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Ketopiperazine-based renin inhibitors: Optimization of the "C" ring

Daniel D. Holsworth,[a,*] Cuiman Cai,[a] Xue-Min Cheng,[a] Wayne L. Cody,[a] Dennis M. Downing,[a] Noe Erasga,[a] Chitase Lee,[a] Noel A. Powell,[a] Jeremy J. Edmunds,[a] Michael Stier,[a] Mehran Jalaie,[a] Erli Zhang,[a] Pat McConnell,[a] Michael J. Ryan,[b] John Bryant,[b] Tingsheng Li,[c] Aparna Kasani,[c] Eric Hall,[c] Rajendra Subedi,[c] Mohammad Rahim[c] and Samarendra Maiti[c]

[a]*Pfizer Global Research and Development, Michigan Laboratories, Department of Chemistry, Ann Arbor, MI 48105, USA*
[b]*Pfizer Global Research and Development, Michigan Laboratories, Department of Pharmacology, Ann Arbor, MI 48105, USA*
[c]*Naeja Pharmaceuticals, Inc., Edmonton, Alta., Canada T6E 5V2*

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Abstract—A systematic investigation of the S_3 sub-pocket activity requirements was conducted. It was observed that linear and sterically small side chain substituents are preferred in the S_3 sub-pocket for optimal renin inhibition. Polar groups in the S_3-sub-pocket were not well tolerated and caused a reduction in renin inhibitory activity. Further, compounds with c$\log P$'s $\leqslant 3$ demonstrated a dramatic reduction in CYP3A4 inhibitory activity.

Hypertension is a leading risk factor for cardiovascular disease, such as congestive heart failure, stroke, myocardial infarction, and is the leading cause of death in the western world.[1] The renin angiotensin system (RAS) is well established as an endocrine system involved in blood pressure (BP) and fluid electrolyte balance (Fig. 1). Renin cleaves the naturally occurring plasma glycoprotein, angiotensinogen, in the rate-limiting step of the RAS to produce the hemodynamically inactive angiotensin I (AI). Angiotensin converting enzyme (ACE) then converts AI to the hemodynamically active angiotensin II (AII) (Fig. 1), which is believed to act by causing the constriction of blood vessels and the release of the sodium-retaining hormone aldosterone from the adrenal gland. It has been proposed that stopping the detrimental effects of the RAS at the most upstream point of the cascade, via renin inhibition, will offer advantages such as enhanced efficacy, improved end organ protection, and fewer side effects than the existing therapies that target downstream events.[2] Thus, renin inhibitors represent a prime therapeutic target for the treatment of hypertension.[3]

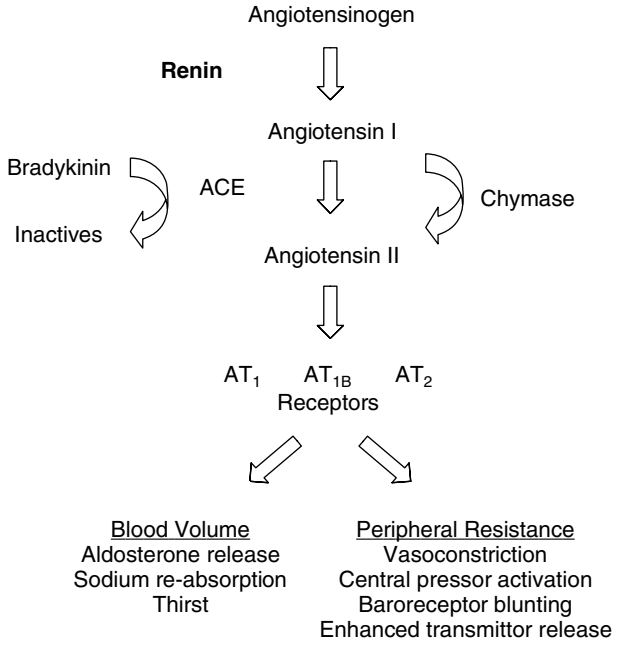

Figure 1. Renin angiotensin system (RAS).

Recently, the first series of small molecule renin inhibitors based on a weakly active *trans*-disubstituted oxoaryl piperidine scaffold was reported.[4] We have utilized

Keywords: Renin inhibitor; Hypertension; Ketopiperazine; CYP3A4.
* Corresponding author. Tel.: +1 734 622 1344; fax: +1 734 622 3909; e-mail: Daniel.Holsworth@pfizer.com

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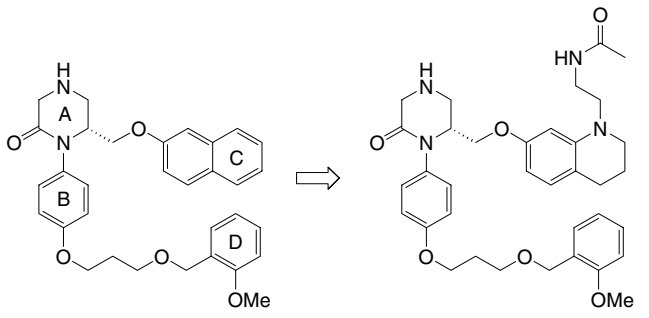
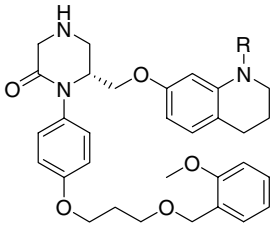


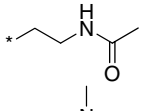
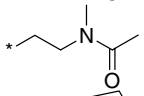
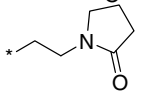
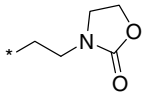
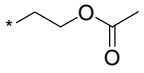
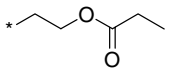
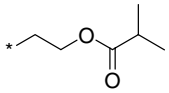
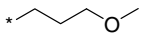
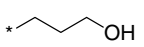
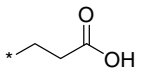
Scheme 1. Modification of the "C" ring of **A** to create analogs that address the S_3 sub-pocket of renin for increased potency, as exemplified by compound **1**.[5a,b]

this scaffold to design novel non-peptidic ketopiperazine-based renin inhibitors, typified by Compound **A** (Scheme 1).[5] Compound **A** possesses moderate affinity for renin, and when administered orally, lowers BP in a hypertensive double transgenic (human renin and angiotensinogen) mouse model.[5a] It has also been shown that replacement of the "C" ring naphthyl of **A** with an *N*-[2-(7-methyl-3,4-dihydro-2*H*-quinoline-1-yl)-ethyl]-acetamide moiety resulted in a marked increase in potency (**1**, IC_{50} = 0.29 nM, Scheme 1, Table 1).[5a] Based on literature X-ray crystal structures, we believe the acetamide moiety penetrates the S_3 sub-pocket of renin, providing the increased affinity.[4] Our earlier structure–activity relationship results indicated that the A, B, and D-ring portions of compound **A** were required for good renin inhibition activity, whereas the C-ring portion was amenable to variation.

Therefore, a structure-based design approach of **1**, guided by literature precedence,[4] was initiated. Compounds **2**–**10** (Table 1) were designed to probe the steric and electronic requirements of the S_3 sub-pocket of renin. In addition, the compounds were evaluated for their

Table 1. "C" Ring SAR of compound **A**



Compound	R side chain	Renin IC_{50} (nM)[5e]	clog *P*	CYP3A4 IC_{50} (BFC, nM)[7]	% Inhibition of CYP 3A4 at 3 μM
1		0.29	5.47	82	92
2		4.0	6.03	18	N/D
3		606	5.86	42	N/D
4		>1000	6.08	3790	N/D
5		0.42	6.44	N/D	87
6		9.0	6.97	N/D	96
7		550	7.28	54	92
8		3.2	6.59	12	96
9		37	5.84	455	71
10		450	3.70	10,000	N/D

N/D, not determined. ∗, designates point of attachment.

propensity to inhibit the cytochrome P450 3A4 isozyme (CYP3A4).[6]

Previous reports had indicated that the presence of at least one hydrogen-bond acceptor in the tetrahydroquinoline nitrogen substituent that extended into the S_3 subpocket was required for increased renin potency.[4e] However, a hydrogen-bond donating NH group was present in all of the published examples, raising the possibility that a dual hydrogen-bond acceptor/donating motif played a large role in the increase in renin potency. Our early work seemed to confirm this, as methylation of the acetamide of **1** resulted in a 14-fold loss of activity (**2**, IC_{50} = 4 nM). Inclusion of the amide functionality into a cyclic lactam or oxazolidone ring also resulted in a significant loss of activity (analogs **3** and **4**). Surprisingly, replacement of the amide NH with an O atom resulted in similar subnanomolar potency (**5**, IC_{50} = 0.42 nM), indicating that the increase in renin potency by binding in the S_3 subpocket observed in previous reports and current work is driven largely by the presence of the hydrogen-bond accepting carbonyl group. We believe that the loss in activity observed in analogs **2**–**4** is due largely to steric hindrance of the bulkier *N*-methyl or cyclic amides with the narrow S_3 subpocket, and not simply to the loss of hydrogen-bond donating capability. The more sterically hindered ester analogs **6** and **7** exhibit drastic reductions in renin potency, again presumably due to steric hindrance with the S_3 sub-pocket.

Since sterically small chains with a hydrogen-bond acceptor moiety seemed to prefer to bind in the S3 subpocket, we investigated changing from a metabolically labile acetate to a propyl methyl ether side chain (**8**, IC_{50} = 3.2 nM), which caused a 10-fold loss of activity. Substitution of the propyl methyl ether side chain with a propyl alcohol resulted in an additional 10-fold loss of affinity (**9**, IC_{50} = 37 nM). X-ray co-crystallographic analysis of **9** in the renin active site (flap open conformation) showed the hydrogen of the hydroxyl group forming a hydrogen bond with a water molecule at the bottom of the S_3 sub-pocket (Fig. 2). This observation may additionally explain why compounds **1** and **5** are potent. X-ray analysis of compounds similar to **1** (flap open conformation), which contain the *N*-[2-(7-methyl-3,4-dihydro-2*H*-quinoline-1-yl)-ethyl]-acetamide moiety, is devoid of a water molecule at the base of the S_3 sub-pocket (results not shown). It is rationalized that the carbonyl of the acetamide moiety of **1** may form a hydrogen bond to TYR-14 NH and the methyl group of the acetamide moiety displaces the water molecule at the base of the S_3 sub-pocket, causing an increase in potency. While the O atom of the propyl methyl ether analog **8** is capable of forming the same hydrogen-bond to the TYR-14 NH group, it is unable to fully displace the water molecule at the base of the S_3 sub-pocket, resulting in 10-fold lower activity.

During this investigation, we observed that most of the analogs were potent inhibitors of CYP3A4, as measured by IC_{50} or single-point % inhibition at 3 μM. However, compounds containing more polar functionalities in the S_3 side chain showed significant reduction in the CYP3A4 inhibition profile (i.e., analogs **4** and **9**). While the 3-hydroxypropyl analog **9** showed relatively modest renin potency, we were encouraged to see that the introduction of a polar hydroxyl group resulted in a >10-fold ratio between CYP3A4 and renin IC_{50} for the first time. Motivated by this favorable renin activity, clog*P* and CYP3A4 profile of **9**, compound **10** was designed to introduce an additional hydrogen-bond accepting carbonyl group. Compound **10**, with a clog*P* of 3.7, did not inhibit CYP 3A4. However, **10** exhibited only modest affinity toward renin (IC_{50} = 450 nM).

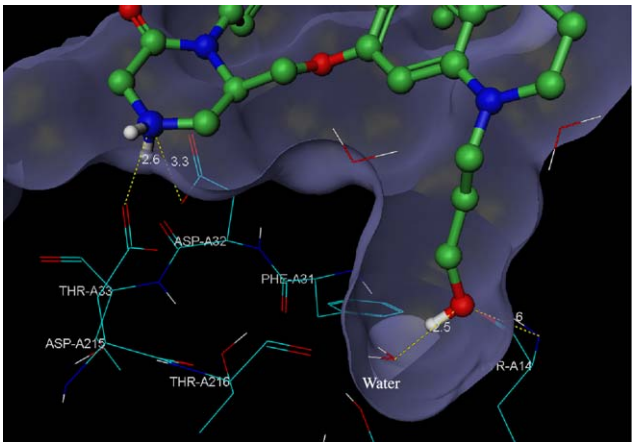


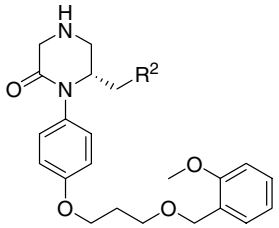
Figure 2. The co-crystallized X-ray structure of **9** is depicted in green.[5b,9] The ligand forms H-bonds to the nitrogen of TYR-14 and a water molecule around the S_3 sub-pocket, while maintaining H-bonds to the aspartic acid residues 32 and 215.

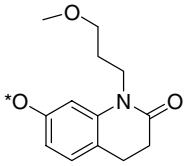
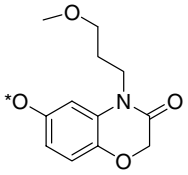
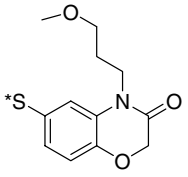
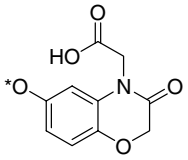
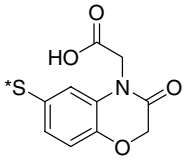
In summary, investigations around the acetamide template (**1**) revealed that linear and sterically small side chain substituents are preferred in the S_3 sub-pocket for renin inhibition, while polar functionalities such as hydroxyl or carboxylic acids provided reduced CYP3A4 inhibition.

Next, heterocycles that have reduced electron density and hydrophobicity relative to the tetrahydroquinoline framework (Table 2) were installed. These analogs were evaluated for their renin and CYP3A4 inhibition.

Replacement of the tetrahydroquinoline with a 3,4-dihydroquinolin-2(1*H*)-one ring gave a slight twofold decrease in activity (**11**, IC_{50} = 7.0 nM) relative to analog **8**, while reducing the electron density of the aryl ring. Incorporation of an additional oxygen atom to form a 1,4-benzoxazin-3-one ring yielded a fivefold increase in binding affinity to sub-nanomolar levels (**12**, IC_{50} = 0.68 nM) relative to **8**. Changing the linkage from oxygen to sulfur yielded an additional fourfold increase in binding affinity (**13**, IC_{50} = 0.18 nM). Modeling of **13** in the renin active site was performed to determine how the 6-sulfanyl-4*H*-benzo[1,4]-oxazin-3-one ring contributed to significantly enhance renin inhibition activity (Fig. 3). Surprisingly, additional hydrogen bond contacts were not observed between the S_3 sub-pocket and the 6-sulfanyl-4*H*-benzo[1,4]oxazin-3-one ring. Instead, we believe that the 6-sulfanyl-4*H*-benzo[1,4]oxa-

Table 2. SAR of the "C" ring



Compound	R_2 side chain	Renin IC_{50} (nM)[5e]	clog *P*	CYP3A4 IC_{50} (BFC, nM)[7]	% Inhibition of CYP 3A4 at 3 μM
11		7	5.05	35	92
12		0.68	4.90	N/D	95
13		0.18	5.34	14	95
14		364	2.06	25,000	N/D
15		1200	2.50	15,000	N/D

These heterocycles were designed to have reduced electron density and reduced hydrophobicity relative to the tetrahydroquinoline framework. Compounds **11**–**13** contained the 3-methoxy propyl side chain due to ease of synthesis and the association with good renin potency (see compound **8**). Compound **14** was designed to exhibit a low clog *P* for reduced CYP 3A4 activity. N/D, not determined. * designates point of attachment.

zin-3-one is a better overall electronic match for the hydrophobic S_3 sub-pocket, and the increase in C–S bond lengths results in better van der Waals contacts with the protein.

Compound **13**, however, was also a potent CYP3A4 inhibitor (IC_{50} (BFC) = 14 nM). Since carboxylic acids in the S_3 sub-pocket were shown to reduce CYP inhibitory activity (along with renin inhibition activity), the oxygen and sulfur analogs (**14** and **15**, respectively) of the 4*H*-benzo[1,4]oxazin-3-one ring system were evaluated. Interestingly, compound **14** (renin IC_{50} = 364 nM) was more active than **15** (renin IC_{50} = 1200 nM) against renin. Both were devoid of activity against CYP3A4.

Compound **1** is a potent renin and CYP3A4 inhibitor. SAR studies based on the "C" ring of the ketopiperazine framework yielded compounds that were modest renin inhibitors devoid of CYP3A4 inhibitory activity. It was shown that linear and sterically small side chain substituents are preferred in the S3 sub-pocket for renin inhibition. Compounds with lower clog *P* values, preferably, less than 3, show reduced CYP3A4 inhibition. Also, polar groups in the S_3 sub-pocket (which were used to reduce the clog *P*) are not well tolerated, causing a drastic reduction in the renin inhibitory activity. Future work will be focused on compounds with increased hydrophilicity, reduced CYP3A4 inhibitory activity, and increased renin inhibitory activity.

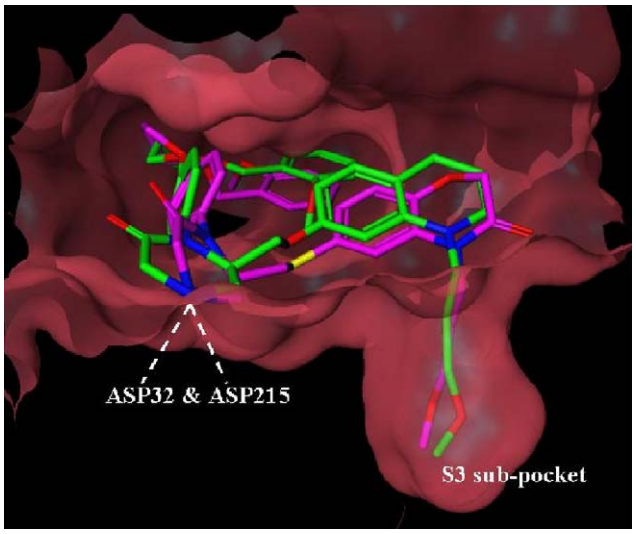


Figure 3. An open-flap X-ray structure of renin was used for docking of molecules **8** (green) and **13** (pink). Random conformations of these ligands were generated using CONCORD and ultimately docked with GOLD[8] software (default parameters were employed) to carryout all the structure-based modeling of this series. The piperazine nitrogen forms two hydrogen bonds to Aspartic acids 32 and 215 within the active site of the renin enzyme.

Acknowledgments

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