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SYNTHESIS AND AFFINITY EVALUATION FOR AT1 RECEPTOR OF PHENYLSALICYLALDOXIME-DERIVATIVES STRUCTURALLY RELATED TO SARTANS

Simona Rapposelli,[*1] Serena Cuboni,[2] Maria Digiacomo,[1] Antonio Lucacchini,[2] Filippo Minutolo,[1] Maria Letizia Trincavelli,[2] and Aldo Balsamo[1]

[1]Department of Medicinal Chemistry, University of Pisa, Via Bonanno, 6 56126 Pisa, Italy rappsi@farm.unipi.it
[2]Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy

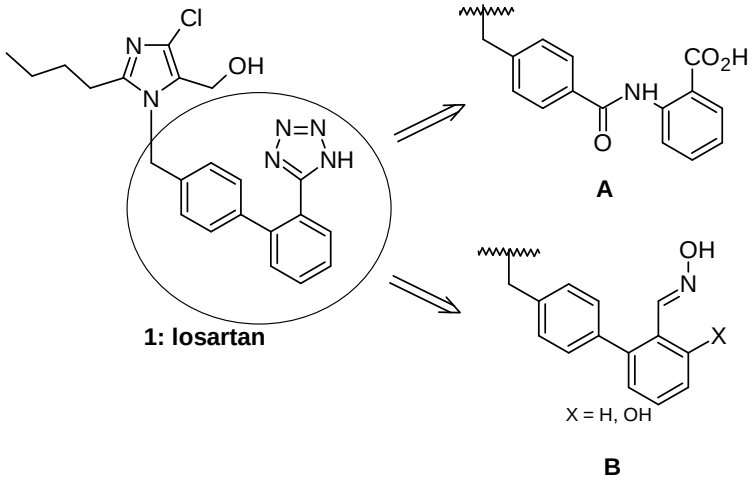
Abstract – In this work we reported the synthesis of new potential AT1 antagonists through the replacement of the biphenyltetrazole portion of the losartan with biphenylaldoximic (**2**) and phenylsalicylaldoximic (**3a**) moieties. Moreover, also the trifluoromethylpyrazole analogue of **3a** (**3b**) was prepared. The new compounds synthesized were evaluated for their AT1 affinity through binding assay carried out on rat liver membranes using [^{125}I]Sar1,Ile8-angiotensina II as radioligand.

INTRODUCTION

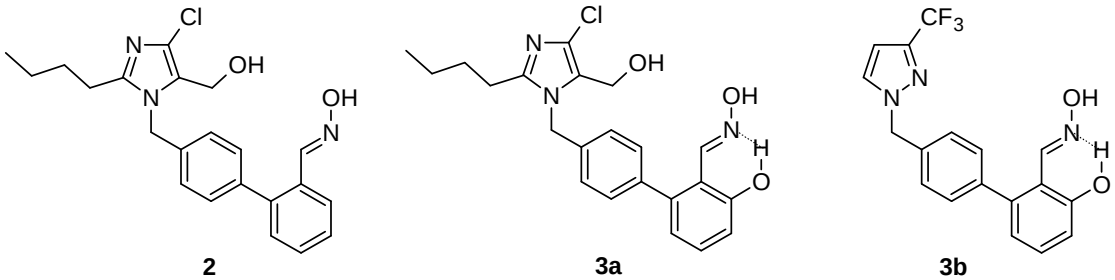
The AT1 antagonists, also known as sartans, constitutes an heterogeneous chemical class of anti-hypertensive drugs, which, together with ACE-inhibitors, seem to represent the first-choice drugs for the therapy of cardiovascular diseases.[1,2] In particular, sartans are antagonists of the angiotensin II at the AT1 receptors and block the angiotensin II action in a potentially more complete way than ACE-inhibitors. This mechanism results in reduced collateral effects with respect to ACE-inhibitors. Indeed, many comparative clinical trials showed that sartans do not induce the cough, a common side-effect of ACE-inhibitors, due to their capacity to preserve kinins from the hydrolisis. The use of these last inhibitors improves levels of kinins, and in particular of bradykinin leading to a stimulation of the cough-centre[3] and a subsequent appearance of the cough as adverse side-effect.

On this basis, in last decades many efforts have been made in the synthesis of new AT1 antagonists in

order to obtain more potent anti-hypertensive drugs possessing better pharmacological profile. In particular, starting from losartan, which constitutes the progenitor of the class of AT1 antagonists, many chemical manipulation such as bioisosteric substitution of imidazole core with other heteroaromatic systems or aliphatic substituents lead to the discovery of new sartans, actually in clinical use. In a previously study (unpublished data), we attended to replace the biphenyltetrazole system of losartan (**1**) with a carboxybenzamidophenyl system, but the AT1 activity was dramatically reduced; this result was tentatively attributed to a modification of the conformational freedom of the new derivatives (**A**) with respect to losartan, which may hinder an optimal fit of the new compounds with the receptor site. Continuing our studies addressed to deepen the knowledge of the importance of some molecular portions of sartans in determining their affinity for AT1 receptor, we decided to verify if the tetrazolic portion of losartan could be substituted by another acidic group, different from those previously used by other authors (carboxylic, sulphonamidic).[4-6]



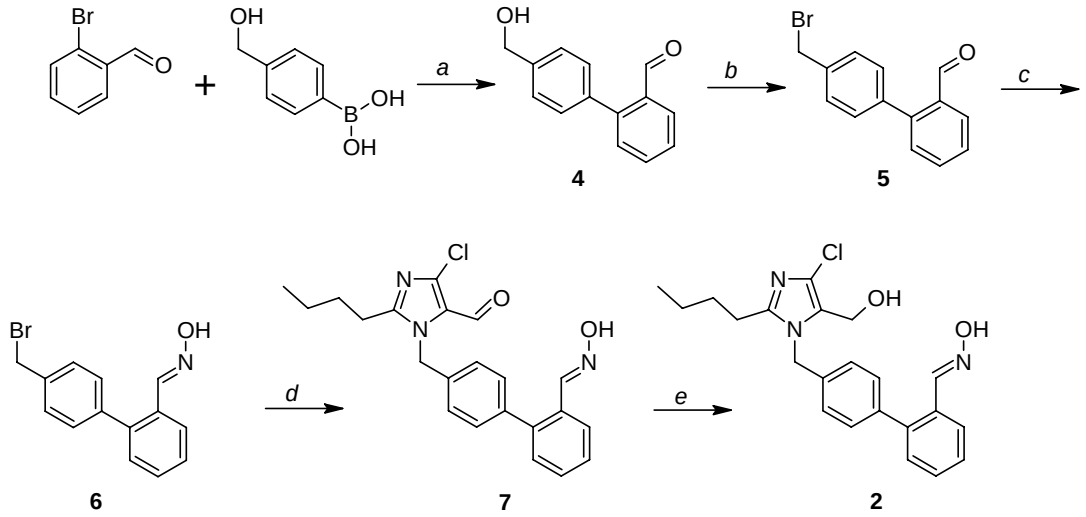
In particular, our attention has been directed to the aldoximic function which possesses a slight acidity, also if considerably lower than that of unsubstituted tetrazole of losartan, and to the couple of the oximic and phenolic functions successfully used in other pharmaceutical classes.[7] Then, we decided to afford the synthesis of new compounds (**B**) in which the biphenyltetrazole portion has been replaced by the biphenylaldoximic (**2**) and phenylsalicylaldoximic (**3a**) ones. Moreover, also the trifluoromethylpyrazole analogue of **3a** (**3b**), has been synthesised.



In **3a** and **3b** the presence of the OH phenolic in the *ortho* position of aldoximic moiety, through the formation of the intramolecular hydrogen bond between the hydroxylic group and the lone pair of the nitrogen atom, is able to stabilize the oxime tautomeric form allowing the formation of a pseudo-phenolic ring, which has furnished interesting results in particular in the field of the estrogen receptors ligands.[7]

The phenylbenzaldoximic derivative **2** was synthesised according the procedure showed in Scheme 1. The palladium-catalyzed cross-coupling reaction of 2-bromo-benzaldehyde with 4-(hydroxymethyl)-phenylboronic acid following the procedure described by Suzuki et al[8] in the presence of a 2M aqueous solution of K_3PO_4 afforded the benzylic alcohol **4** which was converted in the benzylbromide derivative **5** for treatment with CBr_4 and triphenylphosphine. The subsequent condensation of the aldehyde **5** with hydroxylamine hydrochloride in refluxing ethanol gave the corresponding oxime **6** which is almost exclusively of *E*-configuration. This configuration may be assigned on the basis of chemical shift value of the oxime proton which in the aromatic *E*-oximes resonates in the δ 8.00-8.50 range, while in the *Z*-ones resonates generally between 7.3 – 7.60 ppm.

Scheme 1.



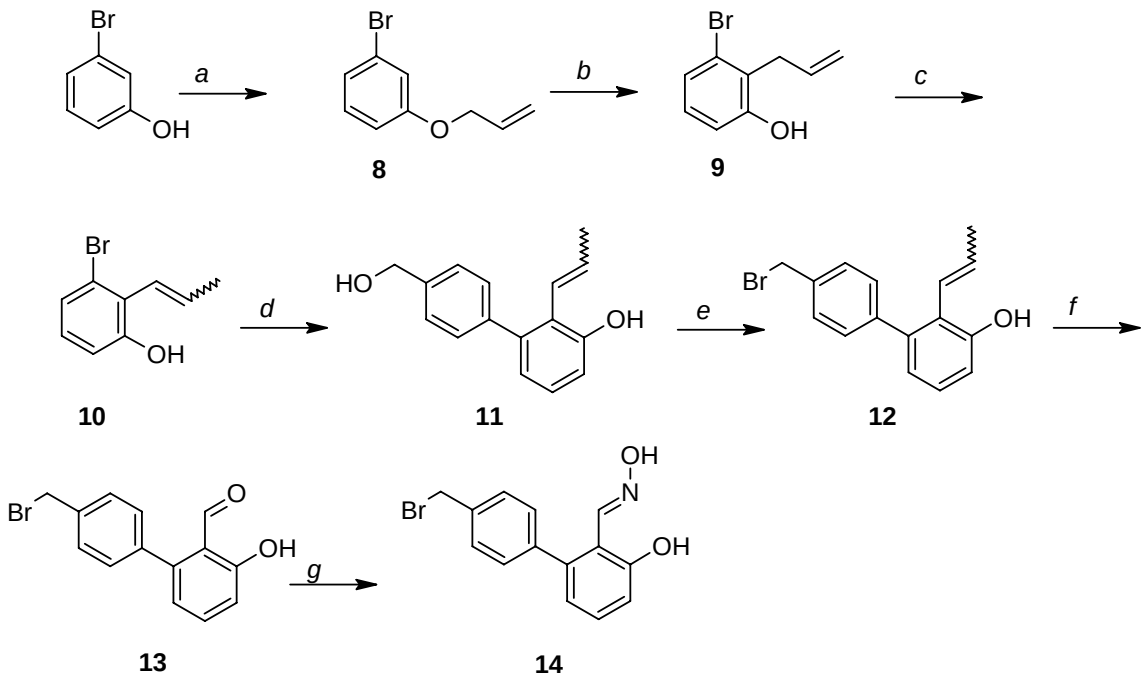
a: 2M K_3PO_4, $Pd(PPh_3)_4$, dioxane, reflux, 2 h; ***b:*** CBr_4, PPh_3, DMF, rt, 4 h; ***c:*** $NH_2OH{\cdot}HCl$, EtOH, 55 °C, 30 min; ***d:*** 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde, DMAC, K_2CO_3, rt, 22 h; ***e:*** $NaBH_4$, MeOH, rt, 1 h.

The alkylation of 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde in the presence of *N*,*N*-dimethylacetamide (DMAC) furnished the imidazole derivative **7** in a ratio 9:1 with its regioisomer N_3-substituted. The regioisomers were assigned by analogy with the results of alkylation reported by Carini et al.[9] Reduction of **7** with sodium borohydride afforded the compound **2.**

The synthesis of derivatives **3a,b** required the preparation of the common intermediate **14** obtained following the procedure reported in Scheme 2. Commercially available 3-bromophenol was treated with

allyl bromide to give allyl ether **8**, which was submitted to a Claisen rearrangement at 200 °C, yielding 2-allyl-3-bromophenol **9** together with the other *ortho*-allyl-substituted derivative (2-allyl-5-bromophenol), from which it was separated by column chromatography. Alkaline isomerization of the terminal double bond of **9** afforded an *E/Z* diastereoisomeric mixture of **10** in a ratio 70/30. Palladium-catalyzed cross coupling reaction of **10** with 4-(hydroxymethyl)phenylboronic acid afforded biphenyl derivative **12**. The hydroxymethyl group was then converted in high yields in its corresponding alkylbromide under mild conditions (Appel reaction). Oxidative cleavage of the double bond of **12**, carried out with sodium periodate in the presence of catalytic amounts of osmium tetroxide, yielded salicylaldehyde **13**, and its condensation with hydroxylamine hydrochloride afforded the oxime-intermediate **14**.

Scheme 2.



a: allyl bromide, K_2CO_3, MeCN, 80 °C; ***b:*** 200 °C; ***c:*** *t*-BuOK, DMSO, 55 °C, 4 h; ***d:*** 4-(hydroxymethyl)phenylboronic acid, $Pd(PPh_3)_4$, 2M K_3PO_4, reflux, 5 h; ***e:*** CBr_4, PPh_3, DMF, rt, 4 h; ***f:*** OsO_4, $NaIO_4$, dioxane-H_2O, rt, 6 h; ***g:*** $NH_2OH{\cdot}HCl$, EtOH, 55 °C, 30 min.

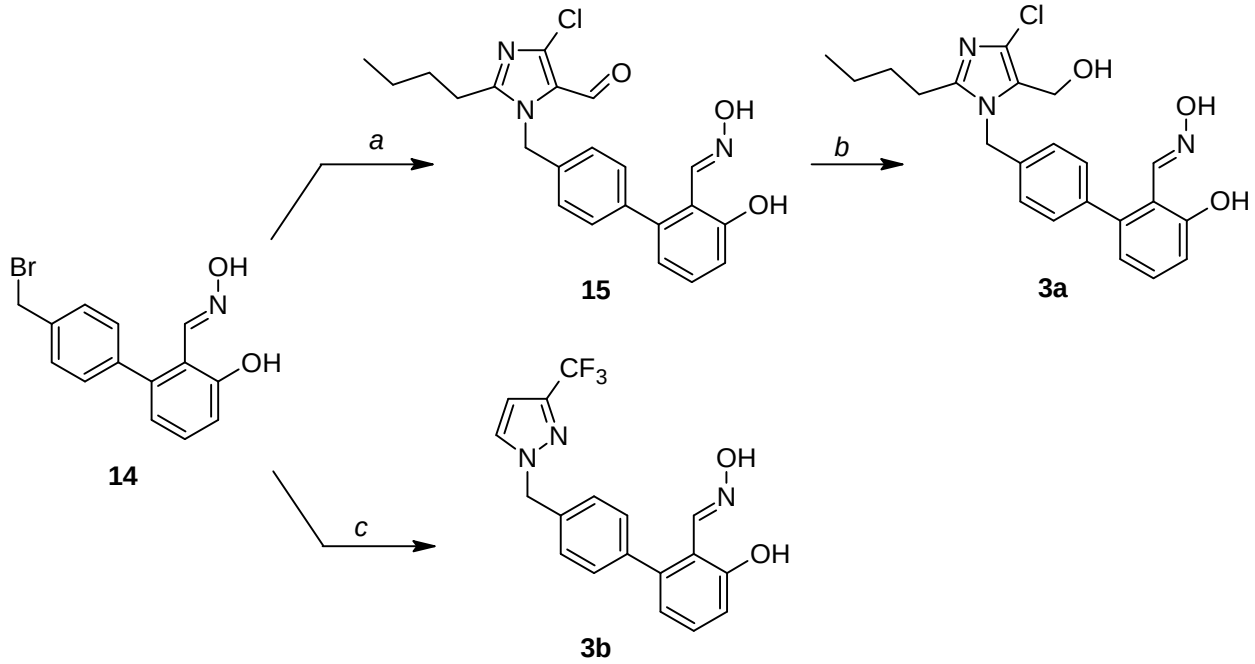
From this compound (**14**) the imidazolic salicylaldoximic derivative **3a** was obtained (see Scheme 3) by reaction with 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde in the presence of DMAC to yield compound **15** which was then submitted to a subsequent reduction with sodium borohydride. The pyrazolic analog of **3a** (**3b**) was obtained by reaction of **14** with 3-trifluoromethylpyrazole to afford almost exclusively the N_1-substituted regioisomer **3b** as indicated from the coupling costant value of the H4-H5 protons (J = 2.6 Hz) which is in accordance with the value reported in literature for analogue structures.[10]

RESULTS AND DISCUSSION

Compounds **2**, **3a** and **3b** have been tested for their AT1 affinity through a binding assay carried out on rat liver membrane using [^{125}I]Sar1,Ile8-angiotensina II as radioligand.

Among the synthesized compounds only the salicylaldoximic derivative **3a** showed a modest affinity towards AT1 receptor with percentage inhibition values of about 40% at 10 μM, while its pyrazole analogue **3b** and the desoxy-compound **2** where practically devoid of any affinity. These results confirm the importance of the heteroaromatic portion for the affinity of sartans towards AT1 receptor, as shown by the complete inactivity of **3b** with respect to its analogue **3a**, but, at the same time, these results also indicate that an acidic function different from those up to now studied, as the salicylaldoximic one of compound **3a** may be able to permit to new compounds a slight interaction with AT1 receptor.

Scheme 3.



a: 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde, DMAC, K_2CO_3, rt, 2 h; ***b:*** $NaBH_4$, MeOH, rt, 2 h; ***c:*** 3-trifluoromethylpyrazole, K_2CO_3, KI, MeCN, reflux, 30 min.

EXPERIMENTAL

Chemistry.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. The elemental compositions of the compounds agreed to within ± 0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40,

0.040–0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualised under a UV lamp. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. Commercially available chemicals were purchased from Sigma-Aldrich.

4'-{[5-(Hydroxymethyl)-2-butyl-4-chloro-imidazolyl]methyl}biphenyl-2-carbaldehyde oxime 2.

Sodium borohydride (5.30 mg; 0.14 mmol) was added to a solution of compound **7** (0.19 g; 0.48 mmol) in MeOH (1.00 mL) cooled to -10 °C. The resulting solution was stirred at -10 °C for 20 min and then was allowed to rt. After 2 h the mixture was quenched by addition of $MeCO_2H$ 50% (0.02 mL) and the resulting emulsion was extracted with AcOEt. The organic layer was washed with H_2O, dried and evaporated. The crude product was purified by trituration with *i*-PrOH affording compound **2** (0.13 g, 0.34 mmol, 70 % yield): ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, *J* = 7.0 Hz, CH_3); 1.29-1.38 (m, 2H, CH_2); 1.58-1.68 (m, 2H, CH_2); 2.55-2.60 (m, 2H, CH_2); 4.51 (s, 2H, CH_2OH); 5.27 (s, 2H, CH_2Ph); 7.04 (d, 2H, *J* = 7.4 Hz, Ar); 7.27-7.38 (m, 5H, Ar); 7.87-7.89 (m, 1H, Ar); 8.03 (s, 1H, CH=N). MS *m/z*: 397 (M^+, 7); 192 (M^+ -imidazole, -OH, 100). Anal. Calcd for $C_{23}H_{24}ClN_3O_4$: C 66.50; H 6.04; N 10.58. Found: C 66.20; H 6.14; N 10.71.

3-Hydroxy-4'-{[5-(hydroxymethyl)-2-butyl-4-chloro-imidazolyl]methyl}biphenyl-2-carbaldehyde oxime 3a.

The compound **3a** was prepared by the method described for **2** starting from compound **15** (0.20 g; 0.48 mmol). The crude product was purified by precipitation from AcOEt/*n*-hexane to give **3a** (0.04 g, 0.09 mmol, 18 % yield): mp 118-120 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 7.1 Hz, CH_3); 1.18-1.41 (m, 2H, CH_2); 1.60-1.72 (m, 2H, CH_2); 2.61 (t, 2H, *J* = 7.6 Hz, CH_2); 4.56 (s, 2H, CH_2OH); 5.28 (s, 2H, CH_2Ph); 6.80 (d, 1H, *J* = 7.3 Hz, Ar); 6.98-7.08 (m, 2H, Ar); 7.26-7.30 (m, 4H, Ar); 8.16 (s, 1H, CH=N). MS *m/z*: 414 (M^+, 3). Anal. Calcd for $C_{23}H_{24}ClN_3O_4$: C 63.77; H 5.80; N 10.14. Found: C 63.51; H 5.77; N 10.10.

3-Hydroxy-4'-{[3-(trifluoromethyl)pyrazolyl]methyl}biphenyl-2-carbaldehyde oxime 3b.

To a solution of **14** (0.21 g; 0.69 mmol) in MeCN (3.4 mL) were added 3-(trifluoromethyl) pyrazole (93 mg; 0.69 mmol), K_2CO_3 (0.16 g; 1.16 mmol) and KI (14 mg; 0.08 mmol). The resulting suspension was refluxed for 30 min and after this period the mixture was cooled to rt and filtered. The solvent was evaporated to give crude product that was purified by column chromatography eluting with AcOEt/*n*-hexane (2:8) afforded **3b** (74 mg, 0.21 mmol, 30 % yield): ^{1}H NMR ($CDCl_3$) δ 5.42 (s, 2H, CH_2Ph); 6.59 (d, 1H, *J* = 2.4 Hz, pyrazole); 6.80 (d, 1H, *J* = 7.4 Hz, Ar); 7.00 (d, 1H, *J* = 8.2 Hz, Ar); 7.26-7.35 (m, 4H, Ar); 7.48 (d, 1H, *J* = 2.4 Hz, pyrazole); 8.17 (s, 1H, CH=N); 10.33 (br s, 1H, OH). MS *m/z*: 361 (M^+, 34). Anal. Calcd for $C_{18}H_{13}F_3N_3O_2$: C 59.83; H 3.60; N 11.63. Found: C 59.59; H 3.59; N 11.58.

4'-(Hydroxymethyl)biphenyl-2-carbaldehyde 4.

To a solution of 2-bromobenzaldehyde (0.50 g; 2.70 mmol) in dioxane (13.7 mL), were added 4-(hydroxymethyl)phenylboronic acid (0.96 g; 6.49 mmol), aqueous K_3PO_4 2M (7 mL) and $Pd(PPh_3)_4$ (0.18 g; 0.15 mmol) and the reaction mixture was refluxed for 2 h. Then the mixture was cooled to rt, diluted with water and extracted with AcOEt. The organic layer was dried and evaporated. The crude product was purified by column chromatography eluting with AcOEt/*n*-hexane (4:6) to give **4** (1.25 g, 5.90 mmol, 91 % yield): ^{1}H NMR ($CDCl_3$) δ 4.80 (s, 2H, CH_2OH); 7.36-7.53 (m, 6H, Ar); 7.61-7.69 (m, 1H, Ar); 8.04 (d, 1H, *J* = 7.1 Hz, Ar); 9.98 ppm (s, 1H, CHO). MS *m/z*: 212 (M^+, 29), 181 (M^+-CH_2OH, 56). Anal. Calcd for $C_{14}H_{12}O_2$: C 79.24; H 5.66. Found: C 78.92; H 5.64.

4'-(Bromomethyl)biphenyl-2-carbaldehyde 5.

To a solution of the benzylic alcohol **4** (0.39 g; 1.82 mmol) in DMF (12.2 mL) cooling to 0 °C, was added CBr_4 (1.35 g; 4.09 mmol) and PPh_3 (1.08 g; 4.09 mmol). The resulting solution was stirred at rt for 4 h, diluted with AcOEt and washed with H_2O. The organic phase was dried and evaporated. The residue was purified by column chromatography eluting with AcOEt/*n*-hexane (2:8) to give **5** (226 mg, 0.75 mmol, 41 % yield): mp 64-66 °C; ^{1}H NMR ($CDCl_3$) δ 4.57 (s, 2H, CH_2Br); 7.35-7.42 (m, 2H, Ar); 7.45-7.55 (m, 4H, Ar); 7.61-7.70 (m, 1H, Ar); 8.03 (d, 1H, *J* = 7.7 Hz, Ar); 9.98 (s, 1H, CHO). MS *m/z*: 276 (M^+, 6), 195 (M^+-Br, 100). Anal. Calcd for $C_{14}H_{11}BrO$: C 60.87; H 3.99. Found: C 60.63; H 3.97.

4'-(Bromomethyl)biphenyl-2-carbaldehyde oxime 6.

To a solution of the aldehyde **5** (0.33 g; 1.24 mmol) in EtOH (22 mL) was added a solution of hydroxylamine hydrochloride (0.12 g; 1.72 mmol) in H_2O (1.4 mL). The reaction mixture was stirred at 50 °C for 1 h, then the solvent was evaporated and the residue was crystallized from MeOH to give **6** (90 mg, 0.31 mmol, 25 % yield): mp 125-127 °C; ^{1}H NMR ($CDCl_3$) δ 4.55 (s, 2H, CH_2Br); 7.75-7.49 (m, 7H, Ar); 7.89 (d, 1H, *J* = 5.7 Hz, Ar); 8.09 (s, 1H, CH=N). MS *m/z*: 290 (M^+, 5), 210 (M^+-Br, 95). Anal. Calcd for $C_{14}H_{12}BrNO$: C 57.93; H 4.14; N 4.83. Found: C 58.16; H 4.16; N 4.85.

4'-{[5-(Carbaldehyde)-2-butyl-4-chloro-imidazolyl]methyl}biphenyl-2-carbaldehyde oxime 7.

To a solution of compound **6** (80 mg; 0.28 mmol) and 2-butyl-4-chloro-5-carbaldehydeimidazole (52 mg; 0.28 mmol) in DMAC (0.6 mL), cooled at -10 °C, was added K_2CO_3 (39 mg; 0.28 mmol). The resulting mixture was stirred at -10 °C for 2 h, and at rt for 20 h. After this period the suspension was filtered and the organic phase was evaporated to give **7** (87 mg, 0.22 mmol, 80 % yield): ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, *J* = 7.2, CH_3); 1.31-1.43 (m, 2H, CH_2); 1.64-1.75 (m, 2H, CH_2); 2.64-2.73 (m, 2H, CH_2); 5.61 (s, 2H, CH_2Ph); 7.12 (d, 2H, *J* = 8.2 Hz, Ar); 7.22-7.30 (m, 3H, Ar); 7.37-7.44 (m, 2H, Ar); 7.89-7.93 (m, 1H, Ar); 8.04 (s, 1H, CH=N); 9.78 (s, 1H, CHO). MS *m/z*: 395 (M^+, 20); 192 (M^+-imidazole, -OH, 100).

1-(Allyloxy)-3-bromobenzene 8.

A solution of 3-bromophenol (4.00 g; 23.12 mmol) in MeCN (18 mL) was treated with K_2CO_3 (0.94 g;

6.80 mmol) and the resulting mixture was heated to 80 °C. Then allyl bromide (0.77 g, 6.40 mmol) was added dropwise and stirring was continued at the same temperature for 1 h. After being cooled to rt, the resulting suspension was filtered and the filtrate was concentrated to give **8** (4.43 g, 20.81 mmol, 90 % yield): ^{1}H NMR ($CDCl_3$) δ 4.50-4.54 (m, 2H, OCH_2); 5.27-5.46 (m, 2H, $=CH_2$); 5.94-6.13 (m, 1H, CH=); 6.82-6.88 (m, 1H, Ar); 7.05-7.18 (m, 3H, Ar).

2-Allyl-3-bromophenol 9.

Compound **8** (4.92 g; 23.12 mmol) was heated to 200 °C for 6 h. After being cooled to rt the residue was subjected to a column chromatography eluting with AcOEt/*n*-hexane (1:9) to give **9** (1.19 g, 5.57 mmol, 24 % yield) ^{1}H NMR ($CDCl_3$) δ 3.63 (d, 2H, *J* = 5.3 Hz, CH_2); 5.08-5.16 (m, 2H, $=CH_2$); 5.88-6.07 (m, 1H, CH=); 6.76 (d, 1H, *J* = 7.9 Hz, Ar); 6.98 (dd, 1H, *J* = 7.9, 8.1 Hz, Ar); 7.17 (d, 1H, *J* = 8.1 Hz, Ar).

3-Bromo-2-propenylphenol 10.

To a solution of 2-allyl-3-bromophenol **9** (0.45 g; 2.10 mmol) in DMSO (7 mL), was added *t*-BuOK (0.59 g; 5.25 mmol), and the resulting mixture was heated to 5 °C for 2 h. Then, after being cooled to rt, the mixture was treated with aqueous HCl (1N) and extracted with Et_2O. The organic phase was dried and evaporated to give a crude residue as a 7:3 *E/Z* diastereomeric mixture **10** (0.42 g, 1.99 mmol, 95 % yield): ^{1}H NMR ($CDCl_3$) δ 1.98 (dd, 3H, *J* = 1.4, 6.4 Hz, CH_3); 5.64 (br s, 1H, OH); 5.98-6.16 (m, 1H, =CH); 6.31-6.39 (m, 1H, CH=); 6.87 (d, 1H, *J* = 8.1 Hz, Ar); 6.98 (dd, 1H, *J* = 7.7, 8.1, Hz, Ar); 7.12 (d, 1H, *J* = 7.7 Hz, Ar). MS *m/z*: 213 (M^+, 100).

4'-(Hydroxymethyl)-3-hydroxybiphenyl-2-propene 11.

Compound **11** was synthesized from **10** (0.59 g; 2.76 mmol) following the same procedure described above for the preparation of **4**. The crude product was dissolved in Et_2O and extracted with an aqueous solution of NaOH (2N) to obtained the corresponding phenolate. Then the aqueous phase was acidified with conc. HCl to pH = 2 and extracted with Et_2O. The organic layer was dried and evaporated to give **11** (0.44 g, 1.82 mmol, 66 % yield): ^{1}H NMR ($CDCl_3$) δ 1.85 (d, 3H, *J* = 4.6 Hz, CH_3); 4.75 (s, 2H, CH_2OH); 5.95-6.08 (m, 2H, CH=CH); 6.86 (d, 1H, *J* = 7.5 Hz, Ar); 6.95 (d, 1H, *J* = 8.1 Hz, Ar); 7.18 (dd, 1H, *J* = 7.5, 8.1 Hz, Ar); 7.32-7.37 (m, 4H, Ar). MS m/z: 240 (M^+, 29), 209 (M^+-CH_2OH, 100). Anal. Calcd for $C_{16}H_{16}O_2$: C 80.00; H 6.67. Found: C 79.68; H 6.64.

4'-(Bromomethyl)-3-hydroxybiphenyl-2-propene 12.

The compound **12** was prepared by the method described for the synthesis of compound **5** starting from compound **11** (0.65 g; 2.70 mmol). The crude product was chromatographed in AcOEt/*n*-hexane (2:8) to give **12** (0.33 g; 1.11 mmol, 41 % yield): ^{1}H NMR ($CDCl_3$) δ 1.86 (d, 3H, *J* = 4.7 Hz; CH_3); 4.55 (s, 2H, CH_2Br); 5.95-6.08 (m, 2H, CH=CH); 6.85 (d, 1H, *J* = 7.5, Ar); 6.95 (d, 1H, *J* = 8.1 Hz, Ar); 7.18 (dd, 1H, *J* = 7.5, 8.1 Hz, Ar); 7.30-7.43 (m, 4H, Ar).

4'-(Bromomethyl)-3-hydroxybiphenyl-2-carbaldehyde 13.

To a solution of compound **12** (0.44 g; 1.44 mmol) in dioxane (22 mL) and H_2O (3 mL) was added $NaIO_4$ (0.70 g; 3.30 mmol) and a 2.5 % solution of OsO_4 in *t*-BuOH (0.04 mL). The reaction mixture was stirred to rt for 2 h, then was diluted with H_2O and extracted with $CHCl_3$. The organic layer was dried and evaporated, and the residue was purified by column chromatography eluting with $CHCl_3$ to give **13** (0.23 g, 0.78 mmol, 54 % yield): ^{1}H NMR ($CDCl_3$) δ 4.56 (s, 2H, CH_2Br); 6.87 (d, 1H, *J* = 7.5 Hz, Ar); 7.01 (d, 1H, *J* = 8.4 Hz, Ar); 7.35 (d, 2H, *J* = 8.1 Hz, Ar); 7.50 (d, 2H, *J* = 8.1 Hz, Ar); 7.54 (dd, 1H, *J* = 7.5, 8.1 Hz, Ar); 9.83 (s, 1H, CHO); 11.90 ppm (br s, 1H, OH). Anal. Calcd for $C_{14}H_{12}BrNO_2$: C 57.73; H 3.78. Found: C 57.49; H 3.76.

4'-(Bromomethyl)-3-hydroxybiphenyl-2-carbaldehyde oxime 14

Compound **14** was synthesized from **13** (95 mg; 0.33 mmol) following the same procedure described above for compound **6**. The crude product was purified by trituration with $CHCl_3$ and evaporation of the organic solvent afforded to derivative **14** (96 mg, 0.31 mmol, 95 % yield): mp 102-104 °C; ^{1}H NMR ($CDCl_3$) δ 4.56 (s, 2H, CH_2Br); 6.83 (d, 1H, *J* = 7.5 Hz, Ar); 7.00 (d, 1H, *J* = 8.4 Hz, Ar); 7.27-7.32 (m, 3H, Ar); 7.47 (d, 2H, *J* = 8.1 Hz, Ar); 8.20 (s, 1H, CH=N); 10.34 ppm (br s, 1H, OH). MS *m/z*: 306 (M^+, 12), 226 (M^+-Br, 100). Anal. Calcd for $C_{14}H_{12}BrNO_2$: C 54.90; H 3.92; N 4.58. Found: C 55.12; H 3.94; N 4.60.

4-Chloro-1-({3'-hydroxy-2'-[*E*-(hydroxyimino)methyl]biphenyl-4-yl}methyl)-2-butyl-5-carbaldehyde imidazole 15

Compound **15** was synthesised from **14** (0.15 g; 0.48 mmol) as previously reported for compound **7**, to give **14** (0.17 g, 0.43 mmol, 89 % yield): ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 7.2 Hz, CH_3); 1.24-1.42 (m, 2H, CH_2); 1.67-1.75 (m, 2H, CH_2); 2.68 (t, 2H, *J* = 7.5 Hz, CH_2); 5.59 (s, 2H, CH_2Ph); 6.76 (d, 1H, *J* = 7.5 Hz, Ar); 6.96 (d, 1H, *J* = 8.2 Hz, Ar); 7.09 (d, 1H, *J* = 7.7 Hz, Ar); 7.23-7.30 (m, 4H, Ar); 8.13 ppm (s, 1H, CH=N). MS *m/z*: 411 (M^+, 3).

Angiotensin II Receptor Binding Assay.

Male Wistar rats were killed by decapitation, and their livers were rapidly removed. Rat liver membranes were prepared by differential centrifugation, as previously described.[11] Briefly, liver was dissected free of fatty tissue and minced accurately with small scissors, and then about 3g of tissue was homogenized by Polytron Ultra-Turrax (maximal speed for 2 x 30s) in ice cold 20 vol of Tris-HCl 5 mM, sucrose 0.25 M (pH 7.4). The homogenate was centrifuged at 750 g for 10 min at 4 °C and the supernatant was filtered through cheesecloth and saved. The pellets were homogenized and centrifuged as before. The combined supernatants were centrifuged at 48,000 g for 15 min at 4 °C. The resulting pellet was resuspended in Tris-HCl 5 mM, sucrose 0.25 M (pH 7.4), and centrifuged as above. The final pellets were used immediately or stored frozen at -70 °C before use. The membrane pellet was resuspended in Tris-HCl 50

mM, NaCl 100 mM, $MgCl_2$ 10 mM, EDTA 1 mM, bacitracin 100 μM, PMSF 100 μM, BSA 0.1% (pH 7.4) to obtain a final protein concentration of 2.5 mg/mL. ATII binding assay was performed incubating aliquots of liver membranes (50 μg) at 25 °C for 180 min in 100 μl assay buffer cointaining 25 pM [^{125}I]Sar^1,Ile^8-angiotensin II (Perkin Elmer life Sciences). Non specific binding was measured in the presence of 1 μM angiotensin II and represented 5-10% of total binding. Binding was terminated by rapid vacuum filtration using GF/C glass fiber filters performing three washes with 4 ml ice cold NaCl 100 mM, $MgCl_2$ 100 mM buffer. Dried filters disks were counted in a gamma-counter with 92% efficiency. The compound % inhibition values were estimated at 10 μM concentration. For all tested compounds IC_{50} values were not calculable.

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