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Identification of a new biaryl scaffold generating potent renin inhibitors

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ABSTRACT

The discovery and SAR of a series of potent renin inhibitors possessing a novel biaryl scaffold are described herein. Molecular modeling revealed that the cyclopropylamide spacer present in **1** can be replaced by a simple, substituted aromatic ring such as a toluene in **2**. The resulting compounds exhibit subnanomolar renin IC_{50} and good oral bioavailability in rats.

The treatment of hypertension, a major risk factor for cardiovascular disease, has been an active area of research for many years. Unfortunately, despite the numerous advances that have been made, most hypertensive patients still can not achieve the targeted 140/90 mmHg recommended by the American Heart Association (AHA) by using the currently available therapies (i.e., diuretics, β-blockers, calcium channel blockers, ACE inhibitors, AT1R antagonist, etc.).[1] However, since the perturbation of the renin–angiotensin–aldosterone system (RAAS) at its various points (Fig. 1) with ACE inhibitors and/or AT1R antagonists have proven to be a viable strategy for the lowering of blood pressure,[2] it therefore serves to reason that the inhibition of the enzyme responsible for the initial and rate-limiting cleavage of angiotensinogen to angiotensin I, should confer the maximum benefit possible with this pathway.[3] Furthermore, it has been hypothesized that renin inhibition should be associated with fewer side effects when compared with other therapies that target downstream events of the RAAS pathway.[4] Indeed, significant time and resources have been invested towards the identification of a renin inhibitor suitable for clinical development. To date, only Aliskiren has received FDA approval (2007) for the treatment of essential hypertension.[5]

Previously, we described the design and optimization of a novel series of substituted amino propanamide renin inhibitors, with compound **1** (Fig 2) as a representative compound.[6] Subsequent molecular modeling[7] and structural biology studies confirmed that the amide functionality is not implicated in any stabilizing hydrogen bond interactions with the renin enzyme but simply serves as a spacer between the terminal P3 aromatic plate and the cationic warhead. Consequently, it was felt that the amide bridge could be effectively replaced by an aryl linker thereby allowing further structural diversification from known renin inhibitors. Furthermore, the resulting biaryl compounds could be accessed using well

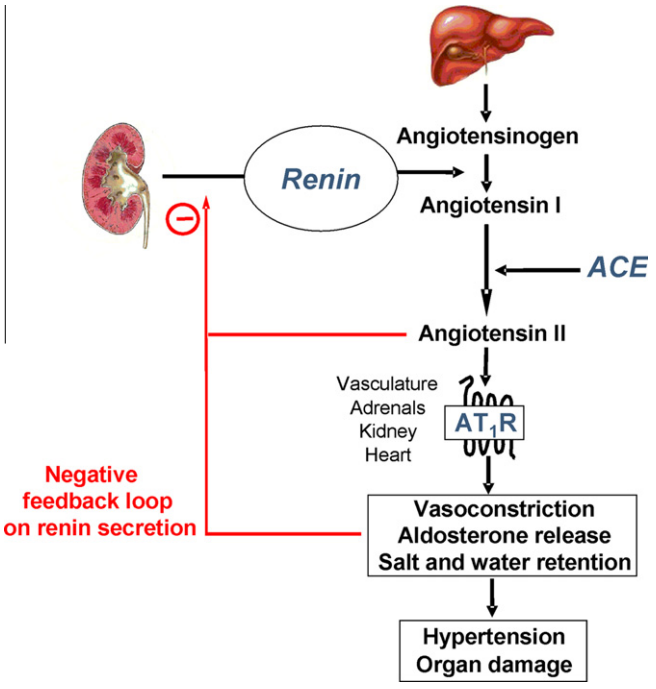

Figure 1. Renin–angiotensin–aldosterone system (RAAS).

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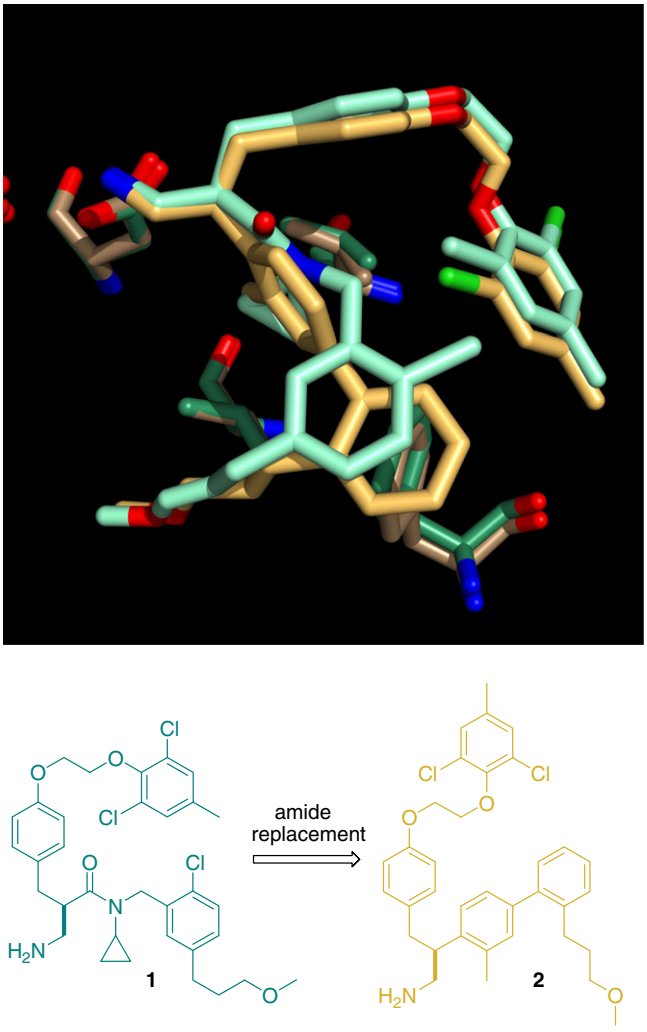


Figure 2. Aryl spacer as amide replacement.

established metal-catalyzed cross-coupling chemistry that is amenable for rapid analogue synthesis.

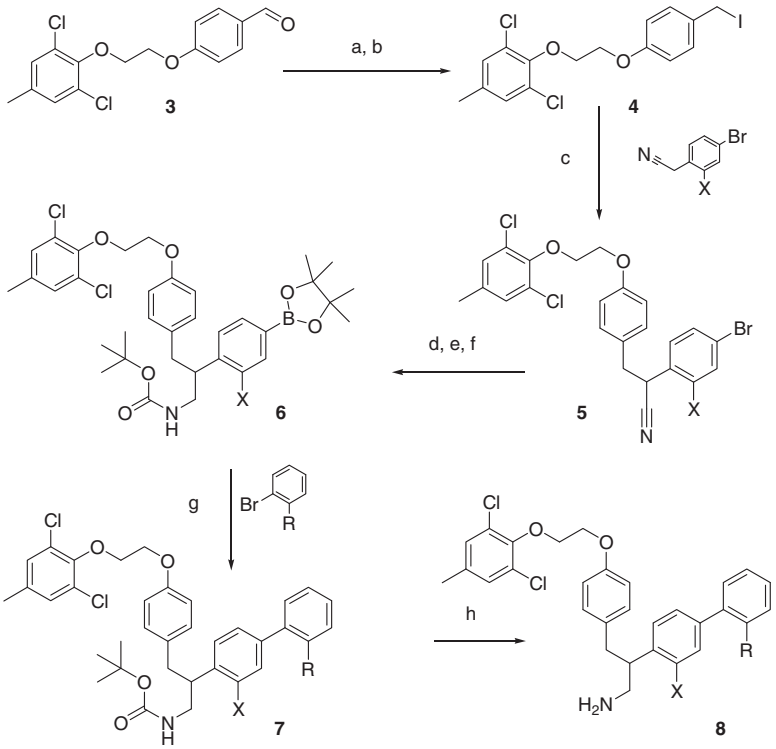
To test this hypothesis, we prepared a series of biaryl analogs using the synthetic route described in Scheme 1. Briefly, sodium borohydride reduction of the known[8] aldehyde **3** and subsequent Arbusov iodination of the resulting alcohol afforded benzyl iodide **4**. Iodide **4** could then be used to alkylate a variety of 2-substituted 4-bromophenyl acetonitriles. The latter compounds were either commercially available or readily prepared from the corresponding benzyl bromides via cyanide displacement. The resulting alkylated nitriles **5** could then be reduced by cobalt borohydride; prepared in situ from cobalt(II) chloride hexahydrate and sodium borohydride, protected as the *tert*-butyl carbamates and converted to the desired boron pinacolates **6** under typical Miyaura conditions.[9] Finally Suzuki cross-coupling of **6** with a suitable 2-substituted phenyl bromide afforded, following BOC-deprotection with hydrochloric acid, the desired amines **8**.

The requisite 2-substituted phenyl bromide for the penultimate cross-coupling step can be obtained using the synthetic route described in Scheme 2. Starting from commercially available alcohol **9**, it can either be converted to the methyl ether **10** by treatment with iodomethane under basic conditions, to the carbamate **11** with sodium cyanate under acidic conditions, or to the nitrile **12** via the Mitsunobu reaction. Starting from known carboxylic acid **13**, it can either be converted to the amide **14** via the acid chloride, or to the ester **18** under Fisher's esterification conditions, or to the alcohol **15** by treatment with borane dimethyl sulfide complex. Alcohol **15** can then be elaborated further to either methyl ether **16** or carbamate **17** using the same reaction conditions described above. Starting from 2-bromophenol (**19**), alkylation with either 1-bromo-3-methoxypropane or 2-bromoethyl methyl ether deliver compounds **20** and **21**, respectively. Finally, acylation of commercially available amine **22** affords the desired acetamide **23**.

Our initial SAR efforts were focused on determining which aryl substituent (i.e., X, Table 1) would best occupy the renin P1 subpocket that was previously filled by the cyclopropyl residue in our earlier amino-propanamide series. The respective ability of these compounds to inhibit the renin enzyme in either buffer[10] or human plasma[11] is shown in Table 1.

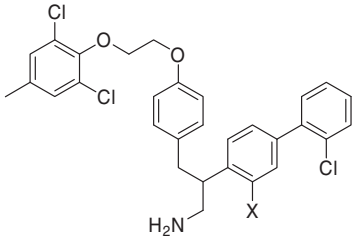
When a simple benzene spacer was used (X = H, **24**), only moderate inhibition of the renin enzyme was observed. The introduction of a small and greasy substituent such as fluorine (**26**) proved to be beneficial for activity whereas the introduction of a larger and more polar methoxy group (**25**) was not. Further optimization revealed that the appendage of a chlorine atom (**28**) could improve the intrinsic potency by another 10-fold but the resulting compound was found to be highly plasma protein bound. A toluene spacer (X = Me, **27**) proved to offer the best compromise between intrinsic potency and plasma shift.

Having established the optimal substitution pattern for the central aromatic ring, we then turned our attention to the optimiza-



Scheme 1. Synthesis of **8**. Reagents and conditions: (a) $NaBH_4$, MeOH; (b) I_2, PPh_3, imidazole, CH_2Cl_2; (c) LiHMDS, HMPA/THF; (d) $CoCl_2{\cdot}6H_2O$, $NaBH_4$, THF/MeOH; (e) $(BOC)_2O$, Hunig's base, CH_2Cl_2; (f) $PdCl_2$(dppf), Bis(pinacolato)diboron, KOAc, DMF; (g) $Pd(OAc)_2$, Ph_3P, Na_2CO_3, *n*-PrOH; (h) HCl, dioxane/CH_2Cl_2.

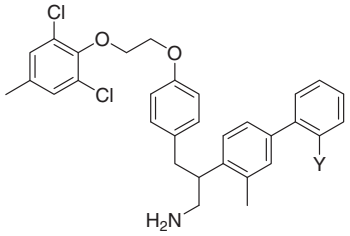
Table 1
In vitro data for compounds **24**–**28**



Compd	X	Renin buffer IC_{50}[a,b] (nM)	Renin plasma IC_{50}[a,c] (nM)
24	H	44.4	—
25	OMe	47.6	3622
26	F	7.0	1151
27	Me	6.0	796
28	Cl	0.52	764

[a] Values are means of at least two experiments.
[b] See Ref. 10 for assay protocol.
[c] See Ref. 11 for assay protocol.

Table 2
In vitro data for compounds **29**–**40**

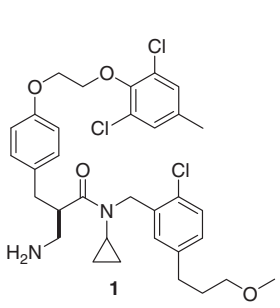


Compd	Y	Renin buffer IC_{50}[a,b] (nM)	Renin plasma IC_{50}[a,c] (nM)
29	CH_2CH_2OMe	19.7	1915
30	$CH_2CH_2CH_2OMe$	0.44	116
31	OCH_2CH_2OMe	7.41	1495
32	$OCH_2CH_2CH_2OMe$	1.7	1158
33	CH_2CO_2Me	12.9	534
34	$CH_2CH_2CO_2Me$	0.09	141
35	$CH_2CH_2CONMe_2$	30.8	1111
36	$CH_2CH_2OCONH_2$	1.02	103
37	CH_2CH_2CN	10.6	544
38	CH_2NHAc	2.1	138
39	CH_2CH_2NHAc	0.04	9.6
40	$CH_2CH_2CH_2NHAc$	0.97	131

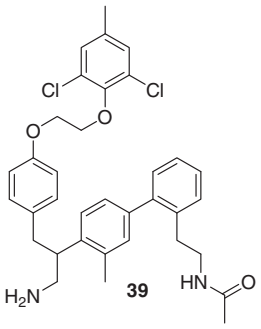
[a] Values are means of at least two experiments.
[b] See Ref. 10 for assay protocol.
[c] See Ref. 11 for assay protocol.

tion of the terminal aromatic ring (Table 2). As was already demonstrated in previous publications,[6,12] maximizing the interaction with the renin P3 sub-pocket is extremely important for renin activity. However, it should be stressed that the SAR established previously in the amide series[6] did not directly translate to the biaryl series. For example, the 2-methoxyethyl $S3_{sp}$ substituent that was found to significantly improve binding to the renin enzyme in the amide series actually led to a threefold loss in potency in the biaryl series (i.e., **29**) when compared to its simple 2-chloro analogue (i.e., **27**). However, further extension of this side chain by one methylene unit to a 3-methoxypropyl group (i.e., **30**) led to a much more potent renin inhibitor. Incorporation of an oxygen linker into the chain (i.e., **31**, **32**) did not prove to be beneficial. On the other hand, replacement of the methoxy capping group by a methyl ester afforded one of our most intrinsically potent biaryl renin inhibitor (i.e., **34**). Unfortunately, the potency of compound **34** was highly shifted in the presence of human plasma. This however, could be readily addressed by switching to a more polar acetamide capping

Table 3
Comparative profile for compounds **1** and **39**



1



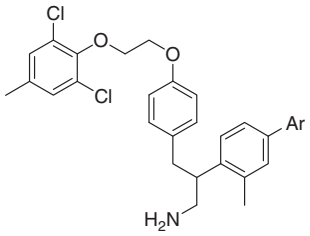
39

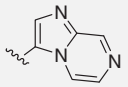
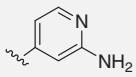
Compd	Renin buffer IC_{50}[a,b] (nM)	Renin plasma IC_{50}[a,c] (nM)	SD rat PK *F* (%), P.O. AUC (μM h), Cl (mL/min/kg), $T_{1/2}$ (h), V_{dss} (L/kg)
1	0.09	2.4	41, 8.3, 35, 6, 12
39	0.04	9.6	23, 5.5, 34, 7, 9

[a] Values are means of at least two experiments.
[b] See Ref. 10 for assay protocol.
[c] See Ref. 11 for assay protocol.

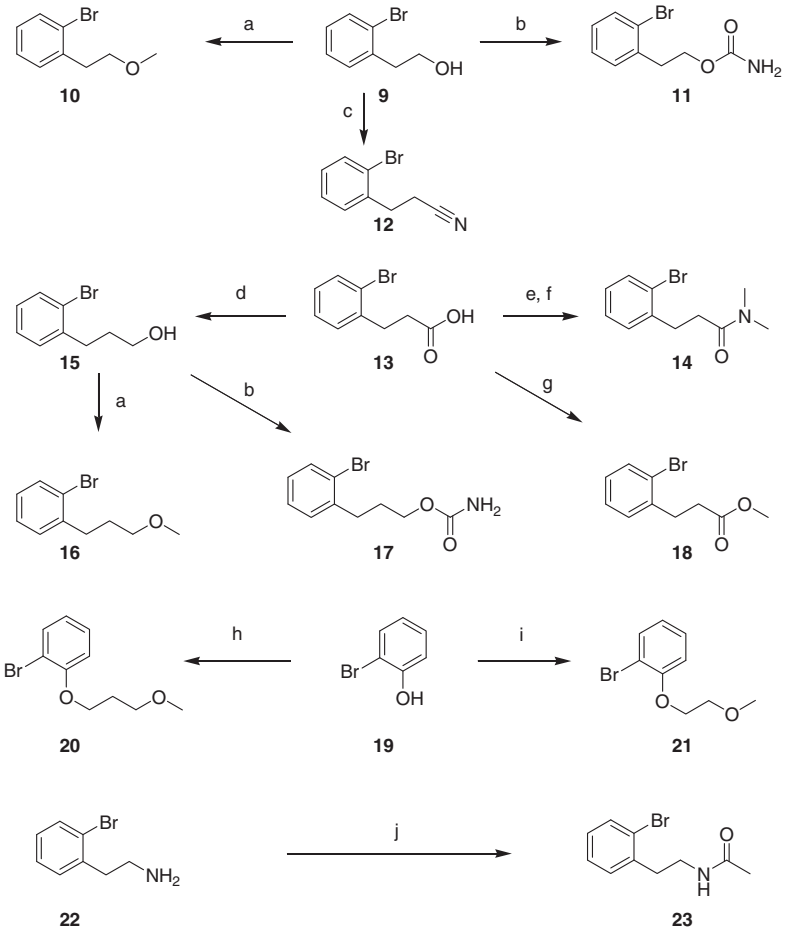
group (compounds **38–40**). With the judicious choice in tether chain length (compound **39**), a renin inhibitor with a low nanomolar IC_{50}, even in the presence of human plasma, was obtained.

Table 4
In vitro data for compounds **27**, **41–43**



Compd	X	Renin buffer IC_{50}[a,b] (nM)	Renin plasma IC_{50}[a,c] (nM)
27	2-ClPh	6.0	796
41		3.9	416
42		1.7	228
43		4.9	614

[a] Values are means of at least two experiments.
[b] See Ref. 10 for assay protocol.
[c] See Ref. 11 for assay protocol.



Scheme 2. Synthesis of aryl bromide intermediates. Reagents and conditions: (a) MeI, NaH, DMF; (b) NaOCN, TFA, Benzene; (c) 1,1′-(azodicarbonyl)dipiperidine, *n*-Bu_3P, acetone cyanohydrin, benzene; (d) BH_3·DMS, THF; (e) oxalyl chloride, DMF (cat.), CH_2Cl_2; (f) Me_2NH, CH_2Cl_2; (g) H_2SO_4, MeOH; (h) 1-bromo-3-methoxypropane, Cs_2CO_3, DMF; (i) 2-bromoethyl methyl ether, Cs_2CO_3, DMF; (j) AcCl, Hunig's base, DMAP, CH_2Cl_2.

The profile of biaryl renin inhibitor **39** is summarized and compared with that of amide renin inhibitor **1** in Table 3. Compound **39** was found to be intrinsically ~twofold more potent against renin than **1**, but was also more plasma shifted. When given orally to Sprague–Dowly rats as the HCl salt at a dose of 30 mpk (0.5% methocel solution, 5 ml/kg) and iv at a dose of 4 mpk (60% PEG 200/water solution, 1 ml/kg), both renin inhibitors exhibited comparable pharmacokinetics, with compound **1** being more orally bioavailable.

Finally a structurally diverse 75-member library was generated from pinacol boronate **6** (X = Me) using rapid parallel synthesis with various aryl halides. The three most promising hits, shown in Table 4, revealed that the terminal 2-chlorophenyl P3 plate in compound **27** can be effectively replaced with hetero-aromatics without loss in renin potency.

In conclusion, with input from molecular modeling and structural biology, we were able to identify a novel series of biaryl renin inhibitors with good in vitro potency and rodent PK. Furthermore with this scaffold, parallel synthesis technology could be employed that allowed us to rapidly modify and tune the physical and chemical characteristics of our renin inhibitors. Other biaryl-based renin inhibitors with different nitrogen bearing warheads, as well as their associated properties, will be disclosed in due course.

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10. Recombinant human renin (3 pg/μM) 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 water), and assay buffer were pre-mix 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 compound and an IC_{50} was determined by curve fitting software.
11. 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 microliters of renin inhibitors, at various concentration 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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