Supporting Information

Antagonism/Agonism Modulation to Build Novel Antihypertensives Selectively Triggering I_1-Imidazoline Receptor Activation

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Chemistry

General. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian Mercury 400 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). IR spectral data (not shown because of the lack of unusual features) were obtained for all compounds reported and are consistent with the assigned structures. Mass spectra were obtained using a Hewlett Packard 1100 MSD instrument utilizing electron-spray ionization (ESI). The microanalyses were performed by the Microanalytical

Laboratory and the elemental composition of the compounds agreed to within ±0.4% of the calculated value. Optical rotation was measured at a 1 g/100 mL concentration (c = 1) with a Perkin-Elmer 241 polarimeter (accuracy ± 0.002 °). All reactions were monitored by thin-layer chromatography (TLC) using silica gel plates (60 F254; Merck), visualizing with ultraviolet light. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040-0.063 mm, Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by ChemBioDraw Ultra (version 11.0) software for systematically naming organic chemicals. The purity of the new compounds was determined by combustion analysis and was ≥ 95%.

Synthetic Procedures

2-(1-(2-(Thiophen-3-yl)phenyl)propan-2-yl)-4,5-dihydro-1*H*-imidazole Oxalate (5).

A mixture of **12** (3.11 g, 13.7 mmol) and sodium methoxide (0.03 g, 1.4 mL) in MeOH (6 mL) was stirred at rt for 18 h. After cooling to 0 °C, a solution of ethylenediamine (0.92 mL, 13.7 mmol) in MeOH (6 mL) was added dropwise under vigorous stirring; after few minutes a solution of 3N HCl in MeOH (4.8 mL, 14.4 mmol) was added dropwise and the mixture was stirred at rt for 18 h and filtered. After removal of the solvent, the residue was dissolved in water, the solution was basified with NaOH and extracted with $CHCl_3$. Removal of the dried solvent gave a residue which was purified by flash chromatography eluting with $CHCl_3$/MeOH/33%

NH_4OH (95:5:0.1) to afford an oil: 2.33 g (yield 63%). ^1H NMR ($CDCl_3$) δ 1.05 (d, 3, C*H*$_3$C), 2.75-3.05 (m, 3, C*H*$_2$C*H*), 3.56-3.78 (s, 4, NC*H*$_2$C*H*$_2$N), 5.05 (br s, 1, N*H*, exchangeable with D_2O), 7.18-7.75 (m, 7, Ar*H*). The free base was transformed into the oxalate salt which was recrystallized from EtOH: m.p. 143-145 °C. Anal. ($C_{16}H_{18}N_2S.H_2C_2O_4$) C, H, N, S.

3-(2-(2-(4,5-Dihydro-1*H*-imidazol-2-yl)propyl)phenyl)pyridine Oxalate (6).

HCl was bubbled through a stirred and cooled (0 °C) solution of **13** (2.12 g, 0.96 mmol) in MeOH (0.08 mL, 0.2 mmol) and dry $CHCl_3$ (3.22 mL) for 45 min. After 12 h at 4 °C, the solvent was removed in vacuo to give an oil (0.30 g, 1.20 mmol) which was dissolved in absolute EtOH (5 mL) and added to a cooled (0 °C) and stirred solution of ethylenediamine (0.22 mL, 3.24 mmol) in absolute EtOH (10 mL). After 1 h, concentrated HCl (0.11 mL) was added to the reaction mixture, which was stored overnight at 4 °C. The mixture was then diluted with absolute EtOH (10 mL) and heated to 70 °C for 5 h. After cooling, the solid was collected and discarded and the filtrate was concentrated and filtered again. The filtrate, evaporated to dryness, gave an oil which was taken up in $CHCl_3$ (20 mL) and washed with 2N NaOH. Removal of the dried solvent gave a residue which was dissolved in MeOH and hydrogenated for 8 h at rt under pressure (50 psi) using 10% Pd/C (0.4 g) as a catalyst. Following catalyst removal and evaporation of the solvent, the residue was purified by flash chromatography eluting with EtOAc/Cyclohexane/MeOH/33%NH_4OH (70:30:10:0.1) to give an oil: 0.46 g (18% yield). ^1H NMR ($CDCl_3$) δ 1.30 (d, 3,

C*H*$_3$C), 2.80-3.05 (m, 3, C*H*$_2$C*H*), 3.70 (s, 4, NC*H*$_2$C*H*$_2$N), 5.22 (br s, 1, N*H*, exchangeable with D_2O), 7.25-8.90 (m, 8, Ar*H*). The free base was transformed into the oxalate salt which was recrystallized from EtOH: m.p. 237-239 °C. Anal. ($C_{17}H_{19}N_3.H_2C_2O_4$) C, H, N.

2'-(2-(4,5-Dihydro-1*H*-imidazol-2-yl)propyl)-[1,1'-biphenyl]-3-ol Oxalate (7).

This was prepared as described for **6** starting from **14** (19% yield). ^{1}H NMR ($CDCl_3$) δ 1.00 (d, 3, C*H*$_3$C), 2.70-3.00 (m, 3, C*H*$_2$C*H*), 3.70 (s, 4, NC*H*$_2$C*H*$_2$N), 4.90 (br s, 1, N*H*, exchangeable with D_2O), 6.55-7.35 (m, 8, Ar*H*), 9.27 (br s, 1, O*H*, exchangeable with D_2O). The free base was transformed into the oxalate salt which was recrystallized from EtOH: m.p. 123-125 °C Anal. ($C_{18}H_{20}N_2O.H_2C_2O_4$) C, H, N.

2-(1-(*o*-Tolyl)propan-2-yl)-4,5-dihydro-1*H*-imidazole Hydrochloride (8).

A solution of ethylenediamine (0.47 mL, 6.50 mmol) in dry toluene (5 mL) was added dropwise to a mechanically stirred solution of 2M trimethylaluminum in hexane (3.5 mL, 6.50 mmol) in dry toluene (5 mL) at 0 °C under a nitrogen atmosphere. After 1 h, the solution was cooled to 0 °C and a solution of **16** (0.76 g, 3.50 mmol) in dry toluene (10 mL) was added dropwise. The reaction mixture was heated to 90°C for 12 h, cooled to 0 °C and quenched cautiously with MeOH (4.5 mL), followed by H_2O (1 mL). After addition of $CHCl_3$ (35 mL) and filtration, the organic layer was extracted with 2N HCl. The aqueous layer, made basic with 10% NaOH, was extracted with $CHCl_3$. Removal of dried solvent gave a residue which

was purified by flash chromatography eluting with $CHCl_3$/MeOH/33%NH_4OH (95:5:0.1) to afford an oil: 1.14 g (72% yield). ^1H NMR ($CDCl_3$) δ 1.20 (d, 3, C*H*$_3$C), 2.33 (s, 3, C*H*$_3$Ar), 2.80-3.10 (m 3, C*H*$_2$C*H*), 3.80 (s, 4, NC*H*$_2$C*H*$_2$N), 5.46 (br s, 1, N*H*, exchangeable with D_2O), 7.10-7.21 (m, 4, Ar*H*). The free base was transformed into the hydrochloride salt, which was recrystallized from EtOH: mp 166-167 °C. Anal. ($C_{13}H_{18}N_2$.HCl) C, H, N.

Resolution of 2-(1-(*o*-Tolyl)propan-2-yl)-4,5-dihydro-1*H*-imidazole (±)-8

Racemic **8** (1 g, 4.95 mmol) in EtOH (30 mL) was treated with a solution of (+)-O,O'-dibenzoyl-D-tartaric acid (1.54 g, 4.3 mmol) in EtOH (35 mL) and left at room temperature for 30 h. The white crystals were crystallized twice from EtOH: 1.1 g yield. The salt was dissolved in water (50 mL) and the ice-cooled solution was made basic with 2 N NaOH and extracted with EtOAc (3 x 30 mL). Removal of dried solvent gave (*S*)-(+)-**8**: 0.4 g; $[\alpha]^{20}_D$ = +22.13 (c 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemic compound **8**. A similar treatment of **8** with (-)-O,O'-dibenzoyl-L-tartaric acid gave the other enantiomer (*R*)-(-)-**8**: $[\alpha]^{20}_D$ = -21.88 (c 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemic compound **8**.

2-(1-(2-Chlorophenyl)propan-2-yl)-4,5-dihydro-1*H*-imidazole Oxalate (9).

HCl was bubbled through a stirred and cooled (0 °C) solution of **15** (0.22 g, 1.20 mmol) in MeOH (0.08 mL, 0.2 mmol) and dry $CHCl_3$ (3.22 mL) for 45 min. After 12 h at 4 °C, the solvent was removed in vacuo to give an oil (0.30 g, 1.20 mmol) which

was dissolved in absolute EtOH (5 mL) and added to a cooled (0 °C) and stirred solution of ethylenediamine (0.22 mL, 3.24 mmol) in absolute EtOH (10 mL). After 1 h, concentrated HCl (0.11 mL) was added to the reaction mixture, which was stored overnight at 4 °C. The mixture was then diluted with absolute EtOH (10 mL) and heated to 70 °C for 5 h. After cooling, the solid was collected and discarded and the filtrate was concentrated and filtered again. The filtrate, evaporated to dryness, gave an oil which was taken up in $CHCl_3$ (20 mL) and washed with 2N NaOH. Removal of the dried solvent gave a residue which was purified by flash chromatography eluting with EtOAc/Cyclohexane/MeOH/33%NH_4OH (70:30:10:0.1) to give an oil: 0.18 g (68% yield). ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3, C*H*$_3$C), 2.95-3.15 (m, 3, C*H*$_2$C*H*), 3.80 (m, 4, NC*H*$_2$C*H*$_2$N), 5.20 (br s, 1, N*H*, exchangeable with D_2O), 7.30-7.55 (m, 4, Ar*H*). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: m.p. 152-153 °C. Anal. ($C_{12}H_{15}N_2Cl.H_2C_2O_4$) C, H, N.

3-(2-Bromophenyl)-2-methylacrylonitrile (10).

A solution of diethyl-(1-cyanoethyl)-phosphonate (1mL, 5.73 mmol) in DME (5 mL) was added dropwise to a suspension of NaH (0.14 g, 5.57 mmol) in DME (5 mL) at 0 °C under a nitrogen atmosphere. After 15 min, a solution of 2-bromobenzaldehyde (Aldrich) (0.63 mL, 5.40 mmol) in DME (5 mL) was added dropwise. The reaction mixture was stirred overnight at rt, cooled at 0°C and quenched with an excess of H_2O. Then it was extracted with EtOAc and the organic layer was dried over Na_2SO_4.

Removal of the solvent gave an oil: 1.02 g (80% yield). ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3, C*H*$_3$C), 7.20-7.90 (m, 5, Ar*H* and C*H*=C).

3-(2-Bromophenyl)-2-methylpropanenitrile (11).

A solution of **10** (0.40 g, 1.8 mmol) in DME (5 mL) was added dropwise to a solution of $NaBH_4$ (0.08 g, 2.14 mmol) and MeOH (0.07 mL, 2.14 mmol) in DME (10 mL). The reaction mixture was stirred at 70 °C for 16 h. After cooling to rt, H_2O (1 mL) was added. Removal of the solvent gave a residue which was purified by flash chromatography eluting with cyclohexane/EtOAc (95:5) to afford an oil: 0.21 g (53% yield). ^{1}H NMR ($CDCl_3$) δ 1.41 (d, 3, C*H*$_3$C), 3.02 (d, 2, C*H*$_2$CH), 4.13 (m, 1, C*H*CH$_2$), 7.08-7.61 (m, 4, Ar*H*).

2-Methyl-3-(2-(thiophen-3-yl)phenyl)propanenitrile (12).

3-Thienylboronic acid (0.95 g, 6.43 mmol), tetrakis(triphenylphosphine)palladium(0) (0.29 g, 0.25 mmol) and 2M Na_2CO_3 (1.31 g, 12.36 mmol) were added to a solution of **11** (1.16 g, 5.2 mmol) in DME (10 mL). The reaction mixture was refluxed in a dark box under a nitrogen atmosphere for 14 h. Then, it was poured into H_2O and EtOAc; the organic layer was washed with iced H_2O and dried over Na_2SO_4. Removal of the solvent gave a residue which was purified by flash chromatography eluting with cyclohexane/EtOAc (70:30) to afford an oil: 0.7 g (59% yield). ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 3, C*H*$_3$C), 2.60 (m, 1, C*H*CH$_2$), 2.83-3.05 (m, 2, C*H*$_2$CH), 7.08-7.40 (m, 7, Ar*H*).

2-Methyl-3-(2-(pyridin-3-yl)phenyl)acrylonitrile (13).

This was prepared as described for **12** starting from **10** and diethyl(3pyridil)borane (48% yield). ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3, C*H*$_3$C), 7.08 (s, 1, C*H*=C), 7.25-7.53 (m, 6, Ar*H*), 8.59 (s, 1, Ar*H*), 8.65 (dd, 1, Ar*H*).

3-(3'-Hydroxy-[1,1'-biphenyl]-2-yl)-2-methylacrylonitrile (14).

This was prepared as described for **12** starting from **10** and 3-hydroxyphenilboronic acid (57% yield). ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3, C*H*$_3$C), 6.80-8.10 (m, 9, C*H*=C and Ar*H*), 9.02 (br s, 1, O*H*, exchangeable with D_2O).

3-(2-Chlorophenyl)-2-methylpropanenitrile (15).

A solution of lithium diisopropylamide 1.8M (3.7 mL, 6.64 mmol) in THF (7 mL) was added dropwise to a solution of 2-chlorohydrocinnamonitrile (Aldrich) (0.68 mL, 4.74 mmol) in THF (10 mL) at 0 °C under a nitrogen atmosphere and the reaction mixture was stirred for 1 h at rt. Methyl iodide (0.99 g, 6.97 mmol) was added and the resulting mixture was stirred for 5h at 50 °C, cooled to 0 °C and quenched with a saturated solution of NH_4Cl. The resulting mixture was extracted with ethyl acetate. Removal of the dried solvent gave a residue which was purified by flash chromatography eluting with cicloexane/EtOAc (98:2) to afford an oil: 0.34 g (yield 40%). ^{1}H NMR ($CDCl_3$) δ 1.38 (d, 3, C*H*$_3$C), 2.95-3.08 (m, 3, C*H*$_2$C*H*), 7.20-7.40 (m, 4, Ar*H*).

Methyl 2-methyl-3-(o-tolyl)propanoate (16).

A solution of 2-methyl-3-(o-tolyl)propanoic acid[23] (0.89 g, 5.00 mmol) in MeOH (20 mL) and H_2SO_4 (1.5 mL) was heated to reflux for 5 h. Removal of the solvent under reduced pressure gave a residue, which was dissolved in water. The aqueous solution, made basic with 2 N NaOH, was extracted with Et_2O. Removal of dried solvent gave a residue which was purified by column chromatography, eluting with cyclohexane/EtOAc (95:5) to give an oil: 0.76 g (79% yield). ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 3, C*H*$_3$C), 2.40 (s, 3, C*H*$_3$Ar), 2.77-3.15 (m 3, C*H*$_2$C*H*), 3.68 (s, 3, C*H*$_3$O), 7.10-7.25 (m, 4, Ar*H*)

Single Crystal X-ray diffraction analysis

Single crystals of (*S*)-(+)-**8** monoacid dibenzoyl-D-tartrate were analyzed by means of X-ray crystallography and a summary of the crystallographic data is reported in Table 2. The structures were solved by direct methods (SIR92).[29] Refinement was performed by means of full-matrix least-squares using SHELX-97 program (SHELXL-97 – A program for Crystal structure refinement. G.M. Sheldrick, University of Goettingen, Germany, 1997, Release 97-2). Anisotropic ADPs were used for all non hydrogen atoms. Hydrogen atoms linked to all atoms, except those of the protonated imidazoline group and of the carboxylic function of the monoacid dibenzoyl-d-tartrate, were introduced in calculated positions and isotropically refined in agreement with the linked atom. The remaining hydrogen atoms were localized in the ΔF map, introduced in the calculation and isotropically refined.

Table 2. Crystallographic data for (*S*)-(+)-**8** monoacid dibenzoyl-D-tartrate

Formula	$C_{31}H_{32}N_2O_8$
F_W	560.59
T/K	100
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a/ Å	7.3644(2)
b/ Å	9.0556(2)
c/ Å	43.738(2)
V/ Å^3	2916.8(2)
Z	4
D_c/g cm^{-3}	1.277
μ mm^{-1}	0.766
Measured Refls	8162
Unique refls, R_{int}	4927, 0.0362
Observed refls [*I*>2sigma(*I*)]	4246
Absorption correction	multi-scan
T_{min}, T_{max}	0.624, 1.000
*R*1[I > 2σ(I)]	0.0635
*wR*2 (all data)	0.1691
parameters, GOF	397, 1.060
$\Delta\rho_{max}$ / e Å^{-3}	-0.400

Description of the crystal structure

The absolute configuration of (+)-**8** has been established by means of X-ray diffraction analysis on the basis of the known configuration of the monoacid dibenzoyl-d-tartrate anion. The asymmetric carbon of (+)-**8** is in *S* configuration.

As clearly shown in Figure 4, both NH groups of the ligand are involved in H-bond interactions with the carboxylic function belonging to two symmetry related anions (H···O 1.87(5) and 2.02(6) Å, Figure 4A). Interestingly, the carboxylic functions of

adjacent monoacid anions give rise to strong omonuclear O-H-O hydrogen bonds which can be described as three-centre-four-electron covalent bonds (O···O distance 2.471(4) Å, O-H 1.21(4) and 1.26(4) Å Figure 4B). As a result, the crystal packing features parallel polymeric chains of dibenzoyl-d-tartrate, which deeply embed the (*S*)-(+)-**8** molecules. Moreover, the overall packing is also stabilized by a dense network of CH···π interactions involving the tolyl as well as the imidazoline groups of the ligand and the benzoyl moiety of the anion.

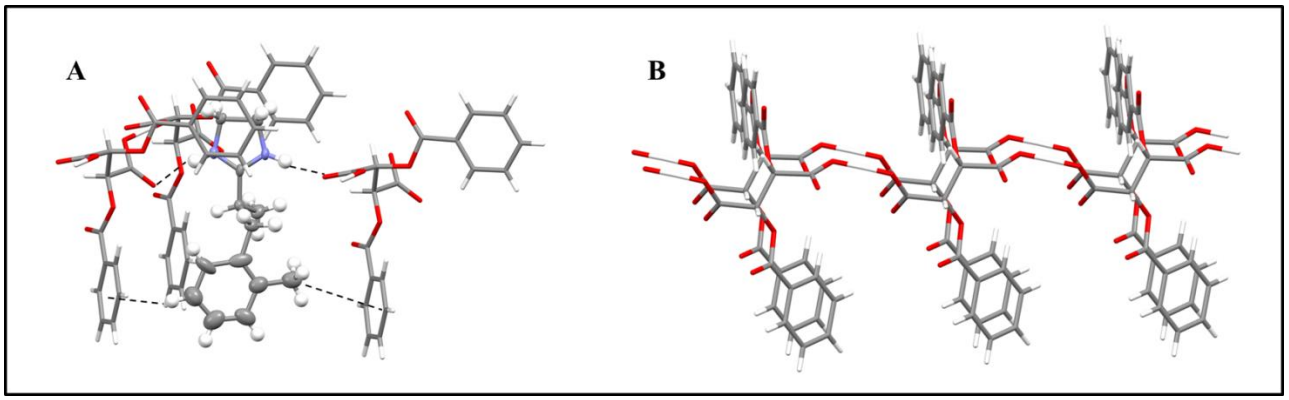

Figure 4. Crystal packing of (*S*)-(+)-**8** monoacid dibenzoyl-D-tartrate: (A) (*S*)-(+)-**8** molecule deeply embedded into the polymeric chains of dibenzoyl-d-tartrate (intermolecular contacts are shown as dashed lines); (B) parallel polymeric chains of dibenzoyl-d-tartrate showing the strong omonuclear O-H-O hydrogen bonds.

In vivo assays

Materials and Methods

Compounds **5**, **8**, (*S*)-(+)-**8**, (*R*)-(-)-**8** and **9**.

The compounds were dissolved in a vehicle solution (20% dimethylsufoxide (DMSO) and 80% sterile saline).

Animals

The experiments were performed on adult male spontaneously hypertensive rats SHR rats (7 weeks old, 223±7 g body weight) obtained from the Charles River Laboratories (Lecco, Italy). All animals were housed at a temperature of 23±1°C in cages for five animals at constant humidity (60%) with alternating 12-hour light/dark cycles, and left to acclimatize to these conditions for a week. All the experiments on animals were performed according to the European and Italian law concerning Animal Use in Experimental Research (Directive 2010/63/EU of the European Parliament and Italian Legislative Decree number 26, March 4, 2014).

Blood pressure and heart rate measurements

Mean arterial blood pressure (MABP) was determined weekly in conscious restrained rats by tail-cuff plethysmography.[30] The instrument used is BP2000 blood pressure analysis system (Visitech Systems, Inc, Apex, NC), designed and built by John E. Rogers and James P. Rogers.[31] This system also measures the frequency of the pulsations of caudal artery (the heart rate is expressed as beats per minute, bpm) with

the use of an optical sensor. Before the beginning of the experiments, rats were conditioned three to four times to the procedure and manipulation.

After habituation to the tail cuff apparatus and stabilization of the blood pressure, the animals were treated with intraperitoneal (ip) administration of each compound at the dose of 30 mg/kg. Controls were treated with the same volume of vehicle. Measurements were repeated at 30, 60, 120 minutes after the single drug administration. The values reported in the graphs represent the mean of five separate determinations.

Statistical analysis

All values are expressed as mean ± SEM. ANOVA was used for statistical analyses, and probabilities of $p<0.05$ were considered statistically significant. Experimental date were carried out using GraphPad Prism 5.0 software (Graphlab Software, San Diego, CA).

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Elemental Analysis of **5-9**

Compd	Formula	Calculated				Found			
		C%	H%	N%	S%	C%	H%	N%	S%
5	$C_{16}H_{18}N_2S.H_2C_2O_4$	59.98	5.59	7.77	8.90	59.87	5.42	7.94	8.72
6	$C_{17}H_{19}N_3.H_2C_2O_4$	64.21	5.96	11.82		64.47	5.74	12.00	
7	$C_{18}H_{20}N_2O.H_2C_2O_4$	64.85	5.99	7.56		64.97	6.12	7.80	
8	$C_{13}H_{18}N_2.HCl$	65.40	8.02	11.73		65.63	7.94	11.98	
9	$C_{12}H_{15}N_2Cl.H_2C_2O_4$	53.77	5.48	8.96		53.52	5.61	8.78	