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Discovery of novel non-peptidic ketopiperazine-based renin inhibitors

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Abstract—Ketopiperazine **2** was designed from a previously published analog. Compound **2** was shown to be a novel, potent inhibitor of renin that, when administered orally, lowered blood pressure in a hypertensive double transgenic (human renin and angiotensinogen) mouse model. Compound **2** was further optimized to sub-nanomolar potency by designing an analog that addressed the S3 sub-pocket of the renin enzyme (**16**).
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1. Introduction

Hypertension (high blood pressure) in humans is defined as a systolic pressure of 140 mmHg or higher, or diastolic pressure of 90 mmHg or higher or both. Pre-hypertensive is defined as having a systolic pressure of 120–139 mmHg or diastolic pressure of 80–90 mmHg or both. Approximately 1 in 4 adults in the United States suffer from hypertension and 45 million suffer pre-hypertension conditions. Interestingly, of the individuals who have hypertension, 30% are not aware they are hypertensive, 25% are on medication but their blood pressure (BP) is not controlled, 34% are taking medication and their BP is under control, and 11% are not taking medication.[1] Hypertension has been shown to precede the development of congestive heart failure in 91% of cases.[2] Further, hypertension is a major risk factor for other cardiovascular diseases such as stroke, myocardial infarction, and is the leading cause of death in the Western World.

The renin angiotensin system (RAS) is well established as an endocrine system involved in BP regulation and fluid electrolyte balance (Scheme 1).[3] The RAS system is stimulated by a number of signals, including a drop in BP, a decrease in circulating volume, or a reduction in plasma sodium concentration. These signals work to stimulate the release of renin, an enzyme synthesized in the juxtaglomerular apparatus of the kidney. Renin cleaves angiotensinogen (a hepatic α-2 globular protein) to form the hemodynamically inactive angiotensin I (AI). Angiotensin converting enzyme (ACE), a monomeric zinc metalloenzyme found in the vascular endothelium, then converts this pro-hormone to angiotensin II (AII), the final active messenger in the RAS pathway. AII inhibits renin secretion by acting directly on the juxtaglomerular cells, forming the basis of a short loop negative feedback mechanism. AII has a number of physiological effects, most importantly as a powerful vasoconstrictor, increasing BP by altering peripheral vascular resistance.

Since angiotensinogen is the only known substrate for renin and cleavage of angiotensinogen by renin is the rate determining step in the renin–angiotensin endocrine system, it is of general consensus that inhibition of renin would be an attractive strategy for the control of hypertension. Furthermore, renin inhibitors would prevent the formation of AI and AII, and, therefore, may act differently from angiotensin receptor blockers (ARB's) and angiotensin converting enzyme (ACE) inhibitors,

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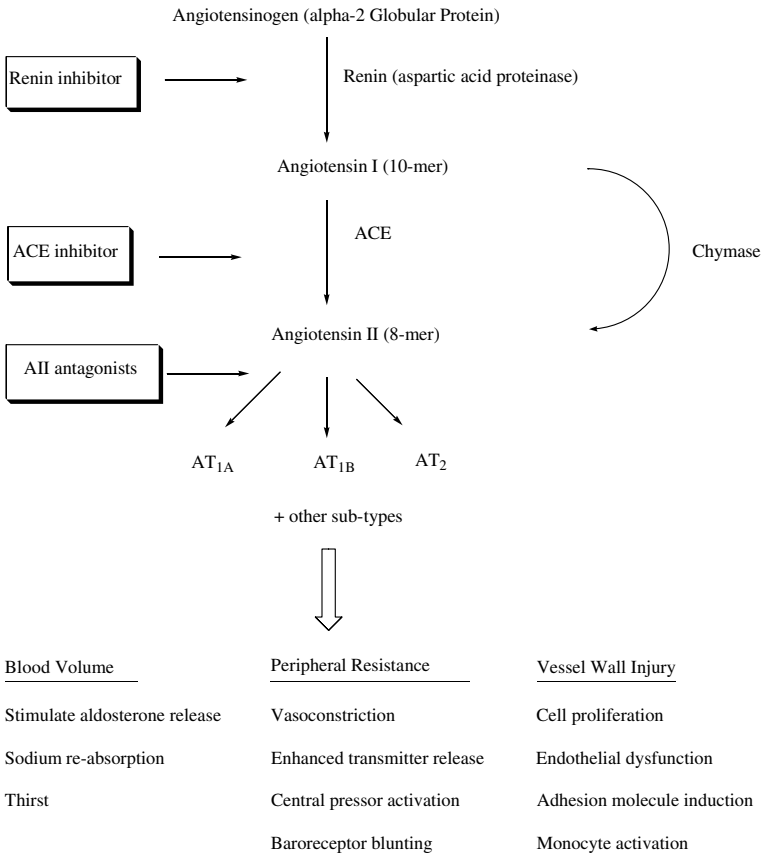


Scheme 1. The Renin Angiotensin System (RAS). Renin is the rate limiting step in the RAS cascade.

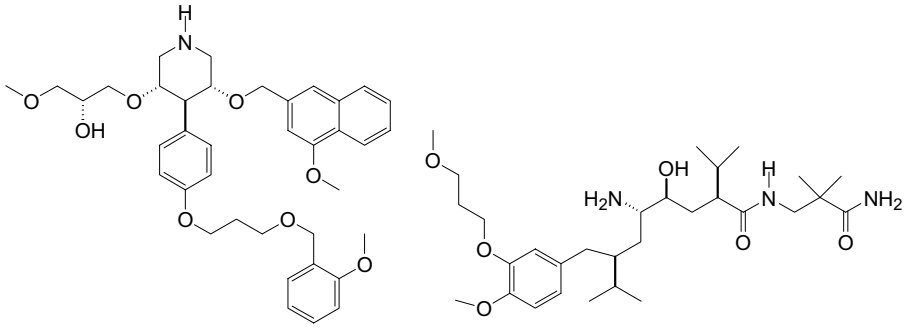


Figure 1. Renin inhibitors by Roche (A) and Novartis (B).

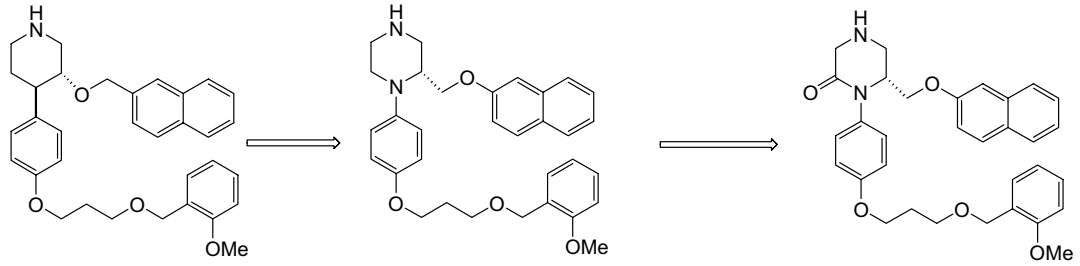
which increase AI levels but do not block ACE-independent AII production. It has been suggested that renin inhibitors may provide better kidney and heart protection than ARB and ACE inhibitors.[4]

Inhibitors of renin were pursued by many companies in the 1980s and 1990s. However, the early inhibitors were peptide based, synthetically challenging, and exhibited poor pharmacokinetic properties such as low oral bioavailability and high clearance, which limited the clinical utility of these agents. Most importantly, however, studies in humans did show that intravenously administered renin inhibitors lowered BP at least as effectively as ACE inhibitors.[5] Therefore, renin remains a viable target, however, compounds with improved properties are needed for this approach to be useful in the clinic.

Recently, Roche[6] and Novartis[7] (Fig. 1) have disclosed non-peptidic renin inhibitors. Although only limited data is available about the Roche series, Novartis's renin inhibitor, Aliskiren, entered phase III clinical trials in March 2004.[4,8]

2. Chemistry

Recently, we have developed a new class of potent non-peptidic renin inhibitors for the treatment of hyperten-

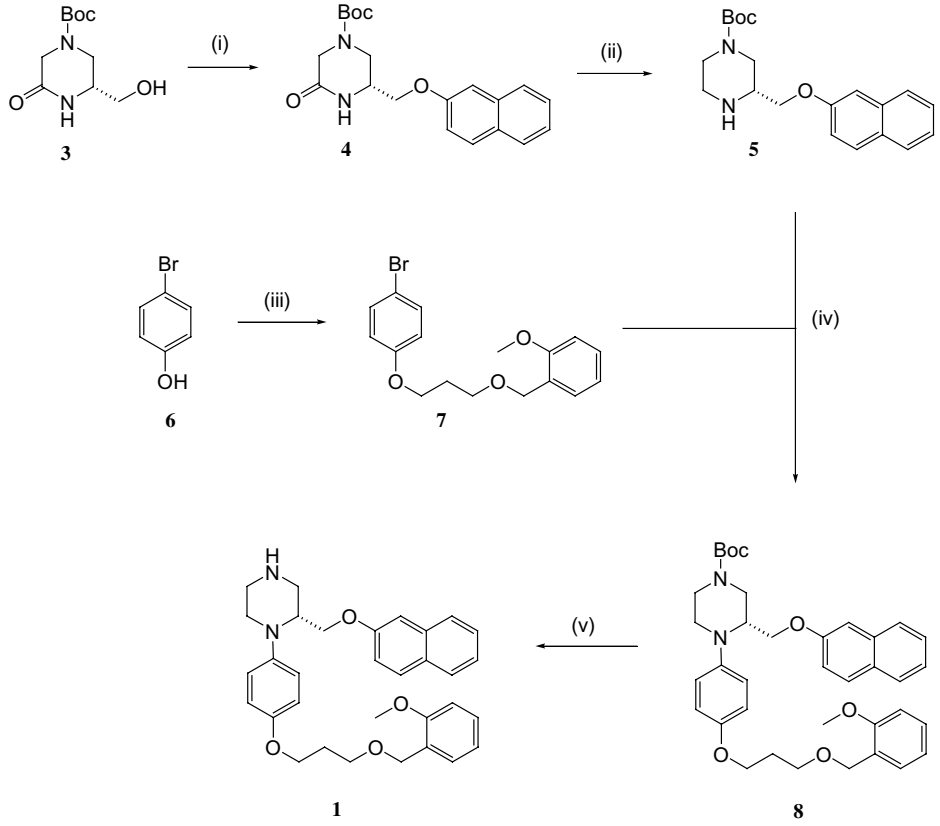


Scheme 2. Evolution of a new class of renin inhibitors based on piperidine template. Ketopiperazine **2** was best in series due to stability and ease of synthesis. Compound **2** was taken further into the optimization process.

sion.[9,10] A previously reported compound[6b,d] (**C**), an analog of **A**, served as the starting point of our efforts (Scheme 2). In an attempt to reduce the length and complexity of the synthesis of the chiral 3,4-disubstituted piperidine (**C**) scaffold, we proposed to prepare compound **1**, which eliminated a chiral center. To avoid synthesis of hemi-aminals, the naphthylmethoxy substituent of **C** was replaced with a naphthyloxy-methylene substituent in **1** (Scheme 2). The synthetic route to produce **1** is outlined in Scheme 3.

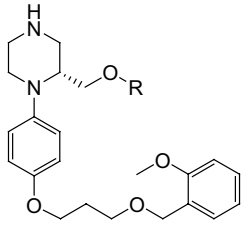
Treatment of the chiral ketopiperazine alcohol **3**[9] with 2-naphthol in the presence of DIAD and polymer-supported PPh_3 provided the desired ether **4** in 49% yield. The yields of the Mitsunobu reaction proved to be variable with increasing scale, and required the slow addition of DIAD to the chilled reaction mixture. Reduction of the ketopiperazine carbonyl was accomplished by treatment of **4** with $BH_3{\cdot}SMe_2$ to afford piperazine **5** in good yield. Aryl bromide **7** and piperazine **5** were coupled in the presence of catalytic $Pd(t\text{-}Bu_3P)_2$ and KO*t*-Bu to yield the protected aryl piperazine **8**. The N-Boc group was removed by treatment with anhydrous HCl generated in situ through the addition of acetyl chloride to anhydrous MeOH to give compound **1** in good yield.

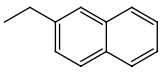
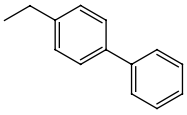
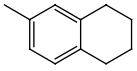
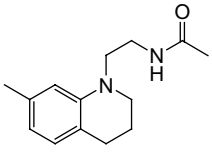
Compound **1** had modest potency against renin (IC_{50}: 180 nM), and represented a reasonable starting point for the development of more potent analogs. Table 1



Scheme 3. Synthetic route to compound **1**. Reagents and conditions: (i) 2-naphthol, DIAD, PS–PPh_3, CH_2Cl_2, 0–25 °C, 49%; (ii) $BH_3{\cdot}SMe_2$, THF, 50 °C, 77%; (iii) 1-(3-iodopropoxymethyl)-2-methoxybenzene, K_2CO_3, CH_3CN, reflux, 71%; (iv) $Pd(t\text{-}Bu_3P)_2$, KO*t*-Bu, toluene, 60 °C, 35%; (v) AcCl, MeOH, 0–25 °C, 89%.

Table 1. SAR of piperazine series based on compound **1**[9a]



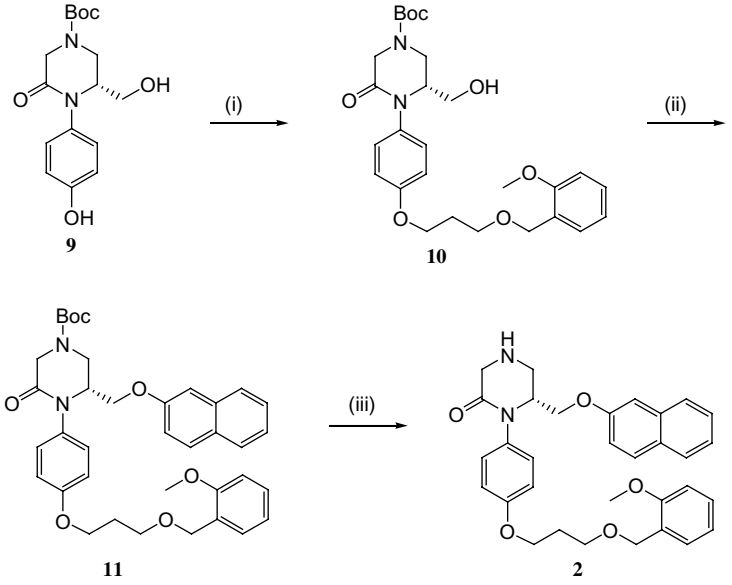
Compound ID	R Side chain	IC_{50} (nM)
12		1700
13		1020
14		440
15		0.50

Compound **15**, an analog designed to penetrate the S3 sub-pocket of renin achieved sub-nanomolar potency.

depicts some SAR within the piperazine series. Installation of a 2-naphthyloxy methylene unit or methoxy biphenyl group (compounds **12** and **13**, respectively), resulted in a drastic decrease in potency. Installation of the 6-methyl- 1,2,3,4-tetrahydro-naphthalene ring (compound **14**) also lowered potency approximately 2-fold. An *N*-[2–(7-methyl-3,4-dihydro-2H-quinolin-1-yl)-ethyl]-acetamide side chain has demonstrated an increase in potency within the piperidine series.[6e] Based on X-ray results with the piperidine series, the acetamide moiety penetrates the S3 sub-pocket of renin, providing the increased affinity. We found this to also translate to the piperazine series (compound **15**), where sub-nanomolar potency was achieved.

Compound **1** and related analogs (Table 1) were found to exhibit a degree of instability over time, possibly as a consequence of air oxidation of the electron rich phenyl ring. In addition, the synthesis was still lengthy, and the Buchwald coupling was problematic on a large scale. Therefore, compound **2** was designed (Scheme 2). It was envisioned that the ketopiperazine template would still possess the basic nitrogen needed to interact with the catalytic aspartic acids within the renin active site,[6d] yet the lone pair of the second nitrogen atom would be delocalized through the carbonyl group. This arrangement was hypothesized to reduce the electron density on the *para*-substituted phenyl ring, and would, therefore, impart additional stability.

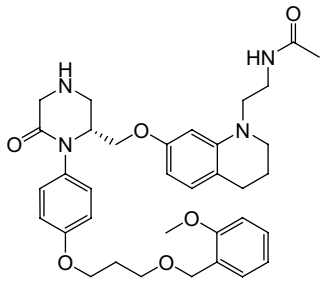
The synthesis of compound **2** began with intermediate **9** (Scheme 4).[11] Alkylation of the phenol with 1-(3-iodopropoxymethyl)-2-methoxybenzene[9b] in the presence of K_2CO_3 provided **10** in excellent yield. Mitsunobu coupling of **10** with 2-naphthol in the presence of DIAD and polymer supported PPh_3 afforded the desired ether **11** in 81% yield. In contrast to our experience with the Mitsunobu coupling of **3**, the reaction between alcohol **10** and 2-naphthol was quite robust, scalable, and high-yielding, indicating that the acidity of the amide NH in **3** played a large role in the non-linear reaction scale variability and lower yields of **4** (Scheme 3). Deprotection of the N-Boc protecting group was again accomplished with in situ generated HCl in cold MeOH



Scheme 4. Synthetic route to compound **2**. Reagents and conditions: (i) 1-(3-iodopropoxymethyl)-2-methoxybenzene, K_2CO_3, CH_3CN, reflux, 89%; (ii) 2-naphthol, DIAD, PS–PPh_3, CH_2Cl_2, 0–25 °C, 81%; (iii) AcCl, MeOH, 0–25 °C, 78%.

to provide compound **2** in good yield. Compound **2** was found to be 3-fold more active against renin in vitro than compound **1** (Scheme 2), as well as more stable and amenable to large scale synthesis.[11] Therefore, compound **2** was chosen as a new framework for the creation of more potent non-peptidic renin inhibitors.

Insertion of the R side chain of compound **15** into the ketopiperazine core, formed compound **16**,[9b] a very potent inhibitor of renin. Based on these data, compounds that possess R side chains designed to address the S3 sub-pocket of renin will be the focus of future work.



Compound **16**, IC_{50}: 0.30 nM

3. In vivo activity

The next crucial step was to determine if whether compound **2** or compound **H** lowered blood pressure in an experimental, whole animal model of renin-dependent hypertension. In this case, compound **2** was evaluated in the in vivo study. Double transgenic mice (h-Ang 204/1 h-Ren 9) that constitutively express both human renin and its substrate angiotensinogen were equipped with telemetry implants for 24 h blood pressure measurements. The mice have mean arterial blood pressures (MABP) that average 155 mmHg, ≈45 mmHg higher than in mice lacking the human genes. To establish a blood pressure control, mice were given vehicle (3% DMA, 97% SBE-CD (40% w/v) in 50 mM lactic acid (10 mL/kg) on two consecutive days before dosing compound **2**. Blood pressures obtained at 15 min intervals from the two vehicle treatment days were then averaged to establish a baseline at each time point. The compound was given by oral gavage (30 mg/kg) on day 3. The change in MABP at each 15 min time point was obtained by subtracting the MABP from the mice treated with compound **2** from the baseline MABP. Compound **2** lowered blood pressure by a maximum of 20 mmHg after 1 h post dose (results not shown). Blood pressure reached baseline levels at 3 h post dose. The observed blood pressure reduction suggests the template may have the potential to be developed into a novel antihypertensive agent.

4. Conclusion

From a previously reported template, a novel non-peptidic ketopiperazine-based renin inhibitor has been created. The ketopiperazine framework (i.e., compound **2**) displays good affinity toward the renin enzyme and lowered blood pressure in the double transgenic (human renin and angiotensinogen) mouse when dosed orally. Further efforts toward the discovery of optimized compounds based on this template will be reported in due course.

5. Experimental

5.1. General

All chemicals, reagents, and solvents were purchased from commercial sources (e.g., Aldrich Chemical Co., Inc., Milwaukee, WI; Mallinckrodt Baker, Inc., Paris, KY, etc.) were available and used without further purification. ^{1}H NMR spectra were obtained on a Varian Unity 400 MHz spectrometer. Elemental analyses were determined on a Perkin–Elmer model 240C instrument. Mass spectral data were obtained on a VG Analytical 7070 E/HF mass spectrometer. Flash column chromatography was performed on silica gel 60, 230–400 mesh, purchased from Mallinckrodt.

5.2. Chemistry

Experimentals for compounds **12**, **13**, **14**, **15**, and **16** are listed in Ref. 9b.

5.2.1. (3*R*)-3-(Naphthalen-2-yloxymethyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (4). An oven-dried, 25 mL round bottom flask was cooled under a N_2 stream and charged with (3*R*)-3-hydroxymethyl-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (100 mg, 0.434 mmol), 2-naphthol (94 mg, 0.65 mmol), and 5 mL of anhydrous dichloromethane. Polymer-supported triphenylphosphine (383 mg, 0.521 mmol, 1.36 mmol/g loading) was added, and the resulting slurry was stirred at rt for 20 min. The mixture was cooled in an ice bath, and diisopropyl azodicarboxylate (141 mg, 0.695 mmol) was added dropwise via a syringe. The yellow slurry was stirred overnight, allowing to warm to rt. The resin was collected on a medium frit and rinsed with dichloromethane (3 × 4 mL). The combined filtrates were washed with aqueous 1 N HCl and 10% NaOH, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (gradient: 60% ethyl acetate/hexanes to 100% ethyl acetate) afforded (3*R*)-3-(naphthalen-2-yloxymethyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (76.3 mg, 49%) as a clear viscous oil that exists as a mixture of carbamate rotamers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39–1.50 (m, 9H), 3.53 (m, 1H), 3.85–4.00 (m, 3H), 4.07–4.20 (m, 3H), 6.60 (s, 1H), 7.11 (m, 2H), 7.36 (dd, J = 7.4, 7.4 Hz, 1H), 7.45 (dd, J = 7.4, 7.4 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H) 7.76 (d, J = 7.6 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H).

5.2.2. (3*R*)-3-(Naphthalen-2-yloxymethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (5). (3*R*)-3-(Naphthalen-2-yloxymethyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (1.55 g, 4.34 mmol) was dissolved in 40 mL of anhydrous tetrahydrofuran under a N_2 atmosphere. A 2.0 M solution of borane dimethyl sulfide complex

in tetrahydrofuran (6.5 mL, 13.0 mmol) was added via syringe, and the reaction mixture was heated in a 50 °C oil bath for 2 h. After cooling to rt, excess hydride was quenched by the careful addition of methanol. The mixture was concentrated under reduced pressure, and then re-dissolved in 75 mL of methanol. An aqueous solution of 10% potassium sodium tartrate (50 mL) was added, and the resulting slurry was heated at reflux for 2 h and then stirred at rt for 18 h. The mixture was concentrated under reduced pressure and partitioned between water and ethyl acetate (3×). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (65/33/2 ethyl acetate/hexanes/methanol then 70/28/2 ethyl acetate/hexanes/methanol) provided (3*R*)-3-(naphthalen-2-yloxymethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (1.149 g, 77%) as a clear glassy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.82 (m, 2H), 2.94 (m, 1H), 3.02 (m, 1H), 3.15 (m, 1H), 3.97 (dd, J = 9.1, 7.7 Hz, 1H), 3.98 (br s, 2H), 4.07 (dd, J = 9.2, 4.1 Hz, 1H), 7.14 (m, 2H), 7.34 (dd, J = 6.9, 6.9 Hz, 1H), 7.44 (ddd, J = 8.0, 6.8, 1.2 Hz, 1H), 7.72 (d, J = 7.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H). HRMS calcd for $[C_{20}H_{26}N_2O_3 + 1]$ 343.2. Found: 343.2 (M+1).

5.2.3. 1-Bromo-4-(3-(2-methoxybenzyloxy)-propyloxy)-benzene (7). A 100 mL round bottom flask was charged with 4-bromophenol (1.87 g, 10.78 mmol), 1-(3-iodopropoxymethyl)-2-methoxybenzene (3.00 g, 9.80 mmol), acetonitrile (20 mL), and K_2CO_3 (1.63 g, 11.8 mmol). The resulting slurry was heated to reflux for 18 h. After cooling to rt, the mixture was filtered through a Celite pad, which was rinsed with additional acetonitrile. The combined filtrates were concentrated under reduced pressure. The residue was dissolved in ethyl acetate, and washed with aqueous 10% NaOH and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (gradient: 5% ethyl acetate/hexanes to 10% ethyl acetate/hexanes) afforded 1-bromo-4-(3-(2-methoxybenzyloxy)-propyloxy)benzene (2.45 g, 71%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 9.0 Hz, 2H), 7.29–7.25 (m, 2H), 6.94 (t, J = 7.4 Hz, 1H), 6.86 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 9.0 Hz, 2H), 4.57 (s, 2H), 4.07 (t, J = 6.2 Hz, 2H), 3.81 (s, 3H), 3.70 (t, J = 6.1 Hz, 2H), 2.10 (quintet, J = 6.1 Hz, 2H). MS calcd for $[C_{17}H_{19}BrO_3 + 1]$: 351.1 (^{79}Br), 353.0 (^{81}Br). Found 351.1 (^{79}Br), 353.0 (^{81}Br).

5.2.4. (3*R*)-4-{4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl}-3-(naphthalen-2-yloxymethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (8). A 50 mL round bottom flask was charged with 1-bromo-4-(3-(2-methoxybenzyloxy)-propyloxy)benzene (1.72 g, 4.88 mmol) and (3*R*)-3-(naphthalen-2-yloxymethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (836 mg, 2.44 mmol). The mixture was dissolved in 10 mL of anhydrous toluene and concentrated under reduced pressure to remove residual water. After the flask was equipped with a magnetic stir bar and a reflux condenser, the flask was purged and back-filled with N_2 twice. Palladium bis(tri-*tert*-butylphosphine) (125 mg, 0.24 mmol), and KO*t*-Bu (301 mg, 2.69 mmol) were added, and the flask was again purged and back-filled with N_2. Anhydrous tetrahydrofuran (10 mL) was added, and the resulting orange slurry was heated in a 75 °C oil bath for 18 h. After cooling to rt, the mixture was diluted with diethyl ether and filtered through a Celite pad, rinsing with diethyl ether. The combined filtrates were concentrated under reduced pressure and purified by flash column chromatography (gradient: 10% ethyl acetate/hexanes gradient to 20% ethyl acetate/hexanes, then 70/28/2 ethyl acetate/hexanes/methanol) to give (3*R*)-4-4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl-3-(naphthalen-2-yloxymethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (530 mg, 35%) as a yellow oil that exists as a mixture of carbamate rotamers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 4H), 1.27 (s, 5H), 2.07 (quintet, J = 5.9 Hz, 2H), 3.16 (br s, 2H), 3.24 (br s, 1H), 3.47 (br s, 1H), 3.69 (t, J = 6.1 Hz, 2H), 3.77 (s, 3H), 3.90 (m, 3H), 4.06 (t, J = 6.1 Hz, 2H), 4.06 (m, 1H), 4.23 (m, 1H), 4.55 (s, 2H) 6.82 (d, J = 8.3 Hz, 1H), 6.87 (m, 2H), 6.91 (d, J = 7.6 Hz, 1H), 6.94 (m, 3H), 7.06 (dd, J = 9.0, 1.8 Hz, 1H), 7.23 (ddd, J = 7.6, 7.6, 1.5 Hz, 1H), 7.31 (dd, J = 7.3, 7.3 Hz, 1H), 7.34 (d, J = 7.3 Hz, 1H), 7.39 (dd, J = 7.8, 7.8 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 8.3 Hz, 1H). MS: *m*/*z* 613.4 (M+1).

5.2.5. (2*R*)-1-{4-[3-(2-Methoxybenzyloxy)-propoxy]-phenyl}-2-(naphthalen-2-yloxymethyl)-piperazine (1). (3*R*)-4-4-[3-(2-Methoxybenzyloxy)-propoxy]-phenyl-3-(naphthalen-2-yloxymethyl)-piperazine-1-carboxylic acid *tert*-butyl ester (530 mg, 0.864 mmol) was suspended in 15 mL of anhydrous methanol under a N_2 atmosphere, and cooled in an ice bath. Acetyl chloride (0.62 mL, 8.64 mmol) was added dropwise over a 2 min interval. The N_2 inlet needle was removed and the resulting green solution was stirred for 12 h, allowing the ice bath to warm to room temperature. Excess acid was quenched by the addition of aqueous saturated $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (95/5 dichloromethane/methanol then 90/10 dichloromethane/methanol) provided (2*R*)-1-4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl-2-(naphthalen-2-yloxymethyl)-piperazine (393 mg, 89%) as a brown viscous oil. $[\alpha]_D$ +89.4 (*c* 1.7, CH_2Cl_2); IR (ATR/diamond) 2950, 2833, 1508, 1461, 1240, 1216, 1180, 1087, 1028, 816 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (br s, 1H), 2.08 (quintet, J = 6.2 Hz, 2H), 3.02 (q, J = 6.6 Hz, 1H), 3.12 (m, 3H), 3.26 (m, 2H), 3.69 (t, J = 6.1 Hz, 2H), 3.78 (s, 3H), 3.85 (dddd, J = 7.3, 3.7, 3.7, 3.7 Hz, 1H), 3.97 (dd, J = 9.0, 3.4 Hz, 1H), 4.06 (t, J = 6.2 Hz, 2H), 4.29 (t, J = 9.0 Hz, 1H), 4.56 (s, 2H), 6.82 (d, J = 8.1 Hz, 1H), 6.86 (m, 2H), 6.91 (dd, J = 7.4, 7.4 Hz, 1H), 6.98 (m, 3H) 7.06 (dd, J = 8.9, 2.6 Hz, 1H), 7.23 (ddd, J = 7.8, 7.8, 1.7 Hz, 1H), 7.30 (ddd, J = 7.3, 6.8, 1.2 Hz, 1H), 7.35 (dd, J = 7.4, 1.6 Hz, 1H), 7.39 (ddd, J = 8.3, 7.1, 1.2 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 9.0 Hz, 1H), 7.73 (d,

J = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 156.7, 154.3, 143.9, 134.6, 129.5, 129.2, 129.0, 128.8, 127.8, 156.9, 126.5, 123.8, 120.6, 120.4, 119.0, 115.6, 110.4, 107.0, 68.0, 67.3, 65.5, 64.4, 56.3, 55.5, 48.1, 47.4, 45.9, 30.0; HRMS calcd for [$C_{32}H_{36}N_2O_4$ + 1]: 513.2753. Found: 513.2742 (M+1).

5.2.6. (3*R*)-3-Hydroxymethyl-4-(4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (10). A solution of 5.0 g (15.5 mmol) of (3*R*)-3-hydroxymethyl-4-(4-hydroxyphenyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester in 30 mL of acetonitrile was treated with 5.70 g (18.6 mmol) of 1-(3-iodopropoxymethyl)-2-methoxybenzene, and 3.22 g (23.3 mmol) of K_2CO_3. The reaction mixture was heated to reflux for 18 h. The reaction mixture was then cooled to rt, diluted with 100 mL of water, and partitioned with 200 mL of ethyl acetate. The organic layer was washed with brine (2100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (gradient: 50% ethyl acetate/hexanes to 100% ethyl acetate) afforded 6.94 g (89%) of (3*R*)-3-hydroxymethyl-4-(4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester that exists as a mixture of carbamate rotamers. $[\alpha]_D$ −19.6 (*c* 5.1, $CHCl_3$); IR (ATR/diamond) 3338, 2972, 2873, 1687, 1639, 1627, 1512, 1462, 1415, 1245, 1150, 1107, 1024, 835, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (br s, 9H), 2.09 (quintet, J = 6.2 Hz, 2H), 2.88 (m, 1H), 3.41 (m, 2H), 3.68 (t, J = 6.1 Hz, 2H), 3.75 (m, 1H), 3.80 (s, 3H), 3.97 (d, J = 18.6 Hz, 1H), 4.08 (t, J = 6.1 Hz, 2H), 4.43 (m, 1H), 4.55 (s, 2H), 6.85 (d, J = 8.1 Hz, 1H), 6.91 (d, J = 9.0 Hz, 2H), 6.92 (m, 1H), 7.07 (d, J = 8.8 Hz, 2H), 7.25 (ddd, J = 8.3, 8.3, 1.6 Hz, 1H), 7.34 (d, J = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.5 (3), 29.8, 55.5, 65.4, 67.0, 68.0, 105.0, 110.4, 115.5, 116.6, 120.6, 126.8, 128.5, 128.8, 129.1, 133.1, 157.3, 158.6; HRMS calcd for [$C_{27}H_{36}N_2O_7$ + 1]: 501.2601. Found: 501.2608.

5.2.7. (3*R*)-4-(4-[3-(2-Methoxybenzyloxy)-propoxy)-phenyl])-3-(naphthalene-2-yloxymethyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (11). A N_2-flushed, round bottom flask, was charged with (3*R*)-3-hydroxymethyl-4-(4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl)-5- oxopiperazine-1-carboxylic acid *tert*-butyl ester (0.500 g, 0.999 mmol), 2-naphthol (216 mg, 1.498 mmol), and dichloromethane (15 mL). Triphenylphosphine supported on polystyrene (0.975 g, 1.598 mmol, 1.64 mmol/g) was added at rt, and the resulting slurry was stirred for 10 min. The reaction mixture was cooled in an ice bath, and diisopropyl azodicarboxylate (0.236 mL, 1.199 mmol) was added dropwise over 2 min. The orange-red reaction mixture was stirred for 18 h, slowly warming to rt. The reaction mixture was filtered, and the resin rinsed with dichloromethane (3×). The crude product was purified by flash column chromatography (gradient: 20% ethyl acetate/hexanes to 50% ethyl acetate/hexanes) to afford 0.507 g (81%) of (3*R*)-4-(4-[3-(2-methoxybenzyloxy)-propoxy)-phenyl])-3-(naphthalene-2-yloxymethyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester as a white solid that exists as a mixture of carbamate rotamers. $[\alpha]_D$ +43.5 (*c* 2.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19–1.37 (m, 9H), 2.01 (quintet, J = 6.0 Hz, 2H), 3.56 (m, 1H), 3.61 (t, J = 6.0 Hz, 2H), 3.71 (s, 3H), 3.64 (m, 1H), 4.02 (t, J = 6.0 Hz, 2H), 4.08–3.98 (m, 3H), 4.42–4.51 (m, 1H), 4.47 (s, 2H), 4.54 (m, 1H), 6.75 (d, J = 8.2 Hz, 1H), 6.83 (d, J = 7.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 7.00 (dd, J = 8.9, 2.4 Hz, 1H), 7.09 (d, J = 8.7 Hz, 2H), 7.16 (dd, J = 7.6, 7.6 Hz, 1H), 7.26 (m, 2H), 7.35 (dd, J = 7.3, 7.3 Hz, 1H), 7.56 (br s, 1H) 7.64 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H); LRMS calcd for [$C_{37}H_{42}N_2O_7$ + 1]: 627.1. Found 627.1 (M+1).

5.2.8. (6*R*)-1-{4-[3-(2-Methoxybenzyloxy)-propoxy]-phenyl}--6-(naphthalen-2-yloxymethyl)-piperazin-2-one (2). (3*R*)-4-(4-[3-(2-Methoxybenzyloxy)-propoxy)-phenyl])-3-(naphthalene-2-yloxymethyl)-5-oxopiperazine-1-carboxylic acid *tert*-butyl ester (55 mg, 87.7 mmol) was dissolved in methanol (2 mL) under nitrogen and cooled to 0 °C. Acetyl chloride (68.9 mg, 0.877 mmol) was added dropwise. The nitrogen inlet needle was removed, and the reaction mixture allowed to warm to rt over a period of 18 h. The reaction mixture was quenched with aqueous saturated $NaHCO_3$. The mixture was extracted with ethyl acetate (3 × 10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The resultant solid was purified by flash column chromatography (gradient: 100% dichloromethane then 95/5 dichloromethane/methanol to 90/10 dichloromethane/methanol) to afford 36.1 mg (78.1%) of 1-{4-[3-(2-methoxybenzyloxy)-propoxy]-phenyl}-6-(naphthalen-2-yloxymethyl)-piperazin-2-one as a semi-solid. $[\alpha]_D$ +36.4 (*c* 1.4, $CHCl_3$); IR (neat) 3353, 2859, 1640, 1509, 1465, 1239, 1176, 1088, 1027, 841 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08 (quintet, J = 6.1 Hz, 2H), 3.38 (d, J = 13.4 Hz, 1H), 3.52 (d, J = 13.8 Hz, 1H), 3.67 (dd, J = 6.3, 1.7 Hz, 1H), 3.70 (dd, J = 6.3, 1.7 Hz, 1H), 3.77 (s, 3H), 4.03 (dd, J = 7.2, 2.2 Hz, 1H), 4.08 (ddd, J = 6.3, 6.3, 2.0 Hz, 1H); 4.27 (t, J = 8.8 Hz, 1H), 4.54 (s, 2H), 6.81 (d, J = 7.6 Hz, 1H), 6.92 (m, 4H), 7.07 (ddd, J = 8.9, 2.7, 2.7 Hz, 1H), 7.15 (dd, J = 8.8, 2.0 Hz, 2H), 7.21 (m, 1H), 7.33 (m, 2H), 7.41 (t, J = 7.4 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.74 (t, J = 9.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7 (*C*H$_2$), 45.8 (*C*H$_2$), 50.78 (*C*H$_2$), 55.3 (*C*H$_3$), 58.6 (*C*H), 65.2 (*C*H$_2$), 66.2 (*C*H$_2$), 66.9 (*C*H$_2$), 67.8 (*C*H$_2$), 106.8 (*C*H), 110.2 (*C*H), 115.4 (2 *C*H), 118.5 (*C*H), 120.4 (*C*H), 124.0 (*C*H), 126.6 (*C*H), 126.7 (*C*), 126.8 (*C*H), 127.7 (*C*H), 128.7 (*C*H), 128.8 (2 *C*H), 128.9 (*C*H), 129.2 (*C*), 129.7 (*C*H), 132.7 (*C*), 134.4 (*C*), 156.0 (*C*), 157.1 (*C*), 158.4 (*C*), 169.1 (*C*); HRMS (ESI) calcd for $C_{32}H_{35}N_2O_5$ 527.2546. Found: 527.2547. Anal. Calcd for $C_{32}H_{34}N_2O_5$: C, 72.86; H, 6.52; N, 5.31. Found: C, 72.57; H, 6.53; N, 5.27.

5.3. In vitro renin IC_{50} determinations

The renin assay utilized a tandem green fluorescent protein (t-GFP) substrate (175 nM) that was hydrolyzed by renin human (50.4 IU/well). The t-GFP substrate contained a nine amino acid (Ile-His-Pro-Phe-His-Leu-Val-Ile-His) recognition sequence for human renin

flanked by two GFP proteins (W1B and Topaz). Human renin cleaves the leucine–valine site of the substrate linker. Tandem GFP Fret assays were carried out in a reaction buffer containing 50 mM Hepes (pH 7.4), 1.0 mM EDTA, 1% PEG (MW800), 1.0 mM DTT, and 0.10% BSA. Once cleaved, the emission ratio changes. The change was monitored by the ratio of 530 nM (topaz) over 475 nM (W1B) with the excitation set at 432 nM and the cutoff at 515 nM. The assay used a 384 well plate format that was read using a Gemini XS fluorometric plate reader (Molecular Devices). Compounds were screened at a starting concentration of 10 μM and used a 4-fold 11-point dilution regimen.

5.4. In vivo efficacy studies

Blood pressure data was obtained by telemetry in conscious, free moving three- to four-month-old double transgenic mice that expressed both human angiotensinogen and renin. The double transgenic mice were derived from a founder colony of eight male mice expressing human angiotensinogen (h-Ang 204/1) and eight female mice expressing human renin (h-Ren 9) obtained through a breeding program conducted at Charles River Laboratories (Wilminton, MA). The double transgenic mice were hypertensive with mean arterial blood pressures (MABP) of 140 mmHg. Both males and females were used. MABP was measured via radiotransmitter (model TA11PA-20, Data Sciences International, Saint Paul, Minnesota) implanted subcutaneously, between the left fore and hind limbs. The radiotransmitter catheter was placed in the left carotid artery. To obtain baseline blood pressure data the mice were dosed via oral gavage with vehicle (3% volume dimethyl acetamide, 97% sulfobutylether-beta-cyclodextrin (40% w/v) in 50 nM lactic acid) for two consecutive days. On the third day, either CI-992 (*N*-(4-morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazoyl)-*N*-[(1*S*,2*R*,3*S*)-1-(*cyclo*-hexyl-methyl)-2,3-dihydroxy-5-methylhexyl]-(HCL))[12,13] or compound **2** was administered at 30 mg/kg. Delta MABP was obtained by subtracting CI-992 or compound **2** dosed blood pressure from baseline blood pressure. Animals were allowed food and water ad libitum. The maximum antihypertensive response occurred within 1 h of a 30 mg/kg oral dose of compound **2**. At the nadir for compound **2**, MABP was normalized, but returned to baseline hypertensive levels approximately 3–4 h later.

References and notes

1. American Heart Association, Statistics: 2004.
2. Kaplan, N. M.; Gifford, R. W. *JAMA* **1996**, *275*, 1557.
3. (a) Oparil, S.; Haber, E. *New Engl. J. Med.* **1974**, *291*, 381, and 446; (b) Brewster, U. C.; Perazella, M. A. *Am. J. Med.* **2004**, *116*, 263; (c) Nicholls, M. G.; Robertson, J. S. *J. Hum. Hypertens.* **2000**, *14*, 649; (d) Zaman, M. A.; Oparil, S.; Calhoun, D. A. *Nat. Drug Discov.* **2002**, *1*, 621.
4. Stanton, A. *JRAAS* **2003**, *4*, 6.
5. Zimmerman, B. G. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 370.
6. (a) Bittner, B.; Chou, R.; Schleimer, M.; Morgenroth, B.; Zell, M.; Lave, T. *Arzneimittel-Forsch./Drug Res.* **2002**, *52*, 593; (b) Vieira, E.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Guller, R.; Hirth, G.; Marki, H. P.; Muller, M.; Oefner, C.; Scalone, M.; Stadler, H.; Wilhelm, M.; Wostl, W. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1397; (c) Oefner, C.; Binggeli, A.; Breu, V.; Bur, D.; Clozel, J.-P.; D'Arcy, A.; Dorn, A.; Fischli, W.; Gruninger, F.; Guller, R.; Hirth, G.; Markl, H. P.; Mathews, S.; Muller, M.; Ridley, R. G.; Stadler, H.; Vieira, E.; Wilhelm, M.; Winkler, F. K.; Wostl, W. *Chem. Biol.* **1999**, *6*, 127; (d) Guller, R.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Hirth, G.; Jenny, C.; Kansy, M.; Montavon, F.; Muller, M.; Oefner, C.; Stadler, H.; Viera, E.; Wihelm, M.; Wostl, W.; Markl, H. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1403; (e) Marki, H. P.; Binggeli, A.; Bittner, B.; Bohner-Lang, V.; Breu, V.; Bur, D.; Coassolo, Ph.; Clozel, J. P.; D'Arcy, A.; Doebeli, H.; Fischli, W.; Funk, Ch.; Foricher, J.; Giller, T.; Gruninger, F.; Guenzi, A.; Guller, R.; Hartung, T.; Hirth, G.; Jenny, Ch.; Kansy, M.; Klinkhammer, U.; Lave, T.; Lohri, B.; Luft, F. C.; Mervaala, E. M.; Muller, D. N.; Muller, M.; Montavon, F.; Oefner, Ch.; Qiu, C.; Reichel, A.; Sanwald-Ducray, P.; Scalone, M.; Schleimer, M.; Schmid, R.; Stadler, H.; Treiber, A.; Valdenaire, O.; Vieira, E.; Waldmeier, P.; Wiegand-Chou, R.; Wilhelm, M.; Wostl, W.; Zell, M.; Zell, R. *Il Farmaco* **2001**, *56*, 21.
7. (a) Meeley, N. E.; Castaner, R. M.; Castaner, J.; Silverstre, J. *Drugs Future* **2001**, *26*, 1139; (b) Wood, J. M.; Maibaum, J.; Rahuel, J.; Grutter, M. G.; Cohen, N.-C.; Rasetti, V.; Vuger, H.; Goschke, R.; Stutz, S.; Fuhrer, W.; Schilling, W.; Rigollier, P.; Yamaguchi, Y.; Cumin, F.; Baum, H.-P.; Schnell, C. R.; Herold, P.; Mah, R.; Jensen, C.; O'Brien, E.; Stanton, A.; Bedigian, M. P. *Biochem. Biophys. Res. Commun.* **2003**, *308*, 698; (c) Stanton, A.; Jensen, C.; Nussberger, J.; O'Brien, E. *Hypertension* **2003**, *42*, 1137.
8. www.speedelpharma.com, *press releases*, July 13, 2004.
9. (a) Cody, W. L.; Edmunds, J. J.; Holsworth, D. D.; Powell, N. A. U.S. Patent 20040204455 A1, 2004; (b) Cai, C.; Clay, E.; Downing, D. M.; Edmunds, J. J.; Holsworth, D. D.; Powell, N. A. Patent WO 2004089915 A1, 2004.
10. Cody, W. L.; Holsworth, D. D.; Powell, N. A.; Jalaie, M.; Zhang, E.; Wang, W.; Samas, B.; Bryant, J.; Ostroski, R.; Ryan, M. J.; Edmunds, J. J. *Bioorg. Med. Chem.* **2005**, *13*, 59.
11. Powell, N. A.; Ciske, F. L.; Clay, E. C.; Cody, W. L.; Downing, D. M.; Holsworth, D. D.; Edmunds, J. J. *Org. Lett.* **2004**, *6*, 4069.
12. Ryan, M. J.; Hicks, G. W.; Batley, B. L.; Rapundalo, S. T.; Patt, W. C.; Taylor, D. G.; Kaiser, J. A. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 372.
13. Patt, W. C.; Hamilton, H. A.; Ryan, M. J.; Painchaud, C. A.; Taylor, M. D.; Rapundalo, S. T.; Batley, B. L.; Connolly, C. J.; Taylor, D. G. *Med. Chem. Res.* **1993**, *2*, 10.