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Discovery of 6-ethyl-2,4-diaminopyrimidine-based small molecule renin inhibitors

Daniel D. Holsworth,[a,*] Mehran Jalaie,[a] Thomas Belliotti,[a] Cuiman Cai,[a] Wendy Collard,[a] Suzie Ferreira,[a] Noel A. Powell,[a] Michael Stier,[a] Erli Zhang,[a] Pat McConnell,[a] Igor Mochalkin,[a] Michael J. Ryan,[a] John Bryant,[a] Tingsheng Li,[b] Aparna Kasani,[b] Rajendra Subedi,[b] Samarendra N. Maiti[b] and Jeremy J. Edmunds[a,†]

[a]*Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*
[b]*Naeja Pharmaceuticals, Inc., Edmonton, Alta., Canada T6E 5V2*

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Abstract—Novel 2,4-diaminopyrimidine-based small molecule renin inhibitors are disclosed. Through high throughput screening, parallel synthesis, X-ray crystallography, and structure based drug design, we have developed the first non-chiral, non-peptidic, small molecular template to possess moderate potency against renin. The designed compounds consist of a novel 6-ethyl-5-(1,2,3,4-tetrahydroquinolin-7-yl)pyrimidine-2,4-diamine ring system that exhibit moderate potency (IC_{50}: 91–650 nM) against renin while remaining 'Rule-of-five' compliant.

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. It is estimated that there are approximately 1 billion people worldwide that suffer from high blood pressure. Despite the current therapies available, approximately 70% of those people with hypertension do not reach their target blood pressure levels. In fact, many patients must take three or more medicines to control their blood pressure, yet some do not respond fully to a combination of treatments. Therefore, opportunities remain for improved treatment options.[1]

The renin–angiotensin system (RAS) is well established as an endocrine system involved in blood pressure (BP) and fluid electrolyte balance. Renin, an aspartyl protease, cleaves angiotensinogen to produce angiotensin I, which is further converted by angiotensin converting enzyme (ACE) to the potent vasoconstrictor, angiotensin II. Since angiotensinogen is the only substrate known for renin and cleavage of angiotensinogen by renin is the rate determining step in the RAS, it is of general consensus that inhibition of renin would be the optimal strategy for the control of hypertension.[2] Renin inhibitors not only should have superior blood pressure reducing effects and thus, exhibit optimal end organ protection, but also should diminish 'dry cough', a reflex demonstrated in some patients who take ACE inhibitors.[3a–c]

Attempts to inhibit renin in the early 1980s centered on high molecular weight transition state mimetics based on the angiotensinogen backbone. These peptidomimetic inhibitors suffered from poor PK properties such as low oral bioavailability, short duration of action, and/or cost of synthesis and all were eventually discontinued. The rationale for the poor PK properties of the peptidomimetic based inhibitors was their large size (600–700 Da) and flexibility (>15 rotatable bonds). Furthermore, cost of goods became an issue as all the compounds contained multiple chiral centers, and required lengthy and inefficient synthesis. These observations led many to believe that renin was an 'undruggable' target since, at the time, only large flexible molecules seemed to inhibit renin at nanomolar levels.[4]

In April 2006, Novartis Pharmaceuticals filed the first NDA for an inhibitor of renin for the treatment of

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* Corresponding author. Tel.: +1 734 622 1344; fax: +1 734 622 3909; e-mail: Daniel.Holsworth@pfizer.com
† Present address: Abbott Labs, Worcester, MA 01605, USA.

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hypertension. Tekturna (Aliskerin) was approved on March 6, 2007 and is the first of a new class of antihypertensive drugs to enter the market in more than a decade.[3d] Based on the positive results exhibited by Tekturna, interest in renin as a viable drug target for the control of hypertension has intensified.[4] Herein, we report our recent efforts in the discovery of novel small molecule renin inhibitors for the treatment of hypertension.

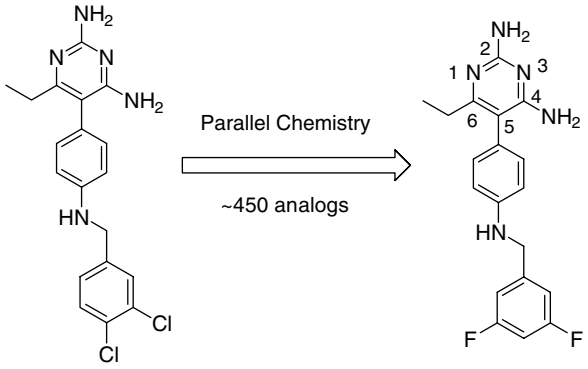
We have described our efforts to develop non-peptidomimetic renin inhibitors based on **1**, a *trans*,*trans*-4-arylpiperidine based scaffold (Scheme 1).[5] These efforts focused on replacing the piperidine C4 chiral center with a nitrogen atom to achieve novel chemical matter, and culminated in **2**.[6] Although **2** lowered blood pressure in the double transgenic mouse when dosed orally,[6a] the ketopiperazine series suffered from cytochrome P450 3A4 inhibition and exhibited a poor PK profile.

Recently, we identified **3** (IC_{50} = 27,000 nM, MW = 387 Da) as a novel small molecule lead from a high throughput screen (HTS) (Scheme 2). In the absence of a crystal structure, parallel chemistry utilizing a reductive amination reaction between aldehydes and 6-ethyl-5-(4′-amino-phenyl)-pyrimidine-2,4-diamine[7] (450 compounds) was conducted to further optimize the template. This exercise led to the identification of **4** (IC_{50} = 4000 nM).

Compound **4** was 7-fold more potent than **3** and had a molecular weight 32 Da less (MW = 355 Da) than the original HTS lead compound. The crystal structure of **4** bound within the active site of renin (2.2 Å, pdb code: 2IKO) revealed that the protein exists in the 'flap-closed' form (Fig. 1). Based on X-ray structure analysis, the 2,4-diaminopyrimidine head group forms a hydrogen bond network with various residues of renin. The 4-amino group can interact with THR-77 and SER-76. The 2-amino group can form bifurcated hydrogen bonds with the side chain of ASP-215 and ASP-32. The pyrimidine ring nitrogen at positions 1 and 3 can utilize interactions with ASP-32 and a water solvent molecule, respectively. The 6-ethyl substituent on the pyrimidine ring extends into the S1 pocket, while the 5-(4-(3,5-difluorobenzylamino)phenyl) moiety occupies a portion of the large S3 pocket and extends into the S4 pocket, toward solvent.

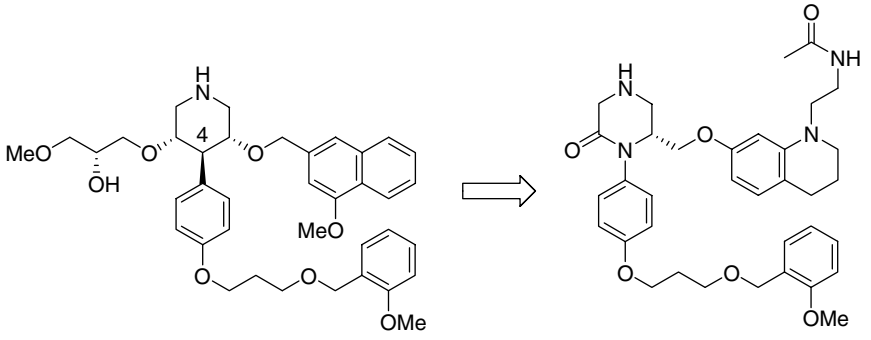
Since the 2,4-diaminopyrimidine moiety can form such an effective network of hydrogen bonds to the renin protein, it was regarded as the 'recognition unit' for binding and was not further manipulated. In order to modify the 5-(4-(3,5-difluorobenzylamino)phenyl) structure to potentially create more active analogs, the binding modes of **2** and **4** to the renin protein were compared (Fig. 2). The conformations of **2** (determined by docking)[8] and **4** (determined by X-ray crystallography) were overlapped. This overlap analysis revealed an opportunity to further modify the 5-(4-(3,5-difluorobenzylamino)phenyl) section of the pyrimidine analog (Fig. 2).

In the ketopiperazine series, sub-nanomolar potency had been achieved by extending a H-bond acceptor moiety (methoxy ether or amide carbonyl) into the S3 subpocket ($S3^{sp}$).[6f] Conformational overlap analysis revealed that replacing the 5-(4-(3,5-difluorobenzylamino)phenyl) section of **4** with either an *N*-3-methoxypropyl-1,2,3,4-tetrahydro-quinoline ring or an *N*-ethyl acetamide-1,2,3,4-tetrahydro-quinoline ring, the proper vector orientation to place the side chains into the $S3^{sp}$



3, IC_{50} = 27,000 nM **4**, IC_{50} = 4,000 nM

Scheme 2. Optimization of **3** by parallel chemistry produced **4**. Numbering of the ring in **4** is provided for clarity.



1, IC_{50} = 0.067 nM **2**, IC_{50} = 0.50 nM
CYP3A4 (BFC), IC_{50} = 82 nM

Scheme 1. Design of ketopiperazine framework based on a 4-arylpiperidine scaffold.

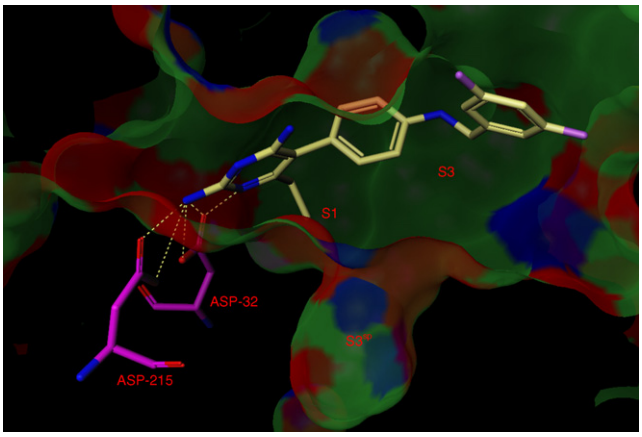


Figure 1. X-ray co-crystal structure of **4** (yellow) bound within the active site of the renin protein. ASP-32 and 215 of the renin protein are shown forming a potential network of hydrogen bonds with the 2,4-diaminopyrimidine moiety of **4**. The protein surface has been colored by atom type (red, O; blue, N; green, C).

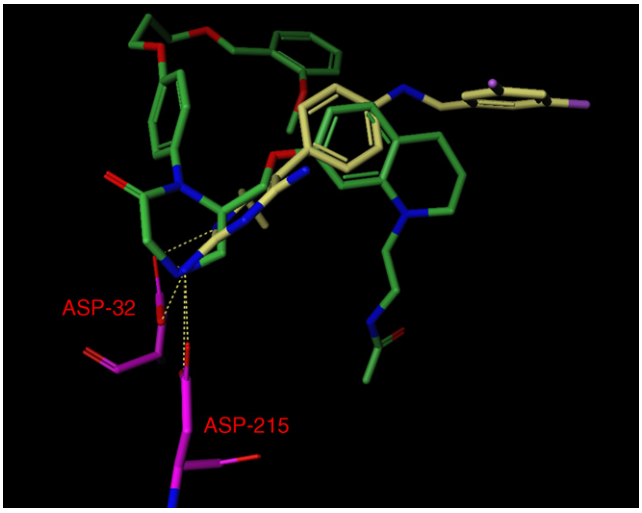


Figure 2. Conformation overlap of **4** (yellow) and **2** (green), bound in the renin active site, as determined by X-ray crystallography (**4**) and docking (**2**). ASP-32 and 215 of the renin protein are shown forming a potential network of hydrogen bonds with the 2,4-diaminopyrimidine moiety of **4** and ketopiperazine ring of **2**. The protein has been removed for clarity.

could be achieved. Based on ease of synthesis, we chose to first synthesize the *N*-3-methoxypropyl-1,2,3,4-tetrahydro-quinoline analog. This modification would result in a new template represented by compound **5** (Scheme 3).

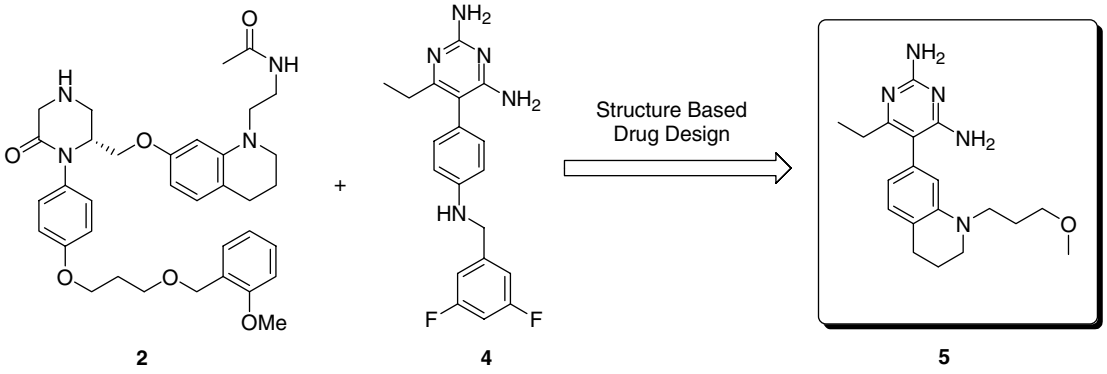
The retrosynthetic analysis of **5** is outlined in Scheme 4. It was envisioned that **5** could be constructed in a highly convergent manner by employing an appropriately functionalized boronate ester (**7**) and 5-bromo-6-ethyl-2,4-diaminopyrimidine[9] (**6**) via a Suzuki reaction (Scheme 4).

The synthesis of boronate ester **7** commenced with the alkylation of 7-benzyloxy-1,2,3,4-tetrahydroquinoline **8** with 3-bromo-methoxypropane in 96% yield (Scheme 5). Next, de-benzylation of **9** was accomplished using trifluoroacetic acid at reflux, to provide the phenol **10** in high yield. The resultant phenol was exposed to triflic anhydride at −78 °C for 10 min to afford triflate **11**. Finally, palladium catalyzed borylation of **11** using bis(pinacolatodiboron) provided **7**.

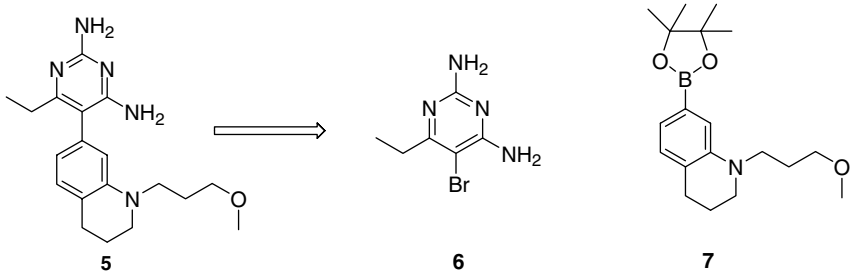
Compound **5** was obtained by reacting **6** and **7** in the presence of tetrakis(triphenylphosphine) palladium (0) and sodium carbonate in a de-gassed solvent system comprising of water/ethanol/toluene (1:1:1).

We were delighted to find that **5** (IC_{50} = 650 nM) exhibited a 6-fold increase in potency against renin, as compared to **4**, while remaining 'Rule-of-five' compliant.[10] X-ray crystallography (2.2 Å, pdb code: 2G21) revealed that **5** bound in the renin active site, as anticipated, in the flap closed form, with the 3-methoxypropyl side-chain extending into the $S3^{sp}$ and potentially making a hydrogen bond interaction with the backbone of TYR-14 (Fig. 3).

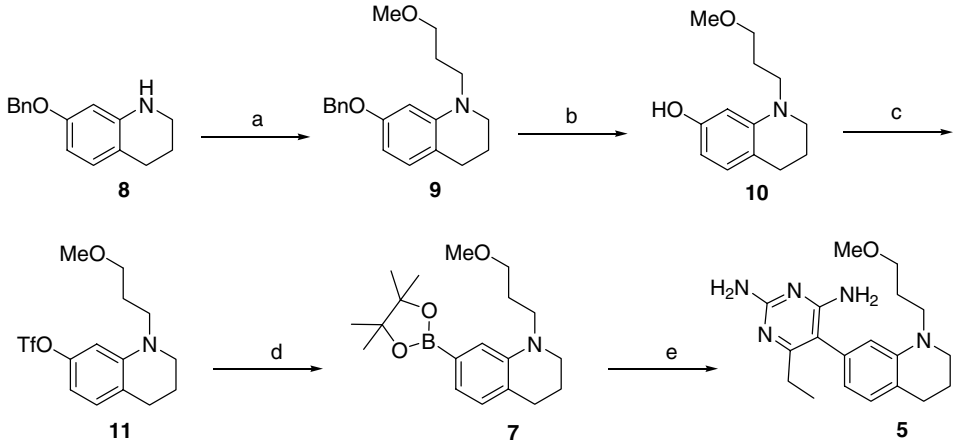
Compound **5** exhibited low molecular weight (341 Da), excellent solubility (59.3 μg/mL), permeability (26.5×10^{-6} cm/s), low *c*log*P* (3.34), and low Cytochrome P450 3A4 inhibition (IC_{50} = 2.8 μM). Furthermore, compound **5** displayed moderate in-vivo elimination half-life (1.11 h), systemic plasma clearance (36.8 mL/min/kg), and volume of distribution



Scheme 3. Scaffold hopping of lead structure **4** to the tetrahydroquinoline framework **5** via structure based drug design and conformational overlap analysis.



Scheme 4. Retrosynthetic approach to compound **5**.



Scheme 5. Reactions and conditions for synthesis of boronate ester **7**. (a) 3-bromo-methoxy propane (2 equiv), sodium carbonate (2 equiv), potassium iodide (0.15 equiv), acetonitrile, reflux, 74 h, 96%; (b) trifluoroacetic acid, reflux, 1 h, 99%; (c) triflic anhydride (1.5 equiv), triethylamine (8 equiv), −78 °C, 10 min, 74%; (d) bis(pinacolatodiboron) (1.1 equiv), potassium acetate (3 equiv), 1,1′-bis(diphenylphosphino)ferrocine palladium (II) chloride (0.2 equiv), dioxane, reflux, 18 h, 90%; (e) 5-bromo-6-ethyl pyrimidine 2,4-diamine (**6**)[8] (1 equiv), $Pd(PPh_3)_4$ (0.10 equiv), Na_2CO_3 (3 equiv), toluene/ethanol/water (1:1:1), reflux 18 h, 32%.

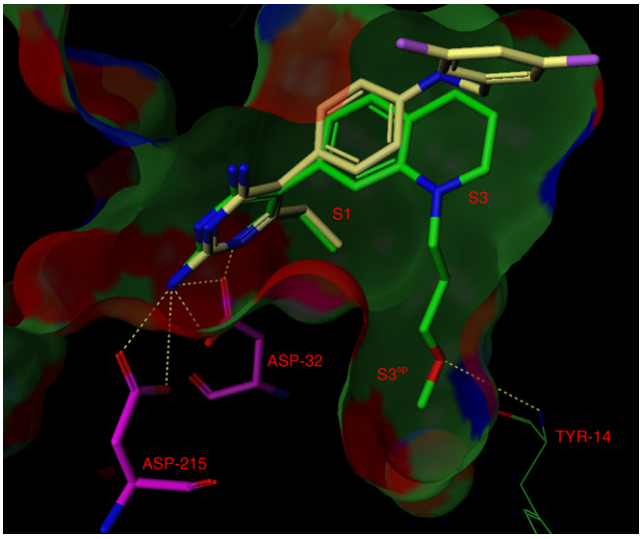


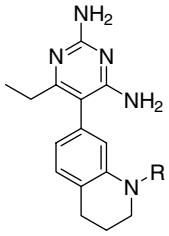
Figure 3. X-ray co-crystal structures of **5** (green) and **4** (yellow), bound in the active site of renin. The 3-methoxypropyl side chain of **5** penetrates into the $S3^{sp}$ of renin. TYR-14 of the renin protein can form a hydrogen bond with the oxygen of the side chain of **5**. ASP-32 and 215 of the renin protein are shown forming a potential network of hydrogen bonds with the 2,4-diaminopyrimidine moiety of **5** and **4**. The protein surface has been colored by atom type (red, O; blue, N; green, C).

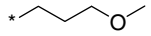
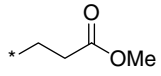
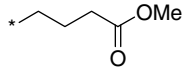
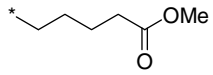
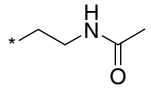
(1.6 L/kg), but exhibited low oral bioavailability (<10%) in Sprague–Dawley rats. Overall, **5** represented a novel, non-peptidic small molecule renin inhibitor and served as an excellent starting point for further investigation.

Next, the introduction of other side chains containing hydrogen bond acceptors that could possibly penetrate into the $S3^{sp}$ was evaluated (Table 1. Compounds **12**–**15**).

Compounds that contained ester side chains (**12**, **13**, **14**) were installed to determine optimal hydrogen bond acceptor location. The activity of the compounds increased as the side-chain length increased. We chose to limit the length of the side chain to five carbons (**14**, IC_{50}: 91 nM), in order to keep the molecular weight low. Compound **14** was 7-fold more active than **5**, with only a 42 mass unit (dalton) increase. Since **12**, **13**, and **14** had metabolic liabilities (Human Liver Microsomal (HLM) half-life of **12**, **13**, **14** = 5 min), **15** was designed. The side chain of **15** contains a hydrogen bond donor and acceptor and was a key side chain in the ketopiperazine series (**2**).[6f] Compound **15** demonstrated an increase in potency (3.5-fold) relative to **5** with a molecular weight of 354 Da and was stable in HLM up to 40 min.[11] Compounds **5**, **12**–**15** were also devoid

Table 1. Brief SAR of compound **5**



Entry no.	R	IC_{50} (nM)	MW	CYP3A4 (BFC) IC_{50} (nM)	*c*log *P*
5		650	341	2800	3.48
12		198	355	10,000	3.33
13		120	369	Not determined	3.65
14		91	383	12,000	3.80
15		178	354	8000	2.36

* point of attachment.

of activity against the aspartic peptidases Cathepsin D, Cathepsin E, and Pepsin (0–5% inhibition at 100 μM).

The identification of non-peptidic small molecule renin inhibitors has historically been a major challenge. Through high throughput screening, parallel synthesis, X-ray crystallography, and structure based drug design we have developed the first non-chiral, non-peptidic, small molecular template to possess moderate potency against renin while remaining 'Rule-of-five' compliant.[9] These novel compounds bind to renin in the 'flap closed' conformation. The 6-ethyl-2,4-diaminopyrimidine moiety serves as the recognition unit by interacting with the catalytic Aspartic acids 32 and 215 within the active site of renin. Further, the compounds take advantage of the $S3^{sp}$ of renin for added potency.[6,12] Future efforts are to optimize potency and pharmacokinetic/dynamic parameters with this novel scaffold.

Acknowledgments

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