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The discovery and preparation of disubstituted novel amino-aryl-piperidine-based renin inhibitors

Wayne L. Cody,[a,*] Daniel D. Holsworth,[a] Noel A. Powell,[a] Mehran Jalaie,[b] Erli Zhang,[b] Wei Wang,[b] Brian Samas,[c] John Bryant,[d] Robert Ostroski,[d] Michael J. Ryan[d] and Jeremy J. Edmunds[a]

[a]*Department of Chemistry, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*
[b]*Department of Discovery Technologies, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*
[c]*Department of Pharmaceutical Sciences, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*
[d]*Department of Cardiovascular Therapeutics, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*

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Abstract—Recently, *trans*-disubstituted oxo-aryl-piperidines have been identified as small molecule nonpeptide renin inhibitors for the modulation of hypertension. Herein, we report on the discovery and preparation of a new class of novel *cis*-disubstituted amino-aryl-piperidines as a mixture of enantiomers that are potent in vitro renin inhibitors and also, possess in vivo antihypertensive activity in a double transgenic mouse model.

1. Introduction

Renin has been identified as a rate limiting enzymatic step in the renin angiotensin cascade. The renin angiotensin system (RAS), is an important factor in the control of blood volume, arterial pressure and cardiac and vascular function. The physiological pathways for RAS have been identified in several tissues, the most critical for the release of renin is found in the kidney. Sympathetic stimulation, renal artery hypotension, and decreased sodium delivery to the kidney has been shown to stimulate renin release. The enzyme, renin, acts on a circulating substrate, angiotensinogen, to undergo proteolytic cleavage to provide angiotensin I (AngI). Angiotensin I is innocuous, but when the two C-terminal amino acids are cleaved by angiotensin converting enzyme (ACE) to angiotensin II (AngII), this compound has been identified as a major factor of hypertension (see Fig. 1). Thus, the actions of renin have been documented to be a major contributor to the development of hypertension. Therefore, inhibitors of renin represent an intriguing and important therapeutic target.[1,2]

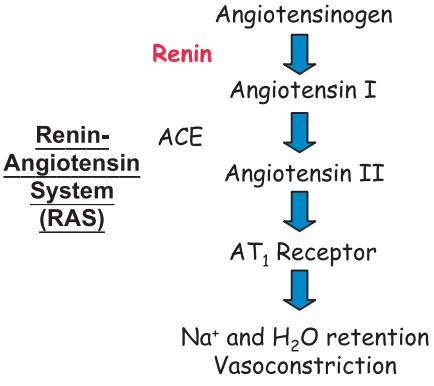

Figure 1. The renin angiotensin system (RAS).

* Corresponding author. Tel.: +1 734 622 7729; fax: +1 734 622 3909; e-mail: wayne.cody@pfizer.com

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Renin is an aspartyl protease, whose active site possesses a long narrow cleft that accommodates seven amino acids of its native substrate angiotensinogen. The enzyme contains a mobile flap, which when closed, holds angiotensinogen in the active site.[1,2] Renin possesses two aspartic acid residues in the active site, that are critical to the observed catalytic activity and cleavage of the Leu10-Val11 bond of angiotensinogen to reveal AngI, which is further processed to AngII.

Earlier efforts on the identification of renin inhibitors, led to the discovery of several peptidomimetic compounds.[1,2] Unfortunately, these compounds suffered from several problems for further development as potential drugs, such as oral bioavailability (i.e., intestinal absorption) and hepatic clearance.[3,4]

Recently, a series of small molecule renin inhibitors have been reported[5–10] based upon a weakly active *trans*-disubstituted amino-aryl-piperidine scaffold that was identified through a high throughput screen (HTS). The potency of the oxo-aryl-piperidine scaffold was optimized to nanomolar levels as typified by compound **1**, which was shown to be a potent active site inhibitor of renin with an IC_{50} of approximately, 1.5 nM (see Fig. 2).

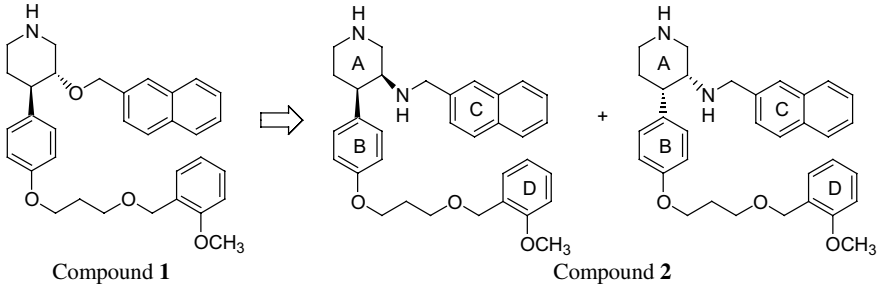
Herein, we report the discovery and development of a novel series of inhibitors of renin utilizing compound **1** as a starting point.[5–10] Compound **2**, exemplifies this new series (Fig. 2) and possesses an affinity for renin of with an IC_{50} of 61 nM. This compound is characterized by a *cis* orientation of the two stereocenters on the amino-aryl-piperidine template, and the incorporation of a secondary amine in place of the exocyclic oxygen of compound **1**. These two modifications have provided a novel series of renin inhibitors. Critical to determining the novelty of this series was providing proof that the relative orientation of the two stereocenters was *cis*. This was achieved through the application of proton nuclear magnetic resonance spectroscopy (^{1}H NMR), small molecule and complexed with renin, X-ray crystallographic studies of compound **2** (see Experimental). The synthetic strategy for the preparation of compound **2** and related analogues, along with the developing structure–activity relationships (SAR) will be further exemplified.

2. Results

The original synthetic strategy for the preparation of compound **2** and related analogues are outlined in Scheme 1. The syntheses of compounds (**9**)[10] and (**10**)[5,6] have been previously reported. To further elaborate, the preparation of the 'A'–'B' ring core (**7**) proceeds smoothly by the palladium(I) catalyzed coupling of the boronic acid (**4**) and triflate (**6**). Subsequent chiral dihydroxylation was successful in moderate yields using AD-mix-alpha[11] to prepare (**8**). Removal of the hydroxyl-protecting group (*tert*-butyl-dimethyl-silane (TBDMS)) was necessary prior to removal of the tertiary alcohol to provide (**9**).[10] Incorporation of the 'D'-ring and corresponding side chain was achieved using 1-(3-iodo-propoxymethyl)-2-methoxy-benzene (see Experimental) in the presence of potassium carbonate (**10**). Further manipulations to chirally incorporate the 'C'-ring were unsuccessful, via the transformation of the alcohol to a primary amine. The alcohol (**10**) could be converted to the ketone (**11**) by pyridinium chlorochromate (PCC) oxidation, albeit in 34% yield. Dess–Martin conditions were also investigated, but provided inferior results. The 'C'-ring was then incorporated via reductive amination to provide the penultimate compound as a mixture of enantiomers (**12**).

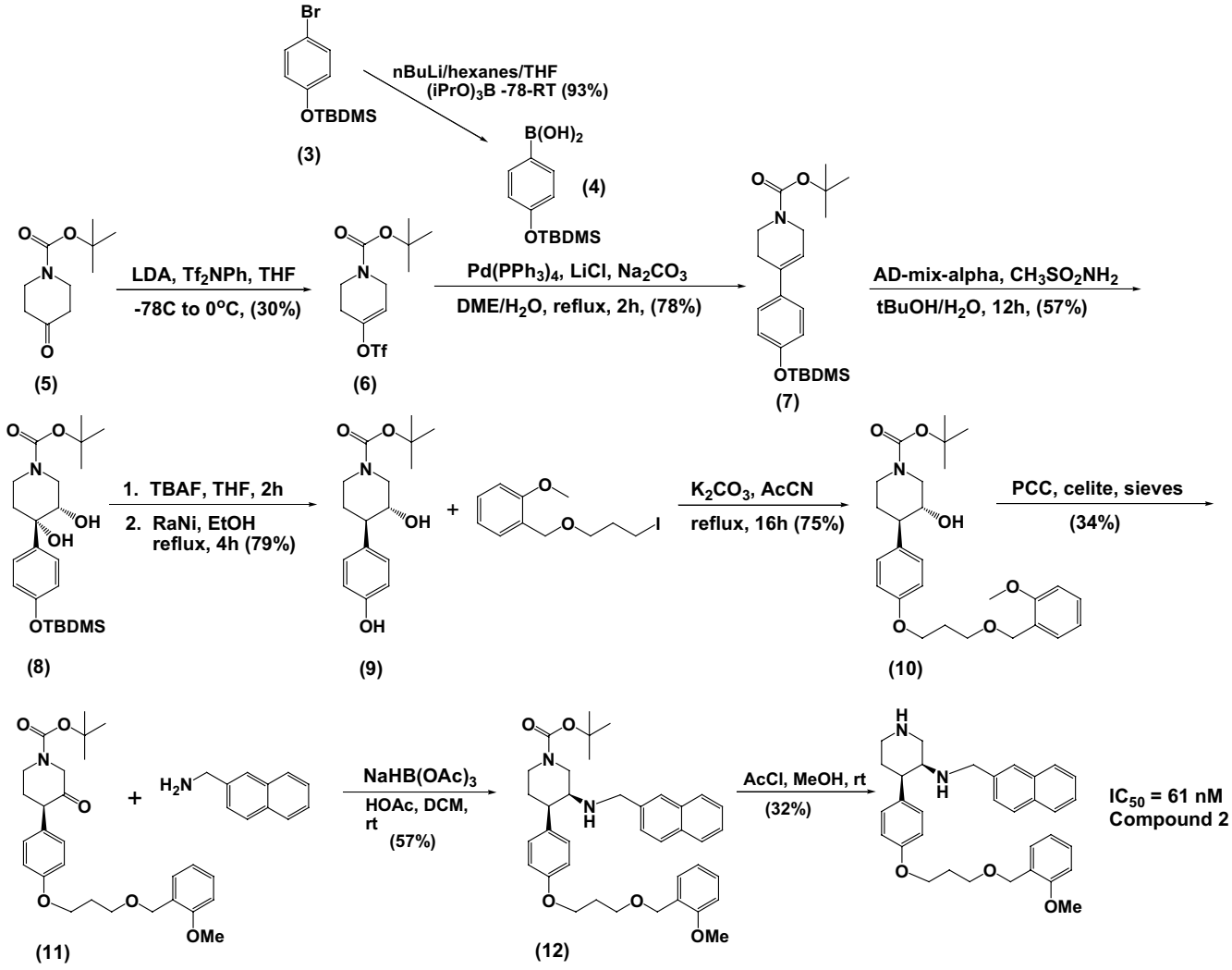
The final compound (**2**) was obtained after removal of the *tert*-Boc protecting group, purification by reversed phase high performance liquid chromatography (RP-HPLC), and subsequent conversion to the free base. The mechanism of the reductive amination would suggest that the relative stereochemistry of the two stereocenters should be *cis*. In fact, the relative stereochemistry was unequivocally determined to be *cis* by spectroscopic and crystallographic methods. None of the *trans* isomers were observed. Therefore, this compound (**2**) and others reported in this manuscript were not further resolved and tested as a enantiomeric mixture. It should be noted that the 'D'-ring is acid sensitive and care must be taken during the *tert*-Boc deprotection step, and conversion to the free base is required for long-term storage.

Due to inconsistent yields with the reductive amination step shown in Scheme 1 and difficult purifications of final products an optimized route was developed, and is shown in Scheme 2. In particular, formation of the benzyl-oxime to provide compound **13**, which was readily hydrogenated in the presence of Raney Nickel to pro-



Compound **1** Compound **2**

Figure 2. Design of compound **2** based upon previously cited literature.[5–10]



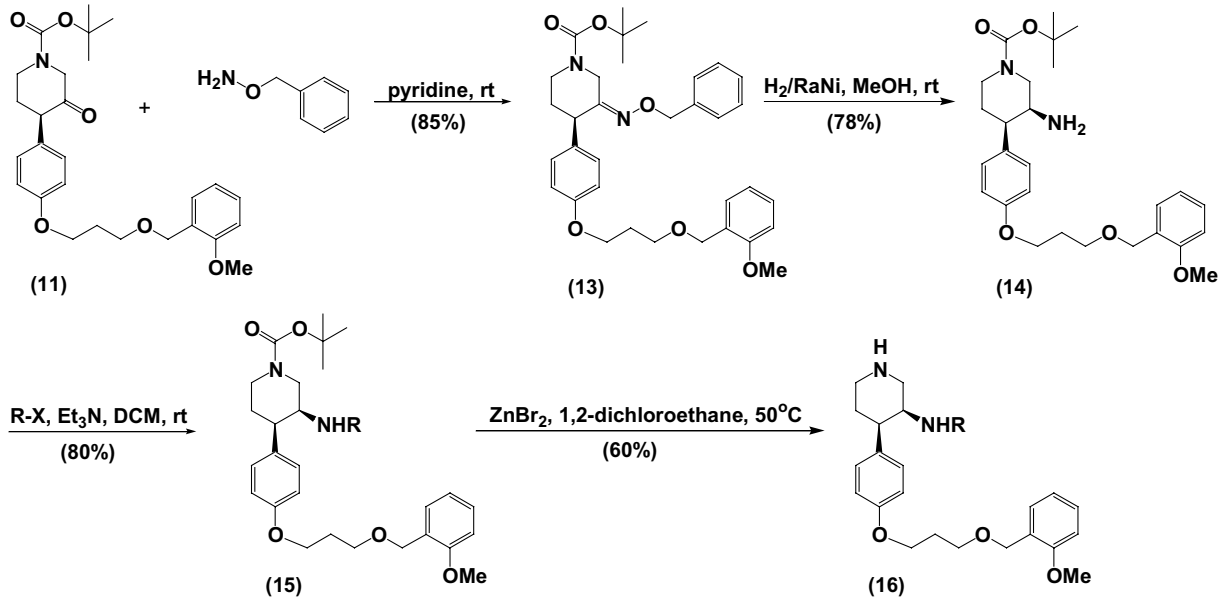
Scheme 1. Compounds **12** and **2** are a mixture of enantiomers.

vide the primary amine (**14**), proved to be more efficient. This intermediate readily undergoes acylations and alkylations in the presence of triethylamine to rapidly provide several *tert*-Boc protected products (**15**). In addition, zinc bromide proved to be a very effective and selective reagent for the removal of *tert*-Boc protecting groups from secondary amines.[12] All of the final compounds were prepared using the above approaches as fully outlined in the Experimental section.

Utilizing the attributes of compound **1**, we have utilized structure based design approaches to prepare a novel template as exemplified by compound **2**. Unlike, the *trans* orientation of compounds previously revealed in the literature[5–10] the 'B'- and 'C'-rings of the current template are orientated in a *cis* manner. In addition, a secondary amine was used to incorporate the 'C'-ring as opposed to an ether linkage, in the previously described series.[5–10] Compound **2** possessed high affinity as an active site inhibitor of renin with an IC_{50} of 61 nM.

The structure–activity relationships (SAR) of the 'C'-ring were explored by the preparation of several analogues (Fig. 3). The methoxy-naphthalene analog (**17**) was essentially equipotent to compound **2**. Compounds **18** and **19** were suggested from previous studies[5–9] and it was suggested that these modifications could greatly enhance efficacy. However, this was not the case in this series. Interestingly, the naphthalene methyl-ester (**20**) and benzyl furan derivative (**21**) were less, but equally efficacious in the submicromolar range however, the indole derivative (**22**) possessed approximately 10-fold less affinity. Compound (**23**) also, possessed similar affinity, but possessed additional interesting properties (see below). In addition, compounds **24** and **25** were ineffective at inhibiting the binding of renin to its substrate at concentrations of 10 μM. Thus, it was clear that a fused bicyclic 'C'-ring was required for affinity as the hydrogen and 3-pyridyl analogues were inactive at concentrations of greater than 10 μM (data not shown).

To determine if compound **2** has antihypertensive activity, it was administered to hypertensive (base line mean arterial blood pressure (MABP) ~140 mmHg) transgenic mice characterized by life-long overexpression of human renin and angiotensinogen. After oral gavage administration (30 mg/kg) to double transgenic mice



Scheme 2. Compounds **15** and **16** are a mixture of enantiomers.

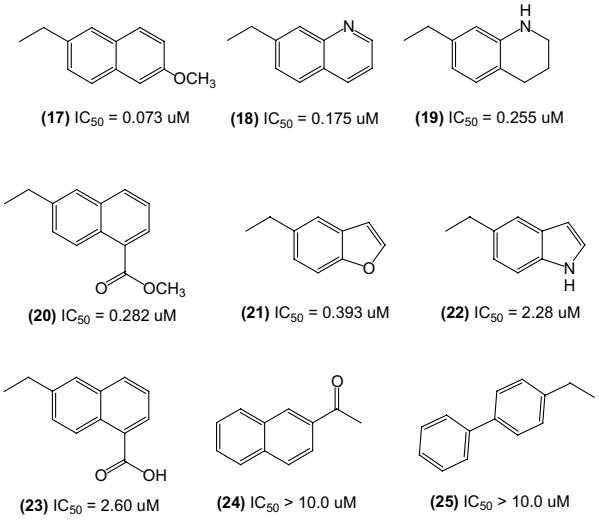


Figure 3. Structure–activity relationships of compound **2**.

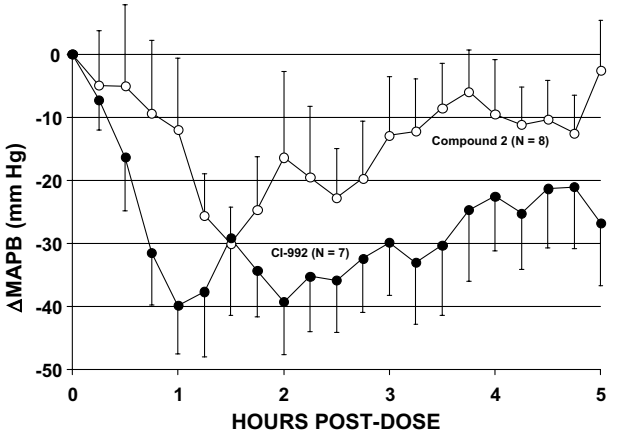


Figure 4. Effects of compound **2** versus CI-992[11,12] on reducing blood pressure in the double transgenic mouse model. Plotted values represent the mean ± SEM.

(Fig. 4) peak antihypertensive activity was observed 1.5 h post-dose. CI-992 (*N*-(4-morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-*N*-[(1*S*,2*R*,3*S*)-1-(*cyclo*-hexylmethyl)-2,3-dihydroxy-5-methylhexyl]-(9Cl)),[11,12] a reference renin inhibitor with documented in vivo antihypertensive activity in this model. The observation of oral antihypertensive activity in a renin-dependent animal model provides evidence that molecules such as compound **2** have potential as a therapeutic for the treatment of hypertension.

An X-ray crystal structure of the piperidine analogue bound to the active site of renin (compound **2**) is depicted in Figure 5. This figure highlights that the piperidine ring conformation plays an important role in positioning the piperidine nitrogen for hydrogen bonding to the catalytic aspartic acids (Asp^{32} and Asp^{215}) of renin, and provides the correct geometry to extend the naphthalene functionality into the S3-subpocket.[10] The X-ray orientation of the ligand reaffirms the roles of the Tyr^{75} and Trp^{39} of renin. Upon the binding of this compound, the hydrogen bonds that hold Tyr^{75} and Trp^{39} together breaks, and the flap region of the protein opens to accommodate the polyether tail of the scaffold. The piperidine-nitrogen provides essential hydrogen bonds to the catalytic aspartic acids. In addition, the naphthalene ring positions itself in the opening of the S3-subpocket as the exocyclic chiral nitrogen hydrogen bonds to Gly^{217}. In addition, this inhibitor binds such that it capitalizes on the Pi–Pi stacking and charged-Pi interactions that are available in the active site. For example, Phe^{112} and Phe^{117} of renin, interacts directly with aromatic ring 'D'-ring and the naphthalene group, respectively.

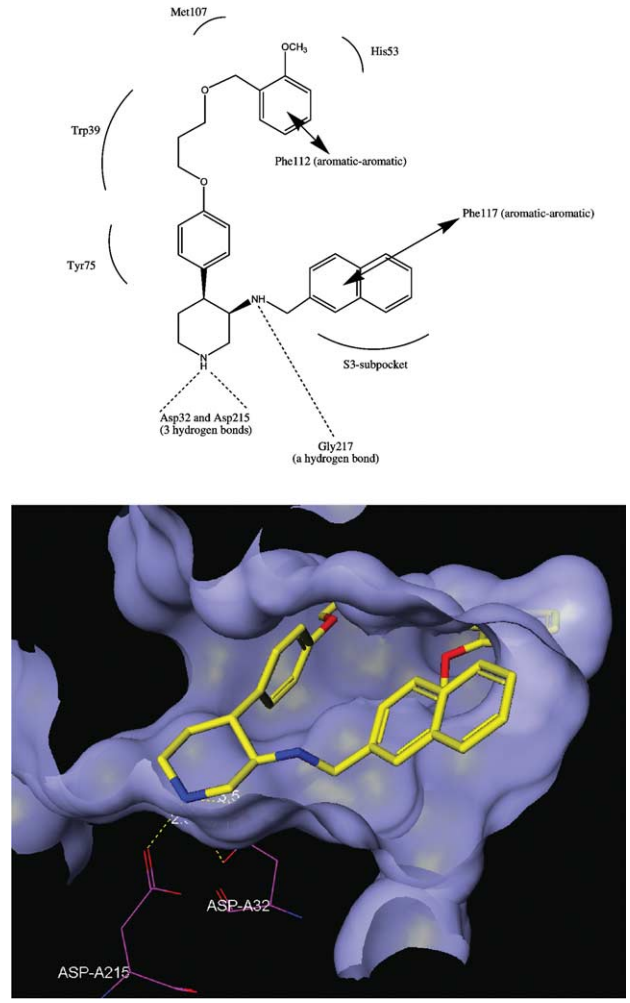


Figure 5. Interactions of an enantiomer of compound **2** with renin as determined from X-ray crystallographic studies.

Unfortunately, this series suffered from submicromolar interactions with Cytochrome (Cyp) P450 isoenzymes, and thus diminishes their potential for further development. Incorporation of an acidic functionality into the 'C'-ring in an effort to neutralize the net charge of the molecule led to the preparation of compound **23**. This compound possessed greatly reduced affinity for selected Cyp enzymes (i.e., micromolar (data not shown)), but unfortunately, was only weakly active as a renin inhibitor (Fig. 2). However, from the data provided it is clear that these prototypic compounds could be used as a starting point for the design of second-generation renin inhibitors.

3. Experimental

3.1. 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.) where available and used without further purification. Compound **9** was prepared as outlined in Ref. 11. All intermediates were characterized by proton nuclear magnetic spectroscopy (^{1}H NMR) and mass spectrometry (MS). All final compounds were determined to be consistent with the proposed structure by ^{1}H NMR, MS, and were greater than 95% pure as determined by analytical RP-HPLC on a C18 column utilizing a gradient of 0.1% trifluoroacetic (TFA) in H_2O to 0.1% TFA in acetonitrile (conditions are elaborated in the detailed Experimental).

3.2. Chemistry

3.2.1. 2-(2-Methoxy-phenyl)-[1,3]-dioxane. 2-Methoxy benzaldehyde (30.0 g, 0.22 mol), propane 1,3-diol (18.44 g, 0.24 mol), and benzene (300 mL) were added to a round bottom flask equipped with a Dean–Stark trap. The reaction mixture was heated to reflux for 5 h and cooled to room temperature. The mixture was diluted with ethyl acetate (300 mL) and the layers separated. The organic layer was washed with water (1 × 300 mL), 1 N HCl (1 × 100 mL), saturated sodium bicarbonate (1 × 100 mL), and brine (2 × 100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain 41.0 g of a yellow solid. The solid was recrystallized from hexanes to obtain white crystals (38.3 g, 89%).

3.2.2. 3-(2-Methoxy-benzyloxy)-propan-1-ol. 2-(2-Methoxy-phenyl)-[1,3]-dioxane (38.3 g, 0.197 mol) was dissolved in toluene (300 mL) under nitrogen. The mixture was cooled to 0 °C and diisobutylaluminum hydride (DIBAL-H) (61.70 g, 0.433 mol) added slowly. Once the addition complete, the reaction mixture was allowed to stir 18 h, slowly warming to room temperature. Ethyl acetate (150 mL) was added to quench the excess DIBAL. A solution of 10% aqueous Rochelle's salt (800 mL) was added and the mixture stirred for 3 h. Once all salts were dissolved, the layers were separated. The aqueous layer was washed with ethyl acetate (2 × 400 mL). To the aqueous layer was added 10% sodium hydroxide solution (150 mL) and the aqueous layer was extracted with ethyl acetate (2 × 150 mL). The organic layers were combined, washed with brine (2 × 150 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to afford a yellow oil (37.92 g, 98%).

3.2.3. Toluene-4-sulfonic acid 3-(2-methoxy-benzyloxy)-propyl ester. 3-(2-Methoxy-benzyloxy)-propan-1-ol (37.9 g, 0.193 mol) was dissolved in dichloromethane (300 mL), dimethylaminopyridine (DMAP) (2.35 g, 0.019 mol), pyridine (16.80 g, 0.212 mol), and *para*-toluenesulfonyl chloride (40.50 g, 0.212 mol) were added. The reaction mixture was heated to reflux for 24 h. The mixture was cooled to room temperature and diluted with dichloromethane (400 mL). The layers were separated and the organic layer was washed with water (2 × 200 mL), 1 N hydrochloric acid (2 × 200 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain 50.0 g of a white solid. The compound was subjected to column chromatography (15–25% ethyl acetate/hexane mixture) to yield a clear oil (18.28 g, 27%).

3.2.4. 1-(3-Iodo-propoxymethyl)-2-methoxy-benzene. Toluene-4-sulfonic acid 3-(2-methoxy-benzyloxy)-propyl ester (18.22 g, 0.051 mol) was dissolved in acetone (100 mL) under nitrogen. Lithium iodide (10.44 g, 0.077 mol) was added, and the mixture heated to reflux for 1 h, cooled to room temperature filtered through a pad of Celite and washed with acetone. The organic layer was concentrated under reduced pressure and redissolved in dichloromethane. The organic layer was washed with water (2 × 100 mL), 10% aqueous sodium bisulfite (2 × 100 mL), brine (2 × 100 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to yield a yellow oil (17.03 g, quantitative).

3.2.5. Naphthalene-2-yl-methyl-amine. Naphthalene-2-carbonitrile (5.57 g, 36.4 mmol) was hydrogenated in the presence of Raney Nickel in methanol and aqueous ammonia. The solution was concentrated under reduced pressure to a red semi-solid that was purified on silica gel (ethyl acetate–methanol (4:1)), combined and concentrated under reduced pressure to a light pink solid (4.21 g, 74%).

3.2.6. 3-Hydroxy-4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidine-1-carboxylic acid *tert*-butyl ester (10). Compound **9**,[14] (6.58 g, 22.4 mmol), 1-(3-iodo-propoxymethyl)-2-methoxy-benzene (8.58 g, 28.0 mmol), and potassium carbonate (4.21 g, 30.5 mmol) were combined in 120 mL of acetonitrile. The solution was brought to reflux, for 16 h. The reaction mixture was cooled, concentrated under reduced pressure, partitioned between ethyl acetate and water (100 mL each), separated, washed with water, brine, separated, dried with magnesium sulfate, filtered, and concentrated to an oil (13.58 g). The oil was purified on silica gel (ethyl acetate–hexanes (1:1)), combined and concentrated under reduced pressure to a clear oil (9.94 g, 94%).

3.2.7. 4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-3-oxo-piperidine-1-carboxylic acid *tert*-butyl ester (11). Compound **10** (9.94 g, 21.1 mmol), PCC (6.80 g, 32.0 mmol), Celite (6.8 g), and crushed 4 Å molecular sieves (6.8 g) were combined in 100 mL of dichloromethane. The solution was allowed to stir overnight, and mass spectra indicated that the reaction was incomplete. An additional 0.75 equiv of PCC, Celite, and molecular sieves were added. The solution was filtered through Celite, washed with ethyl ether, combined, and concentrated under reduced pressure to an oil. The oil was chromatographed on silica (ethyl acetate–hexanes (1:2)), appropriate fractions were combined and concentrated under reduced pressure (3.67 g, 37%).

3.2.8. (3*R*,4*S*)-4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-3-[(naphthalen-2-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester and (3*S*,4*R*)-4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-3-[(naphthalen-2-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester (12). Compound **11** (1.85 g, 3.94 mmol), naphthalen-2-yl-methyl-amine (0.93 g, 5.9 mmol), and acetic acid (0.225 mL, 3.94 mmol), were combined in 20 mL of dichloromethane under argon. After 30 min sodium triacetoxyborohydride (1.3 g, 5.9 mmol) was added and the solution was allowed to stir overnight. The reaction was quenched with saturated sodium bicarbonate, partitioned between ethyl acetate and water (~25 mL each), separated, dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a yellow oil (2.79 g). The oil was purified on silica gel utilizing ethyl acetate and hexanes. Appropriate fractions were combined and concentrated under reduced pressure to a yellow solid (1.36 g, 56%).

3.2.9. (3*R*,4*S*)-(4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-naphthalen-2-ylmethyl-amine and (3*S*,4*R*)-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-naphthalen-2-ylmethyl-amine (2). Compound **12** (200 mg) was dissolved in 5 mL of methanol at 0 °C under argon. Acetyl chloride (230 μL) was added, allowed to stir overnight while warming to room temperature. The reaction was complete by RP-HPLC and was purified directly on a Vydac 218TP1022 column (A: 0.1%TFA/H_2O, B: 0.1%TFA/AcCN, Gradient 10–70% B over 120 min). Appropriate fractions were combined and concentrated under reduced pressure to a white powder. The powder was dissolved in methanol (2 mL) and water was added to the precipitation point, saturated aqueous sodium bicarbonate was added and a precipitate formed that was absorbed on C18, washed with water, and eluted with THF. The effluent was combined with water and lyophilized to afford compound **2** (52.8 mg, 32%). ^{1}H NMR data was collected in two solvents (see below) (400 MHz, methanol-d_4): δ 7.73 (dd, 1H, J = 7.1, 2.7 Hz), 7.62 (d, 2H, J = 8.6 Hz), 7.19 (m, 2H), 7.25 (m, 2H), 7.20 (td, 1H, J = 7.9, 2.0 Hz), 7.07 (d, 2H, J = 8.7 Hz), 7.03 (dd, 1H, J = 8.6, 1.7 Hz), 6.88 (d, 1H, J = 7.9 Hz), 6.86 (t, 1H, J = 7.9 Hz), 6.82 (d, 2H, J = 8.7 Hz); (400 MHz, pyridine-d_5): δ 4.67 (s, 2H), 4.15 (t, 2H, J = 6.6 Hz), 3.89 (d, 1H, J = 14.0 Hz), 3.74 (t, 2H, J = 6.3 Hz), 3.65 (d, 1H, J = 14.0 Hz), 3.63 (s, 3H), 3.29 (dd, 1H, J = 12.6, 1.3 Hz), 3.17 (dm, 1H, J = 12.9 Hz), 2.82 (dt, 1H, J = 12.9, 3.2 Hz), 2.78 (m, 1H), 2.67 (dd, 1H, J = 12.6, 1.9 Hz), 2.64 (dd, 1H, J = 12.9, 2.3 Hz), 2.19 (m, 1H), 2.13 (t, 2H, J = 6.6 Hz), 2.12 (br s, 1H), 1.47 (d, 1H, J = 12.9 Hz), 1.27 (br s, 1H). MS: m/z = 511.2 (M+1).

3.2.10. *C*-(6-Methoxy-naphthalen-2-yl)-methyl-amine. 6-Methoxy-naphthalene-2-carbonitrile (5.00 g, 27.0 mmol) was hydrogenated in the presence of Raney Nickel in methanol and aqueous ammonia hydroxide. The solution was concentrated under reduced pressure to a semi-solid. The semi-solid was partitioned between ethyl acetate and water (50 mL each), separated, washed with water, brine, dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a white solid (4.13 g, 81%).

3.2.11. (3*R*,4*S*)-(4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-(6-methoxy-naphthalen-2-ylmethyl)-amine and (3*S*,4*R*)-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-(6-methoxy-naphthalen-2-ylmethyl)-amine (17). The title compound was prepared as per compound **2** utilizing *C*-(6-methoxy-naphthalen-2-yl)-methyl-amine in the reductive amination step. MS: m/z = 541.2 (M+1).

3.2.12. 7-Trifluoromethyl-quinoline. 4-Chloro-7-trifluoromethyl-quinoline (19.80 g, 100 mmol) was hydrogenated in the presence of 5% palladium on carbon in methanol in the presence of triethylamine. The solution was concentrated under reduced pressure, partitioned between ethyl acetate and water (200 mL each), separated, washed with water (2 × 200 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a yellow solid (15.20 g, 90%).

3.2.13. Quinoline-7-carboxylic acid methyl ester. 7-Trifluoromethyl-quinoline (22.10 g, 0.112 mol) was dissolved in 30% oleum, heated to 150 °C for 2 h. The solution was cooled to 0 °C and 200 mL of methanol was added slowly and the mixture was refluxed overnight. The mixture was cooled to room temperature, concentrated under reduced pressure to an oil, that was neutralized with saturated aqueous sodium carbonate, overlaid with ethyl acetate (100 mL), re-extracted with ethyl acetate (100 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a pink solid (16.10 g, 77%).

3.2.14. Quinolin-7-yl methanol. Quinoline-7-carboxylic acid methyl ester (4.94 g, 26.4 mmol) was dissolved in 70 mL of THF at −20 °C under argon. REDAL (60% in toluene, 12.9 mL, 66 mmol) was added and allowed to stir at −20 °C for 4 h. After warming to room temperature, the reaction was quenched slowly with water, concentrated under reduced pressure, partitioned between ethyl acetate and water (100 mL each), filtered, separated, re-extracted with ethyl acetate (50 mL), separated, dried with magnesium sulfate, and concentrated under reduced pressure. The residue was purified on silica gel with ethyl acetate, appropriate fractions were combined and concentrated under reduced pressure (3.42 g, 82%).

3.2.15. 7-Bromomethyl-quinoline. Quinolin-7-yl methanol (3.25 g, 20.4 mmol) was added to a saturated solution of hydrobromic acid in acetic acid (40 mL). The solution was heated to 70 °C for 4 h, cooled, and concentrated under pressure to a light orange oil (6.18 g, quantitative).

3.2.16. 7-Azidomethyl-quinoline. 7-Bromomethyl-quinoline (2.11 g, 10.0 mmol) was dissolved in 20 mL of DMF and sodium azide (0.975 g, 15.0 mmol) was added and heated to 75 °C for 16 h. The solution was cooled, poured into water (100 mL), extracted with ethyl acetate (2 × 50 mL), washed with water (2 × 50 mL), brine (1 × 50 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a pink oil (1.83 g, 99%).

3.2.17. *C*-Quinolin-7-yl-methyl-amine. 7-Azidomethyl-quinoline (1.76 g, 9.5 mmol) was hydrogenated in the presence of Raney Nickel in methanol. The solution was concentrated under reduced pressure to a yellow oil, dissolved in ethyl acetate (50 mL), extracted with 1 N hydrochloric acid (3 × 50 mL), the pH was adjusted to ~10 with 1 N sodium hydroxide, extracted with ethyl acetate (3 × 50 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a white solid (0.811 g, 54%).

3.2.18. (3*R*,4*S*)-(4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-quinolin-7-ylmethyl-amine and (3*S*,4*R*)-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-quinolin-7-ylmethyl-amine (18). The title compound was prepared by techniques used for the preparation of compound **2** utilizing *C*-quinolin-7-yl-methyl-amine in the reductive amination step. MS: *m*/*z* = 512.2 (M+1).

3.2.19. (3*R*,4*S*)-(4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-(1,2,3,4-tetrahydro-quinolin-7-ylmethyl)-amine and (3*S*,4*R*)-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-(1,2,3,4-tetrahydro-quinolin-7-ylmethyl)-amine (19). 4-{4-[3-(2-Methoxy-benzyloxy)-propoxy]-phenyl}-3-[(quinolin-7-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester (prepared as a precursor of compound **18** (as an enantiomeric mixture), see above) (0.394 g, 0.64 mmol) and nickel(II) chloride hexahydrate (0.077 g, 0.32 mmol) were dissolved in 5 mL of methanol at 0 °C under argon. After 30 min, sodium borohydride (0.100 g, 3.0 mmol) was added in two portions and allowed to stir at 0 °C for 4 h while warming to room temperature. The solution was recooled to 0 °C and another 0.5 equiv of nickel(II) chloride hexahydrate and sodium borohydride were added and allowed to stir overnight while warming to room temperature. The solution was poured into a solution of saturated ammonium chloride (20 mL) and ethyl acetate (40 mL) and stirred vigorously for 15 min, separated, extracted with ethyl acetate (2 × 25 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to afford 4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-3-[(1,2,3,4-tetrahydro-quinolin-7-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester as a clear oil, 0.399 g, (enantiomeric mixture, quantitative). The remaining *tert*-Boc protecting group was removed as in the preparation of compound **2** to yield the title compound. MS: *m*/*z* = 516.3 (M+1).

3.2.20. 6-Methyl-naphthalene-1-carboxylic acid. A mixture of toluene (400 mL, 3.75 mol) and 2-furoic acid (48.0 g, 0.428 mol) was stirred in an ice-bath as 120 g (0.900 mol) of aluminum chloride was added slowly. The mixture was warmed to 60 °C overnight. The solution was poured into cold aqueous 1 N hydrochloric acid, rewarmed to 60 °C and allowed to stir for 6 h. The solution was cooled, separated, washed with water (200 mL), extracted with saturated aqueous sodium biocarbonate (2 × 100 mL), acidified with aqueous 1 N hydrochloric acid, and the light green precipitate was filtered. The precipitate was recrystallized from benzene, filtered, and dried under reduced pressure (8.64 g, 11%).

3.2.21. 6-Methyl-naphthalene-1-carboxylic acid methyl ester. 6-Methyl-naphthalene-1-carboxylic acid was suspended in 30 mL of methanol at 0 °C under argon. Thionyl chloride was added and the solution was allowed to stir overnight while warming to room temperature. The solution was concentrated under reduced pressure,

partitioned between ethyl acetate and water (50 mL each), separated, washed with water, saturated sodium bicarbonate, brine (1 × 50 mL each), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a yellow oil (1.42 g, 87%).

3.2.22. 6-Bromomethyl-naphthalene-1-carboxylic acid methyl ester. 6-Methyl-naphthalene-1-carboxylic acid and *N*-bromosuccinimide were dissolved in carbon tetrachloride (20 mL) at room temperature and (0.10 M equiv) of benzoyl peroxide was added with stirring. The solution was brought to reflux and allowed to stir overnight. The solution was cooled, concentrated under reduced pressure and recrystallized from ethyl acetate–hexanes (1.47 g, 78%).

3.2.23. 4-{4-[3-(2-Methoxy-benzyloxy)-propoxyl]-phenyl}-piperidine-3-one *O*-benzyl oxime (13). Compound **11** (2.95 g, 6.28 mmol) and *O*-benzylhydroxylamine hydrochloride (1.10 g, 6.90 mmol) were combined in 15 mL of pyridine at room temperature under argon and allowed to stir overnight. The solution was concentrated and filtered (2.98 g, 83%).

3.2.24. (3*R*,4*S*)-3-Amino-4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidine-1-carboxylic acid *tert*-butyl ester and (3*S*,4*R*)-3-amino-4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidine-1-carboxylic acid *tert*-butyl ester (14). Compound **13** (2.88 g, 5.02 mmol) and 5.0 g of Raney Nickel were dissolved in 100 mL of THF and placed under a hydrogen atmosphere for 18 h. The solution was concentrated under reduced pressure to a clear oil (2.90 g), which was chromatographed on silica gel (dichloromethane–methanol, 95:5), appropriate fractions were combined and concentrated under reduced pressure (0.795 g, 37%).

3.2.25. (3*R*,4*S*)-4-{4-[-(2-Methoxy-benzyloxy)-propoxyl]-phenyl}-3-[(5-methoxycarbonyl-naphthalen-2-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester and (3*S*,4*R*)-4-{4-[-(2-methoxy-benzyloxy)-propoxyl]-phenyl}-3-[(5-methoxycarbonyl-naphthalen-2-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester. Compound **14** as a mixture of enantiomers (0.312 g, 0.663 mmol) was dissolved in 5 mL of dry THF under argon. Sequentially, 6-bromomethyl-naphthalene-1-carboxylic acid methyl ester (0.280 g, 1.00 mmol) and triethylamine (0.215 g, 2.12 mmol) were added and the solution was brought to reflux overnight. The solution was purified directly on silica (10% ethyl acetate–hexanes to 70% ethyl acetate–hexanes over 45 min), appropriate fractions were combined and concentrated under reduced pressure to yield the title compound (0.225 g, 42%).

3.2.26. (3*R*,4*S*)-6-[(4-{4-[3-(2-Methoxy-benzyloxy)-propoxyl]-phenyl}-piperidin-3-ylamino)-methyl]-naphthalene-1-carboxylic acid methyl ester and (3*S*,4*R*)-6-[(4-[3-(2-methoxy-benzyloxy)-propoxyl]-phenyl}-piperidin-3-ylamino)-methyl]-naphthalene-1-carboxylic acid methyl ester (20). (4-{4-[3-(2-Methoxy-benzyloxy)-propoxyl]-phenyl}-3-[(5-methoxycarbonyl-naphthalen-2-ylmethyl)-amino]-piperidine-1-carboxylic acid *tert*-butyl ester as an enantiomer mixture (0.225 g, 0.336 mmol) was dissolved in 5 mL of dry methanol at 0 °C under argon. After 30 min, acetyl chloride (0.264 g, 3.36 mmol) was added and allowed to stir overnight, while warming to room temperature. The solution was purified directly on a Vydac 218TP1022 column (A: 0.1%TFA/H_2O, B: 0.1%TFA/AcCN, Gradient 10–70% B over 120 min). Appropriate fractions were combined and lyophilized to a white powder. The powder was dissolved in methanol, excess saturated sodium bicarbonate was added, absorbed to C18, eluted with methanol, diluted with water, and lyophilized to yield (67.0 mg, 35%). MH: m/z = 569.3 (M+1).

3.2.27. 1-(2,2-Diethoxy-ethoxy)-4-methyl-benzene. *p*-Cresol (20.0 g, 184.9 mmol), bromoacetaldehyde diethyl acetal (37.2 g, 183.1 mmol), and potassium hydroxide (12.0 g, 183.1 mmol) were combined in 100 mL of dry DMSO and heated to reflux overnight. The solution was cooled, poured over ice containing 3.5 g of sodium hydroxide and diluted to 500 mL with water, extracted with ethyl ether (4 × 100 mL), combined, washed with 1 N sodium hydroxide (1 × 100 mL), water (4 × 100 mL), brine (1 × 100 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a red oil. The oil was passed through a plug of silica (ethyl acetate–hexanes (1:2)), combined, and concentrated under reduced pressure to yield a yellow oil (31.2 g, 76%).

3.2.28. 5-Methyl-benzofuran. (1-(2,2-Diethoxy-ethoxy)-4-methyl-benzene (10.2 g, 45.5 mmol) and poly-phosphoric acid (10.2 g) were combined in 200 mL of benzene and brought to reflux for 3.5 h. The reaction mixture was cooled to room temperature, decanted, concentrated under reduced pressure, and purified on silica (ethyl acetate–hexanes (1:5)). Fractions were combined and concentrated under reduced pressure to yield a yellow liquid (4.61 g, 77%).

3.2.29. 5-Bromomethyl-benzofuran. 5-Methyl-benzofuran (4.50 g, 34.0 mmol) was dissolved in carbon tetrachloride (100 mL) and benzoyl peroxide (200 mg) and *N*-bromosuccinimide (NBS, 6.06 g, 34.0 mmol) were added. The mixture was refluxed for 30 h, cooled to room temperature, concentrated under reduced pressure, and purified on silica (ethyl acetate–hexanes (1:10)). Appropriate fractions were combined and concentrated under reduced pressure to an orange oil that crystallized overnight, which was purified on silica (hexanes), appropriate fractions were combined and concentrated under reduced pressure to a clear oil that crystallized (2.52 g, 35%).

3.2.30. (3*R*,4*S*)-Benzofuran-5-ylmethyl-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-amine and (3*S*,4*R*)-benzofuran-5-ylmethyl-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-amine (21). The title compound was prepared from compound **14** as per compound **20** utilizing 5-bromomethyl-benzofuran in the alkylation step. MH: m/z = 601.3 (M+1). Deprotection of the resulting compound afforded the title compound (31.3 mg, 46%). MH: m/z = 501.1 (M+1).

3.2.31. (1*H*-Indol-5-yl)-menthol. 1*H*-Indole-5-carboxylic acid methyl ester (5.05 g, 28.8 mmol) was dissolved in 20 mL of dry THF at 0 °C under argon. Lithium aluminum hydride (1.0 M) in THF (121 mL, 121 mmol) was added dropwise over 30 min and brought to reflux for 2 h. The solution was cooled, quenched with ethyl acetate, water (100 mL), dichloromethane (100 mL), and 10% aqueous Rochelle's salt (100 mL) was added and filtered. The aqueous residue was extracted with ethyl acetate, washed with brine, dried with magnesium sulfate, filtered, and concentrated under reduced pressure to an oil. The oil was purified on silica (ethyl acetate–hexanes (3:2)), appropriate fractions were combined, concentrated under reduced pressure to a clear oil (3.56 g, 84%).

3.2.32. 1*H*-Indole-5-carbaldehyde. (1*H*-Indol-5-yl)-menthol (0.564 g, 3.83 mmol) was dissolved in 20 mL of dichloromethane at room temperature and manganese oxide (2.78 g, 31.95 mmol) was added. The solution was allowed to stir overnight, filtered through silica gel with ethyl acetate to remove the solids, collected, and concentrated under reduced pressure to a white solid (0.533 g, 96%).

3.2.33. (3*R*,4*S*)-(1*H*-Indol-5-ylmethyl)-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-amine and (3*S*,4*R*)-(1*H*-indol-5-ylmethyl)-(4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-amine (22). Compound **14** (0.129 g, 0.274 mmol), 1*H*-indole-5-carbaldehyde (0.088 g, 0.60 mmol), and acetic acid (15.7 μL, 0.274 mmol) were combined in 10 mL of dichloromethane at room temperature under argon. After 30 min, sodium triacetoxyborohydride (0.130 g, 0.274 mmol) was added and the solution was allowed to stir overnight. The solution was purified directly on silica gel (ethyl acetate–hexanes (1:1)), appropriate fractions were combined and concentrated under reduced pressure to provide an oil (80.1 mg, 49%). Deprotection, purification, and conversion to the free base was performed using techniques for the preparation of compound **20** (14.1 mg, 22%). MS: m/z = 500.2 (M+1).

3.2.34. (3*R*,4*S*)-6-[(4-{4-[3-(2-Methoxy-benzyloxy)-propoxyl]-phenyl}-piperidin-3-ylamino)-methyl]-naphthalene-1-carboxylic acid and (3*S*,4*R*)-6-[(4-[4-(2-methoxy-benzyloxy)-propoxyl]-phenyl}-piperidin-3-ylamino)-methyl]-naphthalene-1-carboxylic acid (23). Compound **20** was dissolved in 4 mL of methanol–water (3:1) at room temperature with stirring. Lithium hydroxide was added and the solution was allowed to stir overnight. The reaction mixture was concentrated under reduced pressure to remove the methanol, absorbed to C18, washed with water, eluted with THF, concentrated, and lyophilized (15.2 mg, 13%). MS: m/z = 555.3 (M+1).

3.2.35. (3*R*,4*S*)-Naphthalene-1-carboxylic acid (4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-amide and (3*S*,4*R*)-naphthalene-1-carboxylic acid (4-{4-[3-(2-methoxy-benzyloxy)-propoxy]-phenyl}-piperidin-3-yl)-amide (24). The title compound was prepared from compound **14** as per compound **20** utilizing naphthalene-1-carbonyl chloride in the alkylation step. MH: m/z = 625.3 (M+1). Deprotection of the resulting compound afforded the title compound (36 mg, 43%). MH: m/z = 525.2 (M+1).

3.2.36. (3*R*,4*S*)-Biphenyl-4-ylmethyl-(4-{4-[3-(2-methoxy-benzyloxy)-propyl]-phenyl}-piperidin-3-yl)-amine and (3*S*,4*R*)-biphenyl-4-ylmethyl-(4-{4-[3-(2-methoxy-benzyloxy)-propyl]-phenyl}-piperidin-3-yl)-amine (25). The title compound was prepared from compound **14** as per compound **20** utilizing 4-chloromethyl-biphenyl in the alkylation step. MH: m/z = 637.3 (M+1). Deprotection of the resulting compound afforded the title compound (23 mg, 49%). MH: m/z = 537.3 (M+1).

3.3. Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)

All final compounds were consistent with the ^{1}H NMR spectra. ^{1}H NMR data for compound **2** were collected on a Varian Inova 400 MHz spectrometer. One-dimensional ^{1}H NMR (400 MHz, methanol-d_4 and pyridine-d_5) peaks were assigned with the aid of COSY and HSQC. Two solvents were to obtain the resolution required to establish and resolve the proton NMR spectrum in the aromatic (methanol-d_4) and the aliphatic (pyridine-d_5) regions. Over 20 cross peaks from eight piperidine protons in the 2D NOESY experiments were observed. These data clearly established that the relative stereochemistry of the two chiral side chains as *cis*. In addition, the coupling constants of the two chiral protons were indicative of a gauche relationship between these protons and were also consistent with the *cis* stereochemistry model.

3.4. X-ray crystallography

The small molecule single crystal structure of compound **2** was solved as the 2:1 (freeform to counterion) edisylate salt. X-ray data was collected at ambient temperature on a Brunker-AXS APEX diffractometer. The structure was solved by direct methods utilizing the SHELXTL suite version 6.10 and clearly established the relative stereochemistry as *cis*.

A binary complex of human renin protein with compound **2** was produced by soaking the ligand in dimethyl sulfoxide with a buffer containing renin crystals. After two days of soaking, crystals were quickly dipped in 20% ethylene glycol containing a cryo-solution and flash-cooled in liquid nitrogen before data collection. X-ray diffraction intensity data were collected under cryogenic conditions at the IMCA-CAT 17-ID beamline at Advanced Photon Source (Argonne National Laboratories, Argonne, IL). The crystal diffracted to 2.1 Å resolution and belongs to the space group $P2_13$ with two molecules per asymmetric unit. The structure of the binary complex was refined starting with previous refined Renin model structure, less the ligand, with program CNX. A rigid-body rotation–translation refinement was initially carried out to place the model structure more accurately in the new unit cell. Crystallographic refinement was continued by conjugated-gradient minimization and individual B factor refinement with

CNX, and model/ligand building was performed with the program QUANTA. The final model includes 668 residues, 175 water molecules, and two molecules (**2**) with an R factor of 23.4% and an R_f of 26.5%.

3.5. In vitro renin IC_{50} determinations

The renin assay utilized a tandem Green Flourescent Protein (tGFP) substrate (175 nM) that was hydrolyzed by renin human (50.4 IU/well). The tGFP substrate contained a nine amino acid (Ile-His-Pro-Phe-His-Leu-Val-Ile-His) recognition sequence for human renin flanked by two GPF 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 fourfold eleven-point dilution regiment.

3.6. 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 five male mice expressing human angiotensinogen (h-Ang 204/1) and six female mice expressing human renin (h-Ren 9) obtained from the University of Iowa. Mice expressing both transgenes were obtained through a breeding program conducted at Charles River Laboratories (Wilmington, 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 a radiotransmitter (model TA11PA-C20, 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-thiazolyl)-*N*-[(1*S*,2*R*,3*S*)-1-(*cyclo*-hexylmethyl)-2,3-dihydroxy-5-methylhexyl]-(HCl))[11,12] 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 2 h of a 30 mg/kg oral dose of either CI-992 or compound **2**. At the nadir, MABP was normalized, but returned to baseline hypertensive levels approximately 6–8 h later.

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