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trans-3,4-Disubstituted pyrrolidines as inhibitors of the human aspartyl protease renin. Part II: Prime site exploration using an oxygen linker

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ABSTRACT

Inhibition of the aspartyl protease renin is considered as an efficient approach for treating hypertension. Lately, we described the discovery of a novel class of direct renin inhibitors which comprised a pyrrolidine scaffold (e.g., **2**). Based on the X-ray structure of the lead compound **2** bound to renin we predicted that optimization of binding interactions to the prime site could offer an opportunity to further expand the scope of this chemotype. Pyrrolidine-based inhibitors were synthesized in which the prime site moieties are linked to the pyrrolidine core through an oxygen atom, resulting in an ether or a carbamate linker subseries. Especially the carbamate derivatives showed a pronounced increase in in vitro potency compared to **2**. Here we report the structure–activity relationship of both subclasses and demonstrate blood pressure lowering effects for an advanced prototype in a hypertensive double-transgenic rat model after oral dosing.

The aspartyl protease renin catalyzes the conversion of angiotensinogen to angiotensin as the first and rate limiting step of the renin–angiotensin–aldosterone system (RAAS). The RAAS is a key regulator of blood pressure and body fluid volume, which renders it an attractive therapeutic target to treat hypertension.[1,2] Inhibition of renin is expected to offer the best option to block the RAAS for two reasons: substrate cleavage by renin is the first and rate limiting step and angiotensinogen is its only known endogenous substrate.[3] Aliskiren[4] (**1**, Tekturna/Rasilez®, approved in 2007, Fig. 1) is the first and only marketed direct renin inhibitor (DRI) for the treatment of hypertension suitable for once-daily oral dosing.[5]

In recent years there has been an increasing number of accounts on non-peptidic DRIs.[6] Recently, we have described the discovery of a class of novel 3,4-disubstituted pyrrolidine-based DRIs, as exemplified by **2** (Fig. 1).[7] This compound showed sub-micromolar in vitro inhibitory potency against recombinant human (rh)-renin (Table 1), high selectivity against other aspartyl proteases and promising oral bioavailability in rat (2 mg/kg i.v./6 mg/kg p.o.: *F* 53%, C_{max} 1310 nM, $T_{1/2\ term}$ 2 h).[7] The X-ray crystal structure of **2** bound to rh-renin revealed that both the isopropyl and the dialkoxy substituted phenyl moiety interact with the hydrophobic contiguous S1–S3–S3^{sp} subpocket, similar to the binding mode observed for aliskiren (**1**).[7] The basic pyrrolidine nitrogen binds to the catalytic dyad Asp_{32} and Asp_{215}, and the benzyl group resides in the prime site. Based on the X-ray structure of **2** it appeared likely that the binding interactions in the prime site could be further optimized since the benzyl group of inhibitor **2** is occupying only the S1′ subpocket. Based on our knowledge of the binding interactions of aliskiren with renin[4c] we assumed that further expansion into the prime site would result in enhanced in vitro potency. Linking the prime site moiety to the pyrrolidine core through an oxygen- or a nitrogen containing linker was expected to offer a facile means to prepare a wide range of substituted prime site derivatives. In the preceding publication in this journal we are reporting on our efforts to link the prime site to the pyrrolidine core via a nitrogen containing linker including amines, amides, carbamates and sulfonamides.[8] These inhibitors had excellent in vitro inhibitory potency against rh-renin. However, they suffered from modest permeability as well as high metabolic

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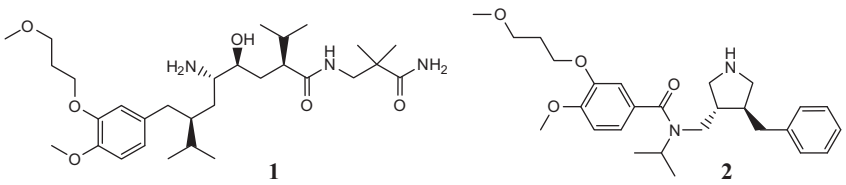
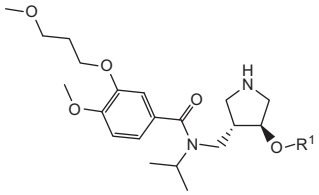
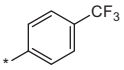
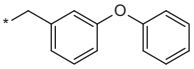
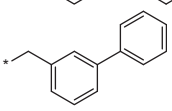
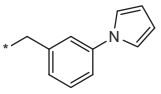
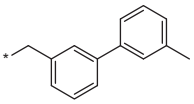
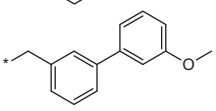
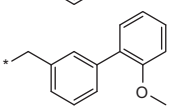
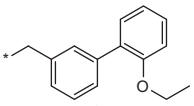
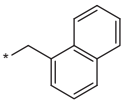
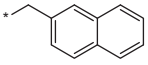


Figure 1. Structures of aliskiren (**1**) and the pyrrolidine lead **2**.

Table 1
In vitro potency for benzyl ether pyrrolidine inhibitors



Compd	R^1	Renin IC_{50}[a] (plasma IC_{50}) (μM)
2	—	0.17[b] (0.3)
9		0.45
10	Benzyl	0.77 (0.85)
11[c]	Benzyl	3.15
12	3-Methoxybenzyl	0.25
13		0.25
14		0.145 (1.1)
15		0.085
16		0.065
17		0.35
18		0.065
19		0.55
20		0.20
21		0.09

[a] Inhibition of rh-renin in a FRET-based enzymatic assay. IC_{50} data represent means of two experiments. All inhibitors are racemic unless otherwise stated.
[b] IC_{50} for the (3*S*,4*S*)-configured eutomer. IC_{50} determined for *rac*-**2**: 0.45 μM.
[c] Relative (3,4-*cis*) configuration at the pyrrolidine ring.

clearance and, as a consequence, showed poor oral pharmacokinetic properties in rat. The present communication summarizes our work on oxygen containing prime-site extensions such as ether or carbamate moieties.

An efficient access to pyrrolidines with an oxygen containing linker attached to the C3-position was established and the synthetic route is outlined in Scheme 1.[9] Racemic *trans*-configured alcohol *rac*-**8** served as common intermediate for the preparation of both the ether and the carbamate extended pyrrolidines. Intermediate **3** was obtained as a racemic mixture of *cis*- and *trans*-stereoisomers in three steps from glycine ethyl ester.[10] Silyl ether **4** was separated into its racemic stereoisomers *rac*-**5** and *rac*-**6** by chromatography on silica gel. Reduction of the nitrile group of *rac*-**6** with DIBAL-H followed by reductive amination of the corresponding aldehyde with isopropylamine/$NaBH(OAc)_3$ afforded *rac*-**7**. Coupling of *rac*-**7** with 4-methoxy-3-(3-methoxy-propoxy)-benzoylchloride[7] followed by TBS deprotection yielded alcohol *rac*-**8**. Racemic pyrrolidines **9**–**21** bearing an ether linker were generally prepared by heating alcohol *rac*-**8** and the corresponding benzyl halide in DMF using NaH as a base, followed by *N*-Boc deprotection under acidic conditions.

Inhibitors with a carbamate linkage were obtained by either reaction of alcohol *rac*-**8** with the respective isocyanate in the presence of $AlCl_3$ as the *Lewis* acid[11] (**22**–**25**, **28**), or by preincubation of *rac*-**8** with triphosgene/DMAP followed by reaction of the intermediate chloroformate with the appropriate amine (**26**, **27**, **29**–**34**), as outlined in Scheme 1. *N*-Boc deprotection afforded the racemic pyrrolidine inhibitors **22**–**34**. *cis*-Configured **11** (Table 1) was accessed from *rac*-**5** following the same synthetic procedures as outlined for the *trans*-configured building block *rac*-**6**. Enantiomerically pure carbamates **24** and **25** were synthesized starting from enantiomerically pure (3*S*,4*S*)-**6** and (3*R*,4*R*)-**6** which were obtained in the following way: the TBS group of *rac*-**6** was cleaved and the resulting racemic alcohol was separated into its enantiomers by preparative HPLC using a chiral stationary phase.[12] The absolute configurations of the two enantiomeric alcohols were determined by X-ray diffraction.[13] The enantiopure alcohols were re-protected with TBS to afford (3*S*,4*S*)-**6** and (3*R*,4*R*)-**6**, and the onward syntheses were carried out as described for *rac*-**6** (Scheme 1).

The in vitro renin inhibitory potency of inhibitors with an ether linkage was assessed in a TR-FRET assay[7] using purified rh-renin, and the data are summarized in Table 1 (lead **2** is shown as reference).

First, it was demonstrated that linking of a prime site 4-(trifluoromethyl)phenyl or a benzyl group to the pyrrolidine ring via an oxygen atom was tolerated: racemic inhibitors **9** and **10** showed in vitro activities comparable to the racemic lead **2** bearing a benzyl group directly attached to the pyrrolidine core. Second, it was confirmed that a relative *trans* configuration at the pyrrolidine ring was favored over the *cis* configuration, as *trans*-benzyl ether **10** was 4 times more potent than its *cis* analog **11**. For our further studies we decided to focus on exploration of prime site benzyl ethers due to their better synthetic accessibility as compared to phenyl ethers such as **9**.

Based on the X-ray structure of **2** we assumed that the benzyl group of **10** resides in the S1′ pocket. Molecular modeling proposed that further extension into the S2′ pocket could be achieved by proper substitution of the 3-position of the phenyl group. Indeed, when a methoxy, phenoxy, phenyl or pyrrole group were attached