rate-limiting step in the RAS cascade, renin inhibition is considered to be an attractive antihypertensive strategy. Renin inhibitors have been predicted to be more efficacious with fewer side effects than ACE inhibitors and AT_1 receptor antagonists, which target downstream events.[3]

Several pharmaceutical companies have attempted to advance renin inhibitors to the clinic. Although potent in vitro renin inhibitory activity was obtained, most of the programs were based on peptidic or peptidomimetic scaffolds. Poor pharmacokinetic properties, including low oral bioavailability and high clearance, and a high cost of goods for large-scale synthesis made these agents unattractive as clinical candidates.[4]

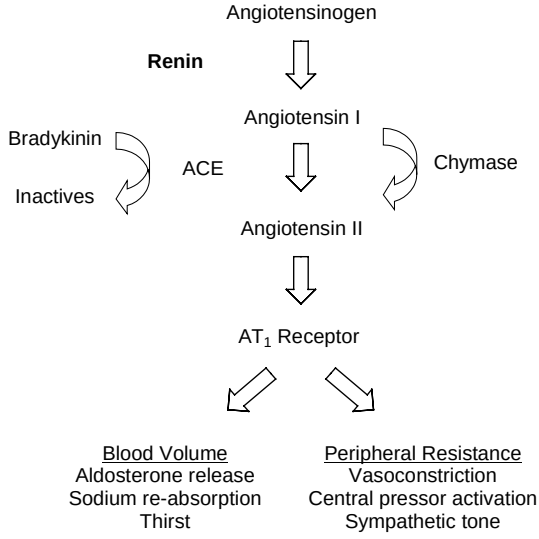

Figure 1. The renin angiotensin system (RAS).

In 1999, the first renin inhibitors based on a *trans*-3-alkoxymethyl-4-arylpiperidine scaffold were disclosed.[5] We have recently disclosed a similar series of non-peptidic renin inhibitors that is based on a 6-alkoxymethyl-1-aryl-2-ketopiperazine scaffold, as exemplified by **1**.[6]

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The ketopiperazine scaffold offers several advantages over the *trans*-3-alkoxymethyl-4-arylpiperidine scaffold, notably the ease of synthesis, removal of a chiral center, and more amenability to modular SAR studies.

Although the ketopiperazine scaffold effected subnanomolar renin inhibition and demonstrated in vivo blood pressure reduction in transgenic mice,[6] these inhibitors suffered from high molecular weights and suboptimal PK properties. Our SAR studies indicated that the A, B, and D-ring portions were required for good renin inhibition activity, whereas the C-ring portion was amenable to variation. Thus, we chose to investigate if the substituted tetrahydroquinoline C-ring in analog **1** could be replaced with a substituted benzyl ether (**2**, Fig. 2), which would reduce molecular weight, increase ease of synthesis, and potentially provide opportunities for improving pharmacokinetic properties.

The analogs **5**–**29** described were synthesized by the route exemplified with analog **5** (Scheme 1). The intermediate alcohol **3** was prepared, as previously described.[6] Deprotonation of the alcohol with NaH, followed by alkylation of the resulting alkoxide with benzyl bromide in the presence of 15-crown-5, yielded the desired benzyl ether **4**. The N-Boc-protecting group was removed by treatment of **4** with cold anhydrous HCl generated by the addition of AcCl to anhydrous MeOH to yield analog **5** in good overall yield. The additional analogs, as given in Table 1, were prepared in a similar fashion using the appropriately

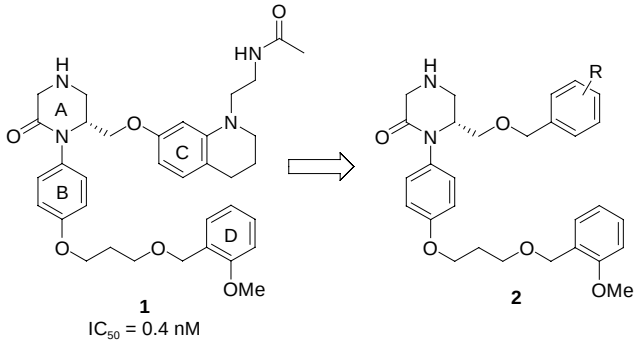
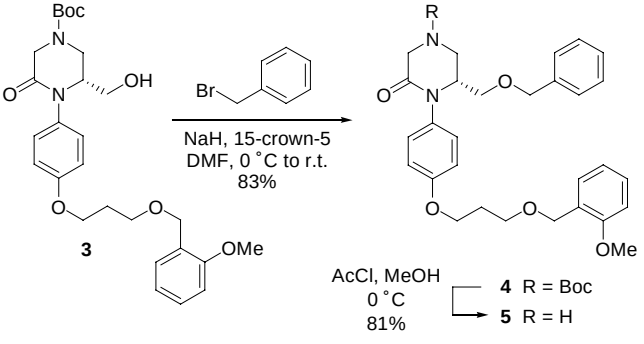
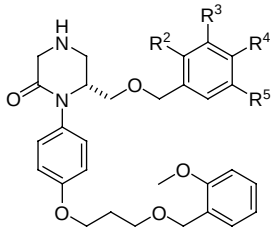


Figure 2. Ketopiperazine-based renin inhibitors.



Scheme 1.

Table 1. Renin inhibition activity for selected analogs



	R^2	R^3	R^4	R^5	IC_{50} (nM)[a]	π[b]	σ[c]	$\pi + \sigma$
5	H	H	H	H	7000	0.00	0	0
6			CF_3		6800	0.88	0.54	1.42
7			OCH_3		3900	−0.20	−0.27	−0.47
8		F		F	1600	0.29	0.4	0.688
9		CN			1200	−0.57	0.56	−0.01
10	OCH_3				900	−0.02	−0.27	−0.29
11		OCH_3			600	−0.20	0.12	−0.08
12			F		450	0.14	0.06	0.2
13	Cl		F		410	0.87	0.44	1.308
14		OCF_3			410	1.04	0.38	1.42
15		CH_3			370	0.56	−0.07	0.49
16			CH_3		340	0.56	−0.17	0.39
17		Cl			330	0.71	0.37	1.08
18			Cl		300	0.71	0.23	0.94
19		CH_3	F		270	0.64	−0.01	0.634
20	F	CF_3			270	1.03	0.49	1.518
21	CF_3				270	1.03	0.6	1.628
22		Cl	CH_3		260	1.21	0.3	1.514
23		F	F		250	0.22	0.4	0.618
24		CF_3			230	0.88	0.43	1.31
25	F				230	0.15	0.06	0.205
26		Cl	Cl		180	1.25	0.52	1.77
27		Cl		Cl	180	1.43	0.75	2.178
28		CF_3	F		140	1.03	0.49	1.518
29		CF_3	Cl		120	1.59	0.66	2.25

[a] IC_{50} values obtained in duplicate using a fluorescent tGFP assay.[6]
[b] Non-literature π values were calculated by the formula π = (c logP of the substituted benzene ring) −2.14.
[c] σ values for disubstituted rings are a sum of the σ values of the individual substituents.

substituted benzyl halide. The heterocyclic analogs given in Table 2 were prepared using the corresponding heterocyclic methylene halide.

Replacement of the tetrahydroquinolinyl C-ring in **1** with a benzyl ether resulted in a dramatic loss of renin inhibition (Table 1, **5** IC_{50} = 7000 nM). However, substitution on the benzyl ether led to greatly improved renin inhibition. Increases in potency were achieved by following the Topliss tree for substituents that have a net +($\pi + \sigma$) effect on the lipophilic and electronic nature of the aromatic ring.[7] For example, introduction of a 4-Cl substituent led to a >20-fold improvement in renin inhibition (**18**, IC_{50} = 300 nM). A further potency gain was achieved by the addition of a second lipophilic electron-withdrawing group at the 3-position, such as the 3,4-dichlorobenzyl ether **26** (IC_{50} = 180 nM), which was almost 1.6-fold more potent than **18**. As predicted by the Topliss tree, the most active compounds in this series were the 3-CF_3-4-fluorobenzyl ether **28** (IC_{50} = 140 nM) and the 3-CF_3-4-chlorobenzyl ether

Table 2. CYP3A4 inhibition and PK profile of selected analogs[a]

	CYP3A4 % inhibition at 3 μM[b]	Solubility[c] (μg/mL)	Caco-2 permeability A to B ($\times 10^{-6}$ cm/s)	Log D at pH 7.4
28	87	7	NT[d]	3.7
29	97	3	NT[d]	3.7
32	44	100	13	2.9

[a] Reported data are an average of results obtained in duplicate.
[b] 7-Benzyloxy-4-(trifluoromethyl)-coumarin (BFC) was used as a fluorogenic substrate for the CYP3A4 isozyme.[9]
[c] Apparent solubility at pH 6.5 measured by laser nepholometric method.
[d] Not tested due to low solubility.

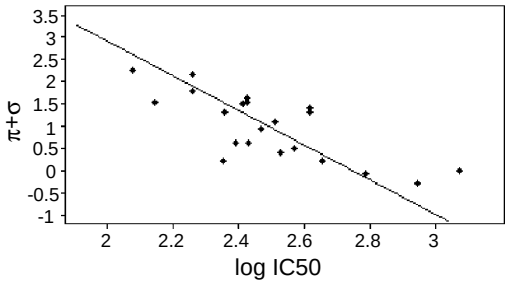


Figure 3. Graph showing the correlation between the log IC_{50} of the analogs in Table 1 and $\pi + \sigma$. Line is an orthogonal straight line fit with $r^2 = 0.58$.

29 (IC_{50} = 120 nM). A graph of the log IC_{50} of the analogs in Table 1 (*X* axis) against $\pi + \sigma$ of the substituents (*Y* axis) indicates a reasonable correlation between potency and a $+(\pi + \sigma)$ effect (Fig. 3).[7] In this series, increased renin inhibition was driven by aryl substituents that led to a net increase in the lipophilic and electron deficient nature of the aromatic ring. This result is not surprising, as previous crystal structures of the ketopiperazine series bound to the renin active site indicate that the benzyl ether occupies a large lipophilic S3 binding pocket.[6]

In conjunction with the benzyl ether SAR, a series of pyridinyl ethers was also examined to explore the effect of basic groups in the S3 pocket (Fig. 4). While introduction of a N atom at the 2- and 4-positions led to inactive compounds (**30** and **31**), the 3-pyridinyl ether **32** inhibited renin with an IC_{50} = 120 nM. This finding was of significant interest since the 3-pyridinyl ether **32** achieved the same renin inhibitory potency as the 3-CF_3-4-chlorobenzyl ether **29**, but with a substantially lower molecular weight (**32** MW = 491 vs **29** MW = 593). Even more interesting was the observation that **32** displayed a significantly less cytochrome P450 3A4 inhibition than either **28** or **29** in our primary screens (Table 2). We believe that the decrease in CYP3A4 inhibition is primarily a result of the lower lipophilicity of **32**, as measured by the logD at pH 7.4.[8] In addition, **32** displayed improved solubility compared to either **28** or **29**, as well as measurable Caco-2 cell membrane permeability. To understand the basis of this remarkable selectivity for the position of the N atom in the benzene ring, we initiated docking studies of **32** in the active site of renin in the flap-open conformation (Fig. 5). [6,10] These studies indicated that the 3-pyridinyl ether extended into the S3 subpocket, where it could potentially make hydrogen bond contacts with the Ser219 hydroxyl and amide NH along the rim of the S3 subpocket. Analogs **30** and **31** are less active because they are unable to make these potential hydrogen bonds.

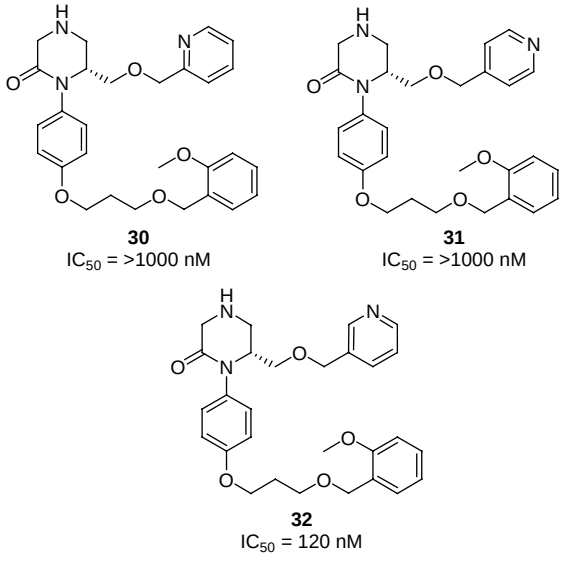


Figure 4. Pyridinyl ether structure–activity relationships.

We recently reported that both enantiomers of the ketopiperazine scaffold with bicyclic C-ring portions exhibited equipotent renin inhibition and followed similar SAR trends.[10] To examine if this trend held good for benzyl ethers, we prepared several compounds of the *S* configuration (Table 3). Surprisingly, the benzyl ether analogs of *S* configuration were found to be much less potent

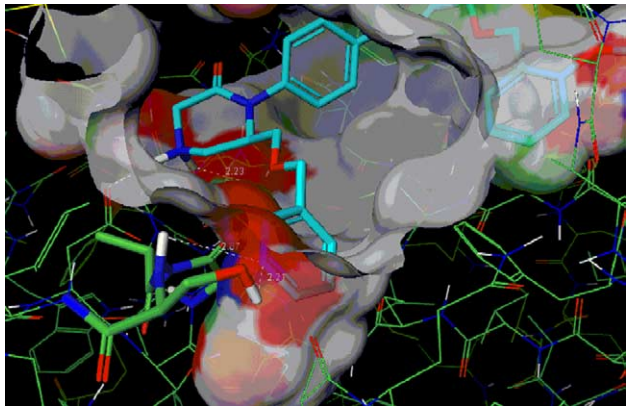
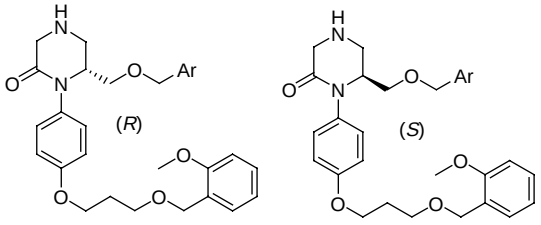
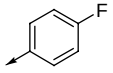
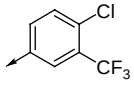


Figure 5. View of analog **32** (blue atom coloring) docked in the flap-open conformation of the renin active site.[6,10] Protein surface is colored by atom (red, oxygen; blue, nitrogen). Residue Ser219 is indicated by the green stick atom coloring.

Table 3. Comparison of the activity of ketopiperazine benzyl ether enantiomers



Ar group	*R* analog	IC_{50} (nM)	*S* analog	IC_{50} (nM)
	**12**	450	**33**	5590
	**29**	120	**34**	594
	**32**	12	**35**	>10,000

against renin. Although the 4-Cl-3-CF_3-benzyl ether **34** (*S* configuration) was only 5-fold less active than the corresponding analog with *R* configuration **29**, both the 4-fluorobenzyl ether **33** and 3-pyridinyl ether **35** showed a >10-fold difference in renin inhibition activity compared to the corresponding *R* configuration analogs **12** and **32**. A vector change in the orientation of the C6 hydroxymethyl linker is required to orient the bicyclic C-ring in the S3 pocket in the analogs with *S* configuration.[10] We theorize that a similar vector change cannot be induced in our C6 benzyloxymethyl analogs because the benzyl ether does not occupy enough steric space in the S3 pocket and the hydroxymethyl linker between the ketopiperazine and benzyl ether rings contains too many degrees of rotational freedom.

In summary, we have reported our efforts to replace the bicyclic C-ring portion of the ketopiperazine scaffold with benzyl ethers in an effort to achieve renin inhibition potency, while reducing molecular weight and for ease of synthesis. Increased renin potency was guided by following the Topliss tree for substituents that give a net increase in lipophilicity and electron deficiency ($\pi + \sigma$) of the C6 benzyloxymethyl moiety to match the lipophilic and electronic nature of the S3 pocket. It was also discovered that equivalent renin inhibition potency could be obtained through the use of a 3-pyridinyl ether, resulting in a substantial reduction in molecular weight, decreased CYP3A4 inhibition, and improved cell membrane permeability and aqueous solubility.

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