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New arylsparteine derivatives as positive inotropic drugs

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Abstract

Positive inotropic agents are fundamental in the treatment of heart failure; however, their arrhythmogenic potential and increased myocardial oxygen demand strongly limit their therapeutic utility. Pursuing our study on cardiovascular activities of lupin alkaloid derivatives, several 2-(4-substituted-phenyl)-2-dehydrosparteines and 2-(4-substituted-phenyl)sparteines were prepared and tested for inotropic and chronotropic activities on isolated guinea pig atria. Four compounds (6b, 6e, 7b, and 7f) exhibited significant inotropic activity that, at the higher concentrations, was followed by negative inotropism or toxicity. Compound 6b (2-(4-tolyl)sparteine) exhibited a steep dose-dependent inotropic activity up to the highest concentration tested (300 μM) with an Emax of 116.5 ± 3.4% of basal force, proving less potent but more efficacious in comparison to the highest concentrations tested of digoxin and milrinone having Emax of 87.5 ± 3.1% and 52.2 ± 1.1%, respectively. Finally, docking studies suggested that the relevant sparteine derivatives could target the σ1 receptor, whose involvement in cardiac activity is well documented.

Introduction

Congestive heart failure (CHF) is a chronic and progressive disorder of left ventricular myocardial remodeling, which may lead to acute decompensation, resulting in a major cause of death in patients with heart disease. To relieve symptoms and to improve cardiac function, several classes of drugs are available, such as diuretics, ACE inhibitors, angiotensin receptor blockers, β-adrenergic receptor antagonists, and others. To increase the impaired contractile ability of heart, digitalis glycosides (digoxin and others) are used since long time, while some modifications of its structure have been also explored. A set of 17-alkylsparteines (from 17-methyl to 17-hexylsparteine) (5), R = alkyl showed improved inotropic effects in electrically stimulated guinea pig atria. According to Engelmann et al., in electrically driven guinea pig atria, sparteine produced a maximal increase (+42%) of contractile force at 200 μM concentration, while the 17-ethylsparteine, at the same concentration, displayed a +60% increase that reached +81% at the highest tested concentration (800 μM), resulting the most active 17-alkylsparteine derivative as inotropic. On the contrary, 17-butyl and 17-penty1sparteines were the best as antiarrhythmic agents. The replacement of the 17-alkyl substituents with heteroaryl-
heteroaryalkyl- and benzyl-moieties led to compounds with antiarrhythmic activity and reduced oxygen consumption by myocardium, so that 17-(3-methoxybenzyl)sparteine (5, R = 3-methoxybenzyl) was suggested for the therapy of coronary insufficiency\(^\text{15a,15b}\) (Figure 1).

Some 2-alkyl- and 2-arylderivatives of 2-dehydrosparteine (6, \(R = \text{H, OCH}_3\)) and sparteine (7, \(R = \text{H, OCH}_3\)) were recognized to display 10- to 30-fold higher antifibrillatory activity on isolated frog heart in comparison to sparteine\(^{16,17}\), but the eventual inotropic activity was not investigated (Figure 1).

Finally, through a pharmacological screening of assorted quinolizidine derivatives performed, some years ago, by "Panlabs Inc." (Bothell, WA), we observed that the 2-(4-fuorophenyl)sparteine displayed a significant positive inotropic activity on electrically driven guinea pig atria. Therefore, pursuing our long standing study about cardiovascular activity of derivatives of lupin alkaloids\(^{16,22}\), we deemed interesting to extend the study of inotropic activity to twelve 2-aryl-2-dehydrosparteine and 2-arylsparteine of structures 6 and 7, respectively, with \(R = \text{H, F, Cl, OCH}_3, \text{CH}_3\), and \(\text{CF}_3\) (Figure 2), thus including the four compounds previously investigated by Winterfeld et al.\(^{16,17}\) for the antifibrillatory activity.

**Materials and methods**

**General**

Chemicals, solvents, and reagents used for the syntheses were purchased from Sigma-Aldrich (St. Louis, MO), Fluka (Newport News, VA), or Alfa Aesar (Ward Hill, MA), and were used without any further purification. Column chromatography (CC): silica gel (Merck, Darmstadt, Germany). Mps: Büchi apparatus (Büchi, Flawil, Switzerland), uncorrected. \(^1\)H NMR and \(^{13}\)C NMR spectra: Varian Gemini-200 spectrometer (Varian Medical Systems (VAR), Palo Alto, CA); CDCl\(_3\); \(\delta\) in ppm rel. to Me\(_4\)Si as an internal standard; \(J\) in Hz. Elemental analyses were performed on a Carlo Erb EA-1110 CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmacy of Genoa University.

**Extraction of (±)-lupanine**

The ground seeds of *Lupinus albus* L. (1 kg) were extracted with light petroleum ether in a Soxhlet apparatus for 24 h to eliminate the lipidic materials. Successively the air dried material was further extracted with methanol for about 36 h. The methanol solution was filtered and concentrated to small volume in a Büchi Rotavapor (Büchi, Flawil, Switzerland). To the concentrated extract, 300 mL of water followed by 150 mL of 2 N hydrochloric acid were added and the acidic solution was filtered and extracted three times with ether to eliminate all non-basic compounds. The acidic solution was basified with 6 N sodium hydroxide solution and extracted with chloroform (5 \(\times\) 100 mL). After drying (Na\(_2\)SO\(_4\)), the chloroform was evaporated in Rotavapor obtaining 22.7 g of an oil that partially crystallized standing in cold. The addition of a little of acetone allowed the filtration of the crystals that were recrystallized from acetone yielding 8.3 g of pure (±)-lupanine melting at 95–97 °C. The joined acetone solution was evaporated to dryness and the residue was chromatographed on basic alumina (220 g) eluting with chloroform (15 \(\times\) 40 mL). The elimination of the solvent left 6.65 g of crystals that were rinsed with a little of acetone yielding 5.5 g of (±)-lupanine with m.p. 93–94 °C. Therefore, the total yield of (±)-lupanine was 1.38% in respect to the seeds used.

**2-(4-Substituted-phenyl)-2-dehydrosparteines (6). (General method)**

A solution of aryl magnesium bromide (20.1 mmol) was prepared by reacting at r.t. Mg turnings (0.51 g, 21.0 mmol) in dry Et\(_2\)O (10 mL), activated by methyl iodide and iodine, with a solution of the proper aryl bromide (20.1 mmol) in dry Et\(_2\)O (10 mL). Then a solution of lupanine (2 g, 8.1 mmol) in dry Et\(_2\)O (50 mL) was added. After being refluxed for 2 h, to the cooled (0–5 °C) reaction mixture, 50 mL of a solution of 2N HCl were added, then the resulting mixture was washed with Et\(_2\)O in order to remove the aromatic compounds derived from the exceeding arylmagnesium bromides. The acidic solution was basified with a solution of 6N NaOH and...
extracted with Et₂O. The dried organic layer (Na₂SO₄) was evaporated, leaving an oily residue that was purified by CC (SiO₂/ Et₂O + 2%DEA) and, when necessary, crystallized from the proper solvent.

2-Phenyl-2-dehydrosparteine (6a) Yield: 35%. Mp 99–100 °C (acetone) [lit. 16a]: 103–105 °C. ¹H NMR (200 MHz, CDCl₃): 1.10–2.48 (m, 18 H), 2.70–3.04 (m, 4 H), 4.41–4.53 (m, 1 H, C(3)), 7.18–7.43 (m, 5 H, ArH). Anal. Calcd for C₂₁H₂₈N₂: C, 70.19; H, 7.22; N, 7.44. Found: C, 70.53, 71.76, 34.99, 33.23, 31.83, 26.61, 25.67, 24.67, 23.92, 21.77. ¹³C NMR (50 MHz, CDCl₃): 146.87, 142.96, 127.56, 126.98, 123.87, 102.98, 63.46, 61.27, 54.70, 53.78, 51.73, 34.99, 33.23, 31.83, 26.61, 25.67, 24.67, 23.92, 21.77. Anal. Calcd for C₂₁H₂₉NF₂: C, 77.26; H, 8.34; N, 8.58. Found: C, 77.21; H, 8.35; N, 8.49.

2-(4′-Fluoroaryl)-2-dehydrosparteine (6b) Yield: 49%. Mp 124–125 °C (EtO). ¹H NMR (200 MHz, CDCl₃): 1.10–2.47 (m, 18 H), 2.70–2.95 (m, 4 H), 4.44–4.56 (m, 1 H, C(3)), 6.92–7.07 (m, 2 H, ArH), 7.21–7.37 (m, 2 H, ArH). ¹³C NMR (50 MHz, CDCl₃): 163.18, 158.31, 146.90, 135.27, 128.38, 101.33, 63.39, 61.27, 54.68, 53.66, 51.81, 35.06, 33.34, 31.89, 26.67, 25.81, 24.76, 23.96, 21.70. Anal. Calcd for C₂₁H₂₉F₂N₂O: C, 53.78, 51.73, 34.99, 33.23, 31.83, 26.61, 25.67, 24.67, 23.92, 21.77. ¹³C NMR (50 MHz, CDCl₃): 162.96, 158.11, 140.63, 127.74, 67.46, 65.28, 63.53, 57.44, 54.70, 52.69, 36.08, 35.11, 33.48, 32.76, 28.85, 26.80, 24.87, 23.90, 23.48. Anal. Calcd for C₂₁H₂₉F₂N₂: C, 76.90; H, 8.90; N, 8.53. Found: C, 76.79; H, 8.97; N, 8.50.

2-(4′-Chlorophenyl)-2-dehydrosparteine (6c) Yield: 37%. Mp 130–133 °C (EtO). ¹H NMR (200 MHz, CDCl₃): 1.09–2.40 (m, 18 H), 2.68–2.94 (m, 4 H), 4.46–4.57 (m, 1 H, C(3)), 7.27 (pseudo s, 4 H, ArH). ¹³C NMR (50 MHz, CDCl₃): 146.84, 137.75, 131.41, 128.10, 2.69, 101.76, 101.17, 63.38, 61.27, 54.67, 53.71, 51.77, 35.03, 33.34, 31.87, 26.67, 25.74, 24.75, 23.95, 21.72. Anal. Calcd for C₂₁H₂₉Cl₂N₂: C, 73.56; H, 7.92; N, 8.17. Found: C, 73.22; H, 8.01; N, 8.11.

2-(4′-Methoxyphenyl)-2-dehydrosparteine (6d) Yield: 38%. Oil (lit. 16b): oil, b.p. 194–202 °C, high vacuum). ¹H NMR (200 MHz, CDCl₃): 1.00–3.35 (m, 22 H), 3.88 (s, OCH₃), 4.45–4.67 (m, 1 H, C(3)), 6.92 (d, J = 10.0 Hz, 2 H, ArH), 7.36 (d, J = 10.0 Hz, 2 H, ArH). Elemental analysis produced results not well fitting for the formula C₂₂H₃₂N₂O, due to the instability of the compound.

2-(4′-Toly)-2-dehydrosparteine (6e) Yield: 39%. Mp 102–103 °C (acetone) [lit. 23]: 113–115 °C. ¹H NMR (200 MHz, CDCl₃): 1.09–2.44 (m, 18 H and 2.34, s, CH₃, superimposed), 2.68–3.03 (m, 4 H), 4.46–4.56 (m, 1 H, C(3)), 7.12 (d, J = 9.8 Hz, 2 H, ArH), 7.23 (d, J = 9.8 Hz, 2 H, ArH). ¹³C NMR (50 MHz, CDCl₃): 147.85, 136.40, 135.36, 127.56, 126.73, 100.68, 63.28, 61.27, 54.68, 51.85, 35.09, 33.40, 31.98, 26.73, 25.88, 24.84, 23.97, 21.76, 20.10. Anal. Calcd for C₂₂H₂₈N₂: C, 81.94; H, 9.38; N, 8.69. Found: C, 82.15; H, 9.45; N, 8.70.

2-(4′-Trifluoromethyl)-2-dehydrosparteine (6f) Yield: 28%. Mp 98–99 °C (acetone). ¹H NMR (200 MHz, CDCl₃): 1.05–2.47 (m, 18 H), 2.69–3.03 (m, 4 H), 4.46–4.54 (m, 1 H, C(3)), 7.44 (d, J = 9.2 Hz, 2 H, ArH), 7.58 (d, J = 9.0 Hz, 2 H, ArH). ¹³C NMR (50 MHz, CDCl₃): 146.87, 142.96, 127.56, 126.98, 123.87, 102.98, 63.46, 61.27, 54.70, 53.78, 51.73, 34.99, 33.23, 31.83, 26.61, 25.67, 24.67, 23.92, 21.77. Anal. Calcd for C₂₂H₂₉F₂N₂: C, 70.19; H, 7.22; N, 7.44. Found: C, 70.00; H, 7.19; N, 7.40.

2-(4′-Substituted-phenyl)sparteine (7). (General method) A solution of the suitable 2-dehydrosparteine derivative (1.6 mmol) in 20 mL of EtOH was hydrogenated at r.t. and atmospheric pressure in the presence of 10% Pd/C (0.1 g). After about 1 h, the calculated volume of H₂ was absorbed. The catalyst was removed and the solvent was evaporated in vacuo, affording a residue that was crystallized from the indicated solvent.

2-Phenylsparteine (7a) Yield: 62%. Mp 91–92 °C (Et₂O) [lit. 15a]: oil, b.p. 160–161 °C, high vacuum]. ¹H NMR (200 MHz, CDCl₃): 1.04–2.18 (m, 20 H), 2.37–3.08 (m, 5 H), 7.19–7.55 (m, 5 H, ArH). Anal. Calcd for C₁₇H₂₈N₂: C, 81.24; H, 9.74; N, 9.02. Found: C, 81.39; H, 9.84; N, 9.08.

Docking studies

All the compounds were built, parameterized (Gasteiger–Huckel method) and energy minimized within MOE using MMFF94 force-field. For the newly synthesized sparteine derivative 7b–f the two R and S enantiomers were taken into account and built in silico.
Docking calculations within the X-ray structure of human sigma-1 receptor (pdb code = 5HK1) were performed using the MOE-DOCK tool, implemented in MOE. The compound best-docked pose, evaluated in terms of “London dG”, was refined by energy minimization (MMFF94) and rescored according to “Affinity dG” (kcal/mol of total estimated binding energy). The final score (based on the latest refinement step which has been applied) namely S score, was considered to prioritize any ligand conformation.

In addition, the best docking geometry was refined by ligand/protein complex energy minimization (CHARMM27) and successfully assessed using a short ~1 ps run of molecular dynamics (MD) at constant temperature, followed by an all-atom energy minimization (LowModeMD implemented in MOE software). This kind of module was allowed to perform an exhaustive conformational analysis of the ligand–receptor binding site complex, as we already discussed about other case studies, where it proved to be useful for a preliminary evaluation of docking poses26.

**Pharmacological studies**

**Animals**

Dunkin–Hurtley male guinea pigs (300–500 g), obtained from Harlan Italy (S. Pietro al Natisone, Italy), were kept in controlled environmental conditions (temperature: 23 ± 2 °C; light–dark cycle: 7 a.m. to 7 p.m.). Animals had free access to a standard laboratory diet and to water. All animal-use procedures described in this paper were in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals and comply with the ethical principles and guidelines adopted by the European Community, law 86/609/CEE. The experimental protocol was approved by the local veterinary committee.

In order to obtain myocardial tissues depleted in endogenous catecholamines, the animals were treated daily for 2 d with reserpine (2 mg/kg i.p.) and anesthetized with methoxyflurane before sacrifice.

**Assessment of inotropic and chronotropic activities on isolated atrial preparations**

The atria were separated from ventricles and suspended vertically in a 30 mL organ bath containing a physiological salt solution constantly gassed by 95% O2 and 5% CO2, at 30 °C. The bathing solution contained (mM): NaCl 120, KCl 2.7, CaCl2 1.36, MgCl2 0.09, NaH2PO4 0.4, NaHCO3 12 and glucose 5.5 (pH = 7.5). The resting tension was adjusted at 10.0 mM and the developed tension was recorded isometrically by means of a high-sensitivity transducer (Ugo Basile, type DYO Comerio, Italy) connected to a PC-based Acqknowledge acquisition system (BIOPAC Systems, Inc., 42 Aero Camino Santa Barbara, CA). The drugs were added to the perfusion fluid after 90 min of equilibration. Since the atria were isolated from reserpine treated animals, before the beginning of experiments, the depletion of catecholamines was verified by lack of any positive inotropic effect induced by tyramine (2 μg/mL). Experiments were performed only in preparations that did not respond to tyramine. Test compounds and milrinone were added cumulatively and the effects caused by each drug concentration were recorded up to the maximum response before a higher concentration was added. The effects of each compound on the force of contraction and the frequency were expressed as the percent increase/decrease over controls (Δ%). Compounds were dissolved in physiological solution or in the stoichiometric 0.5 N HCl, while milrinone was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the medium did not influence the basal activity of the atrial preparations. The statistical comparisons between treatment and control data were performed by ANOVA followed by Bonferroni t test; a value of $p < 0.05$ was considered statistically significant.

**General pharmacological screening**

For in vivo tests, compounds were generally administered orally (p.o.) by gavage; they were prepared as aqueous solutions or finely homogenized suspensions in “tween 80” (2%). In a few cases, the substances were introduced intraperitoneally (i.p.). Groups of three or five animals (mice or rats) were used. For in vitro assays, sometimes it was necessary to increase the solubility by means of DMSO in a concentration not interfering with the tests (0.1% for platelet aggregation and 0.5% for all the others).

Doses (mg/kg) or concentrations (μg/mL) indicated in the following methods were the highest commonly utilized; when significant activity was detected, lower doses or concentrations were tested in order to define the minimal effective ones.

**Maximal tolerated dose, autonomic signs, and Irwin test**

Three mice were dosed at 300 mg/kg p.o. and 100 mg/kg i.p. for observation of acute toxic symptoms or autonomic effects during the subsequent 72 h. If none was noted, pharmacological evaluation proceeded employing doses and concentrations for each test based on appropriate multiple of doses required by suitable reference compounds. If acute toxicity was observed initially, the maximal tolerated dose was determined and pharmacological screening doses were reduced proportionally.

Before and 1 h after dosing mice with test samples, 10 parameters indicating motor stimulation (irritability, hyperactivity, increased palpebral size, increased startle response, increased response to touch, increased exploration, piloerection, strand tail, tremors, convulsions) were measured. Normalcy for each is 0; maximum abnormal condition for each score 2 x 10 x 3 mice = 60. Scores greater than 12 denote significant stimulation. Similarly, 10 parameters (pinna reflex, spontaneous activity, palpebral size, startle response, touch response, reactivity, placing, righting reflex, exploration, and ataxia) were measured for behavioral depression. Each parameter scores 2 points for normalcy for a total of 2 x 10 x 3 = 60 points possible. Scores below 40 denote significant behavioral depression.

**Blood pressure**

Two spontaneously hypertensive rats (SHR) with systolic blood pressures ranging between 180 and 220 mmHg were used. Blood pressure was determined by tail-cuff method in a temperature-controlled environment (32 ± 1 °C) before, 1, 2, and 4 h after test substance administration p.o. (100 mg/kg). Reduction in systolic blood pressure by more than 10% at any two of the aforementioned three consecutive time intervals is considered significant.

**Heart rate**

The same SHRs employed in the preceding test were used. Heart rate was recorded by ECG, immediately after blood pressure recordings, and before 1, 2, and 4 h post-treatment p.o. (100 mg/kg). An increase or a decrease in heart rate greater than 20% from pretreatment control readings indicates significant tachycardia or bradycardia, respectively.
**Antiarrhythmic activity**
The substance was administered *i.p.* (30 mg/kg) to a group of 3 mice 30 min before exposure to deep chloroform anesthesia and observed during the following 15 min period. The absence of ECG recorded cardiac arrhythmias and heart rate above 200 beats/min (usually 400–480 beats/min) in at least two mice indicates significant protection.

**In vitro inotropic effect**
Test substance (10 μg/mL)-induced variation in contractile force of electrically stimulated (95% of maximum, 150 beats/min) guinea pig left atria, bathed in physiological salt solution containing one third normal calcium concentration at 32 °C, by more than 40% indicates significant (±) inotropic activity. Only inamrinone sensitive preparations were used.

**In vitro chronotropic effect**
Test substance (10 μg/mL)-induced change in atrial rate in spontaneously beating guinea pig atria, bathed in physiological salt solution at 37 °C, by more than 10% is considered significant.

**Effects on diuresis, saluresis, and kaluresis**
Groups of three overnight-fasted rats were used. Each group was hydrated with distilled water (25 mL/kg, *p.o.*) administered together with test substance (30 mg/kg, *p.o.*) or vehicle. Urine volume was measured over the ensuing 6 h period and analyzed for Na⁺ and K⁺ contents, expressed as μeq/100 g body weight. A greater than 50% increase or decrease in urine volume in test versus control animals was considered a significant effect. A greater than two-fold increase of Na⁺ and K⁺ excretion in test versus control animals was considered a significant saluretic or kaliuretic effect.

**In vitro tracheal relaxation**
The strip preparations of isolated guinea pig trachea was used to study the contractile tension, placed in physiological salt solution at 37 °C under 4.9 mN. Test substance (100 μg/mL) inhibition of tracheal tone by more than 50%, relative maximal relaxation induced by 0.3 μg/mL epinephrine, indicates significant activity. If test substance-induced relaxation is antagonized more than 50% by propranolol (2 μg/mL), a well known β₂-adrenoceptor blocker, an agonist action is indicated.

**Anti-inflammatory activity**
In a group of three overnight-fasted rats, test substance was administered *p.o.* (100 mg/kg) 1 h before right hind paw intra-plan- tar injection of carrageenan (0.1 mL, 1% suspension). Inhibition of paw edema by more than 30% 3 h after carrageenan administration indicates acute anti-inflammatory activity.

**Analgesic activity**
**Writhing test:** Test sample was administered *p.o.* (100 mg/kg) to a group of 3 mice 1 h before injection of phenylquinone (2 mg/kg, *i.p.*), greater than 50% inhibition of the number of twists per group of animals observed during 5–10 min after phenylquinone, relative to a vehicle-treated group, indicates possible analgesic activity.

**Formalin test:** Test sample was administered *p.o.* (30 mg/kg) to a group of 5 mice 1 h before sub-planter injection of formalin (0.02 mL, 1% sol.) into the right hind paw. Reduction of the induced paw licking time recorded during the following 20–30 min period by more than 50% indicates analgesic activity.

**In vitro platelet aggregation inhibition**
**Sodium arachidonate induced aggregation:** Test substance (10 μg/mL) inhibition by more than 50% of maximum non-reversible platelet aggregation (rabbit platelet-rich plasma, RPP) induced by sodium arachidonate (50 μg/mL) indicates significant activity.

**ADP induced aggregation:** Test substance (100 μg/mL) inhibition by more than 50% of maximum non-reversible platelet aggregation (RPP) induced by adenosine diphosphate (ADP, 0.4–0.8 μg/mL) indicates significant activity.

**PAF induced aggregation:** Test substance (10 μg/mL) inhibition by more than 50% of maximum non-reversible platelet aggregation (RPP) induced by platelet activating factor (PAF-acether, 10–20 ng/mL) indicates significant activity.

**Results and discussion**

**Synthesis**
The required compounds were prepared by reacting (±)-lupanine (extracted from seeds of *Lupinus albus*) with the proper 4-substituted phenylmagnesium bromides.

The initially formed carbinolamines could not be isolated even in the mildest working up procedures. The reduction of 2-dehydrosapentine derivatives (6) was commonly performed by catalytic hydrogenation (*Pd/C*), or by the action of sodium borohydride in the case of compound 6c, in order to avoid the chlorine hydroge- nolysis. The methoxy derivative 6d was rather unstable and was immediately converted into the saturated compound 7d.

**Biological results**

**Pharmacology: general considerations**
Ten compounds (6b, 6c, 6e, 6f, and 7a–f) were tested in vitro for inotropic and chronotropic activities on spontaneously beating atra obtained from guinea pigs pretreated with reserpine in order to avoid the influence of endogenous catecholamines released from nerve terminals (*Tables 1* and *2*). Two compounds could not be tested either for shortage of sample (6a) or for its chemical instability (6d).

**Scheme 1:** Reagents and conditions: (a) dry Et₂O; reflux; 2 h; (b) dil. HCl; (c) H₂/Pd-C, EtOH; r.t.; (d) NaBH₄, EtOH; reflux, 4 h. (±)-lupanine, for the sake of simplicity, only one enantiomer is shown.
Developed tension (Δ%)  

| Comp. | R= | 10⁻⁷ M | 3 x 10⁻⁷ M | 10⁻⁶ M | 3 x 10⁻⁶ M | 10⁻⁵ M | 3 x 10⁻⁵ M | 10⁻⁴ M | 3 x 10⁻⁴ M |
|-------|----|---------|------------|--------|------------|--------|------------|--------|------------|
| 6b†  | F  | 1.82 ± 0.02 | 3.01 ± 0.03 | 7.07 ± 0.04 | 15.88 ± 0.07 | 29.44 ± 0.11 | 32.88 ± 0.14 | 28.57 ± 0.12 | 57.14 ± 0.22 |
| 6c†  | Cl | 3.70 ± 0.13 | 7.40 ± 0.11 | 3.70 ± 0.09 | -3.70 ± 0.08 | -14.80 ± 0.12 | -29.60 ± 0.14 | tox     | tox        |
| 6e†  | CH₃ | 4.10 ± 0.01 | 10.64 ± 0.05 | 19.38 ± 0.07 | 28.44 ± 0.12 | 26.99 ± 0.17 | 24.69 ± 0.16 | -29.76 ± 0.13 | 58.33 ± 0.24 |
| 6f†  | CF₃ | 0.65 ± 0.01 | 0.78 ± 0.02 | 0.09 ± 0.01 | -9.02 ± 0.02 | -12.93 ± 0.04 | -33.09 ± 0.15 | -48.21 ± 0.57 | -74.10 ± 0.86 |
| 7a†  | H  | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.56 ± 0.09 | 2.75 ± 0.10 | 0.72 ± 0.03 | -3.73 ± 0.08 | tox     | tox        |
| 7b†  | F  | 1.50 ± 0.23 | 2.14 ± 0.18 | 5.17 ± 0.24 | 9.53 ± 0.24 | 20.85 ± 0.36 | 29.99 ± 0.37 | tox     | tox        |
| 7c†  | Cl | 0.00 ± 0.00 | 0.00 ± 0.00 | -4.17 ± 0.24 | -16.67 ± 0.18 | -29.17 ± 0.20 | tox     | tox        |
| 7d†  | OCH₃ | 0.01 ± 0.01 | 0.34 ± 0.02 | 0.83 ± 0.03 | 1.65 ± 0.04 | 1.39 ± 0.04 | -5.01 ± 0.07 | tox     | tox        |
| 7e†  | CH₃ | 1.97 ± 0.15 | 2.55 ± 0.17 | 3.27 ± 0.09 | 9.76 ± 0.10 | 38.81 ± 0.35 | 68.96 ± 1.15 | 110.71 ± 2.77 | 116.50 ± 3.41 |
| 7f†  | CF₃ | 1.64 ± 0.16 | 8.63 ± 0.21 | 17.45 ± 0.17 | 27.02 ± 0.21 | 43.08 ± 0.40 | 41.32 ± 0.25 | tox     | tox        |
| M†   | nt | nt       | 16.69 ± 0.95 | 30.24 ± 0.75 | 38.68 ± 1.25 | 46.16 ± 0.80 | 52.70 ± 0.83 | 52.25 ± 1.13 | tox     |

Test compounds were added cumulatively to the bathing fluid, and inotropic effect was recorded for 5 min after they reached maxima, before adding a higher concentration. The value of basal force of contraction was 7.23 ± 0.32 mN. Results are means ± SEM of 6–10 experiments. Negative value indicates negative inotropism (decrease of developed tension). nt: not tested; tox: toxicity.

Inotropic and chronotropic activities

As it is illustrated in Table 1, the dehydrosparteine derivatives 6b and 6e showed a concentration-dependent positive inotropic effect that at the higher concentrations of 100 and 300 μM was converted to negative inotropism. A similar positive inotropism was observed for compounds 7b and 7f which, however, at 100 and 300 μM induced overt toxicity characterized by the appearance of arrhythmias and functional derangement leading to the irreversible stop of atrial contractions. It is worth noting that, at the highest non-toxic concentration (30 μM), compound 7b displayed an inotropic effect (+30%) comparable with that previously observed on electrically driven atria of non-reserpinized guinea pig (+56%, see Table 3).

Moreover, the so far unpublished results of the previous pharmacological screening of compound 7b, in comparison with milrinone, were included, providing some additional information on other cardiovascular and related activities and on toxicity of this kind of compounds (Table 3).

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Inotropic and chronotropic activities

As it is illustrated in Table 1, the dehydrosparteine derivatives 6b and 6e showed a concentration-dependent positive inotropic effect that at the higher concentrations of 100 and 300 μM was converted to negative inotropism. A similar positive inotropism was observed for compounds 7b and 7f which, however, at 100 and 300 μM induced overt toxicity characterized by the appearance of arrhythmias and functional derangement leading to the irreversible stop of atrial contractions. It is worth noting that, at the highest non-toxic concentration (30 μM), compound 7b displayed an inotropic effect (+30%) comparable with that previously observed on electrically driven atria of non-reserpinized guinea pig (+56%, see Table 3).

At concentrations up to 30 μM, compounds 6b, 6e, and 7b exhibited only modest (-6%) positive or negative chronotropic activities (Table 2), while compound 7f exhibited moderate positive chronotropism (up to +19%). At higher concentrations, all the cited compounds showed either negative chronotropism or toxicity (Table 2).

Very interestingly, the 2-(4-tolyl)sparteine 7e exhibited a steep enhancement of contractile strength up to the maximal concentration tested (300 μM), with an E_max of 116.5 ± 3.4% of the basal contractile force. This powerful positive inotropism was associated with only a moderate increase of the beating rate that reached +27.3 ± 0.3% at the highest concentration (300 μM).

The remaining compounds, including 2-phenylsparteine 7a and 2-(4-methoxyphenyl)sparteine 7d (tested in the past16,17 for anti fibrillatory activity), were endowed with modest positive inotropism at the lower concentrations, followed by increasing negative inotropism or toxicity at the higher concentrations. Similar effects were observed concerning the beating frequency.

Further, the inotropic activity of 7e was compared with that of two well-known inotropic drugs digoxin and milrinone. Figure 2 reports the concentration-effect curves of the three inotropic agents showing that digoxin was the most potent, increasing the basal contractile force by 50% at 0.9 μM concentration, while compound 7e was the most efficacious with an E_max = 116% at 300 μM concentration. Milrinone was moderately potent but the least active. Concentrations of digoxin higher than 3 μM could not be tested because of toxicity.
Comparing chronotropism of the three agents, it was observed (Figure 3) that digoxin increased the frequency up to 25% on basal value at the concentration of 3 μM; furthermore, higher concentrations induced high toxicity with arrhythmias and atrial block. Instead, the compound 7e slightly increased frequency, much less than digoxin, and also less than milrinone without ever causing severe cardiac toxicity (Figure 3). Anyhow, among the 2-dehydrosparteines (6), the highest and lowest inotropic activities were found for the fluorophenyl (6b) and the trifluoromethylphenyl (6f) derivatives, respectively. On the contrary, among the saturated compounds (7), the fluorophenyl (7b) was less active than the trifluoromethylphenyl (7f) derivative that was the most active of the whole set of compounds up to the 10 μM concentration, beyond which it was largely surmounted by the methylphenyl analog 7e. Indeed, the concentration–effect curve of 7f is rather flattened and parallel to that of milrinone up

The structural features that characterized the studied compounds (the presence or the absence of the enaminic double bond and consequent variation of the configuration of the tetra-cyclic system; nature of the substituent on the aromatic moiety) clearly influence both inotropic and chronotropic activities. On the whole, the 2-dehydrosparteine derivatives were more prone to produce negative inotropism and, even more, negative chronotropism, in comparison with the saturated compounds. However, the influence on the activity exerted by the presence of the enaminic double bond is not constant in the four couples of compounds bearing the same substituent on the aromatic moiety (compare 6b/7b; 6c/7c; 6e/7e; 6f/7f). The influence on the activity of the structural features is also variable in relation to the tested concentrations, because they may exert different effects in relation to permeability of cardiac tissue and intracellular targets responsible for the force and the frequency of cardiac contraction.

### Table 3. General pharmacological screening of compound 7b and milrinone (M).

| Test                              | Dose (mg/kg) or Conc. (μg/mL) | Considered parameter                  | Significant criterion | 7b | M | Additional reference drug* |
|-----------------------------------|-------------------------------|---------------------------------------|-----------------------|----|---|---------------------------|
| MTD and Irwin test**              | p.o. 300                      | Number animals (died/treated)         | 3/3 nt                | 3/3 3/3 (48h) | 3/3 3/3 (48h) | Lidocaine: 3/3 |
|                                  | 100                            |                                       | 3/3 3/3 (48h)         | 3/3 3/3 (48h) | 3/3 3/3 (48h) | Quinidine: 3/3 |
|                                  | 30                             |                                       | 0/3 3/3 3/3           | 0/3 3/3 3/3 | 0/3 3/3 3/3 | Furosemide: 5 mg/kg: 3.0 |
|                                  | i.p. 100                       | N° protected animals/treated          | >1/3                  | 0/3 nt 0/3 | 0/3 nt 0/3 | Triamterene (2.5 mg/kg): 2.6 |
| Blood pressure                   | p.o. 30 3-10                   | Δ% after 1 and 4 h                     | >10% -4/0             | -13/13 3/3 3/3 | -13/13 3/3 3/3 | Trequinsin: +48 |
|                                  | 10                             |                                       | 3/3 +31 -3            | +60 +45 +45 | +60 +45 +45 | Amiloride: 20 mg/kg: 1.6 |
|                                  | 5                              |                                       | 3/3 +31 -3            | +60 +45 +45 | +60 +45 +45 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 3                              |                                       | 3/3 +31 -3            | +60 +45 +45 | +60 +45 +45 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 2.5                            |                                       | 3/3 +31 -3            | +60 +45 +45 | +60 +45 +45 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 1                              |                                       | 3/3 +31 -3            | +60 +45 +45 | +60 +45 +45 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
| Saluresis                         | p.o. 30 3-10                   | Urine Na1 μeq treated/control         | >2                    | 4.5 4.1 1.8 | 4.5 4.1 1.8 | Furosemide (5 mg/kg): 3.0 |
|                                  | 10                             |                                       | 4.1 nt                | 0.5 nt 0.5 | 0.5 nt 0.5 | Triamterene (2.5 mg/kg): 2.6 |
|                                  | 3                              |                                       | 3.4 nt                | 0.5 nt 0.5 | 0.5 nt 0.5 | Triamterene (2.5 mg/kg): 2.6 |
| Diuresis                          | p.o. 30 3-10                   | Urine vol. treated/control            | >1.5                  | 2.2 2.0 1.8 | 2.2 2.0 1.8 | Amiloride (20 mg/kg): 1.6 |
|                                  | 10                             |                                       | 2.0 nt                | 0.4 nt 0.4 | 0.4 nt 0.4 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 3                              |                                       | 1.8 nt                | 0.4 nt 0.4 | 0.4 nt 0.4 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 2.5                            |                                       | 1.8 nt                | 0.4 nt 0.4 | 0.4 nt 0.4 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 1                              |                                       | 1.8 nt                | 0.4 nt 0.4 | 0.4 nt 0.4 | Hydroflumethiazide (2.5 mg/kg): 1.7 |
|                                  | 30                             | Relaxation (%)                        | >50% nt               | nt 100 nt 100 | nt 100 nt 100 | Theophylline: 60 |
|                                  | 1.0                            | nt 100 nt                            | nt 50                 | nt 23 nt 23 | nt 23 nt 23 | Theophylline: 60 |
|                                  | 0.5                            | nt 100 nt                            | nt 23                 | nt 23 nt 23 | nt 23 nt 23 | Theophylline: 60 |
| Anti-inflammatory                 | p.o. 100                       | Inhibition (%)                        | >30% nt               | 42 nt 42 nt 42 nt | 42 nt 42 nt 42 nt | Aspirin (150 mg/kg): 40 |
| (paw edema)                      | 30                             | nt 100 nt                            | nt 50                 | nt 23 nt 23 | nt 23 nt 23 | Aspirin (150 mg/kg): 40 |
|                                  | 10                             | nt 100 nt                            | nt 23                 | nt 23 nt 23 | nt 23 nt 23 | Aspirin (150 mg/kg): 40 |
|                                  | 0.5                            | nt 100 nt                            | nt 23                 | nt 23 nt 23 | nt 23 nt 23 | Aspirin (150 mg/kg): 40 |
|                                  | 30                             | nt 100 nt                            | nt 23                 | nt 23 nt 23 | nt 23 nt 23 | Aspirin (150 mg/kg): 40 |
|                                  | 10                             | nt 100 nt                            | nt 23                 | nt 23 nt 23 | nt 23 nt 23 | Aspirin (150 mg/kg): 40 |
|                                  | 0.5                            | nt 100 nt                            | nt 23                 | nt 23 nt 23 | nt 23 nt 23 | Aspirin (150 mg/kg): 40 |
| Phenyquinone writhing            | p.o. 300                       | Writhing inhibition (%)               | >50% nt               | nt 42 nt 42 nt | nt 42 nt 42 nt | Aspirin (50 mg/kg): 68 |
|                                  | 30                             | nt 100 nt                            | nt 42                 | nt 42 nt 42 nt | nt 42 nt 42 nt | Aspirin (50 mg/kg): 68 |
|                                  | 10                             | nt 100 nt                            | nt 42                 | nt 42 nt 42 nt | nt 42 nt 42 nt | Aspirin (50 mg/kg): 68 |
|                                  | 0.5                            | nt 100 nt                            | nt 42                 | nt 42 nt 42 nt | nt 42 nt 42 nt | Aspirin (50 mg/kg): 68 |
| Formalin algesia                 | p.o. 30 3-10                   | Licking time reduction (%)            | >50% nt               | 89 nt 89 nt 89 nt | 89 nt 89 nt 89 nt | Aspirin (50 mg/kg): 68 |
|                                  | 10                             | nt 100 nt                            | nt 89                 | nt 89 nt 89 nt | nt 89 nt 89 nt | Aspirin (50 mg/kg): 68 |
|                                  | 0.5                            | nt 100 nt                            | nt 89                 | nt 89 nt 89 nt | nt 89 nt 89 nt | Aspirin (50 mg/kg): 68 |

nt: not tested.

*When not otherwise specified, the drug reference dosage corresponds to that of column 2.

MTD: maximal tolerated dose; neither significant motor stimulation nor behavioral depression were observed.

Not blocked by propranolol.

Only compounds active in the writhing test were assayed.
to 30 μM (Table 1), while that of compound 7e is characterized by its steepness, paralleling (at the higher concentrations) that of digoxin (Figure 2).

**Pharmacological screening of compound 7b and milrinone**

The most interesting results of the general pharmacological screening of compounds 7b in comparison with milrinone are illustrated in Table 3.

First of all, it is observed that the sparteine derivative was moderately toxic, but less than milrinone. Indeed, in both cases at the dose of 300 mg/kg p.o. and 100 mg/kg i.p. all treated mice died, but at lower dose (30 mg/kg p.o. and i.p.), no death was observed with the sparteine derivative within the 72 h of observation, whereas with milrinone all animals died within 48 h (Table 3). For comparison, in guinea pig, sparteine sulfate exhibited an i.p. MLD between 42 and 55 mg/kg77, and digoxin exhibited an oral DL50 = 3.5 mg/kg. In the Irwin test, compound 7b and milrinone showed neither significant motor stimulation nor behavioral depression.

Concerning the cardiovascular system, it is observed that the 2-(4-fluorophenyl)sparteine 7b did not exhibit any activity on the blood pressure and the heart rate in spontaneously hypertensive rats, while milrinone showed a moderate reduction of pressure. Somewhat surprising, 7b did not display significant antiarrhythmic activity in the chloroform induced arrhythmia assay. In previous studies16,17, the analogous compounds 6a, 6d, 7a, and 7d, as well as sparteine, were found to display antifibrillatory activity on isolated frog heart.

More importantly, a net positive inotropic activity was found for compound 7b and milrinone on isolated, electrically driven guinea pig left atria. Negative chronotropic action (comparable to that of quinidine) was observed for the sparteine derivative on spontaneously beating guinea pig right atria, while milrinone displayed positive activity on beating frequency. While the results reported in Tables 1 and 2 were obtained from reserpinized guinea pig atria, those reported in Table 3 were from untreated tissue and the endogenous amines can affect the observed inotropic and chronotropic activities of 7b and milrinone.

The positive inotropism of compound 7b is related neither to β-adrenergic receptor activation, since it was not blocked by propranolol nor to phosphodiesterase inhibition, since the reduction of tone in guinea pig tracheal strips was not observed, even at 30 μg/mL concentration, while milrinone clearly reduced the tone of trachea still at 1 μg/mL (−50%). High positive inotropic activity on electrically driven guinea pig left atria (from non-reserpinized animal) was observed19 for a couple of cytisine derivatives (8 and 9; Figure 4). Also in this case, the inotropic activity was not blocked by propranolol, and no reduction of spontaneous tone of trachea strips was observed.

Compound 7b exhibited potent saluretic and diuretic activities (Table 3) that were quite higher than those expressed by furosemide, triamterene, amiloride, and hydroflumethiazide. These activities were not shared by milrinone.

The combination of diuretic activity with positive inotropism may result very valuable to relieve symptoms of CHF; therefore,
the presence of diuretic and saluretic activities should be investigated for the whole set of sparteine derivatives.

Finally, 7b exhibited moderate analgesic (writhing test and formalin algesia) and anti-inflammatory (carragenin induced edema) activities that were not shared by milrinone. The anti-inflammatory activity seems unrelated to Cox inhibition, since inhibition of arachidonic acid (AA) induced platelet aggregation was not observed; milrinone completely inhibited the AA induced aggregation even at 0.1 μg/mL concentration.

Thus, the combination of polycyclic alkaloidal frameworks with particular aromatic moieties, as in compounds 6–9, seems suitable to generate positive inotropism through a mechanism not related to adrenergic stimulation or PDE inhibition. The common structural feature of compounds 6–9 resembles that of some arylalkyl quinolizidines and some arylalkyl amines, like BD-737, BD-1008, BD-1063, and the butyrophenone antipsychotics (Figure 5) that are characterized by high affinity to sigma-1 receptor.

Sigma receptor ligands present well-documented effects on cardiac muscle, thus the possibility that the inotropic activity of compounds 6–9 is chaperoned by interaction with sigma receptor is tentatively advanced. This hypothesis is supported by the excellent fitting of molecules 7b and 7e on the binding site of sigma 1 receptorial protein (see further). Anyhow, further investigations are needed to define the mechanism of the inotropic activity of the relevant sparteine derivatives.

**Docking studies**

Sigma ligands are characterized by a rather large variety of structures and up to now, the development of potent sigma-1 ligands was efficiently driven by computational methods, based on homology studies of the target and also fulfilling a pattern of pharmacophore features exhibited by several series of derivatives, as discussed in the literature. Indeed, most of sigma-1 ligands were characterized by the positive ionizable group and hydrogen bond acceptor functions connecting at least two hydrophobic cores. Thus, for sigma-1 ligands, a recurrent binding mode was proposed in literature, suggesting key contacts with an aspartic acid residue (D126), as confirmed by mutagenesis experiments.

More recently, the X-ray crystallographic structure of the human sigma-1 receptor became available, definitively providing more reliable avenues to pave the way for the rational design of further molecules (pdb code = 5HK1; resolution = 2.51 Å). This computational work was aimed to explore the reliability of the sigma-1 protein as putative biological target to be proposed for the newly derivatives here discussed. The main issues to be addressed were to clarify, through docking studied of the two series of compounds 6–7, the role played by the sparteine nucleus and by the phenyl ring with respect to the co-crystallized ligand PD144418, bearing a 1-propyl-3-[3-[p-toly]isoxazol-5-yl]-1,2,5,6-tetrahydropyridine structure (Table 4).

As shown in Figure 6, PD144418 was engaged in salt bridges involving the protonated nitrogen atom of the tetrahydropyridine group and D126, E172, giving a good validation of the aforementioned homology models and mutagenesis data. On the contrary, the propyl chain and the phenyl ring detected hydrophobic contacts with the surrounding amino acids.

According to our calculations, the most promising compounds 7b and 7e (the R enantiomers proved to be preferred) overlapped the sparteine nucleus onto the tetrahydropyridine and oxazole

| Compound     | S Score (kcal/mol) |
|--------------|--------------------|
| 6b           | –96.312            |
| 6c           | –91.364            |
| 6e           | –95.678            |
| 6f           | –98.364            |
| 7a           | –121.543           |
| 7b           | –112.003           |
| 7c           | –114.301           |
| 7d           | –112.378           |
| 7e           | –123.137           |
| 7f           | –121.312           |
| PD144418     | –132.632           |
| sparteine    | –88.721            |

Figure 6. X-ray crystallographic data of the human sigma-1 receptor and of the ligand PD144418. The most important residues are labeled and colored in orange.
moieties of the reference compound (Figure 7). As a consequence, the required contacts with D126 and E172 occurred. In addition, the overall sparteine architecture displayed hydrophobic contacts with V84, W89, F107, Y120, and F184, as exhibited by the reference compound oxazole ring and tetrahydropyridine moiety. Moreover, the phenyl ring of 7b and 7e proved to highly mimic the role of the one of PD144418, establishing Van der Waals contacts with L95, A98, L182, and π–π stacking with Y103 and Y206. Interestingly, this kind of positioning proved to be allowed only when small lipophilic substituents decorate the phenyl ring para position, being projected toward a small cavity delimited by the aforementioned residues L95, A98, and L182. Indeed, the analogs 7e and 7f were endowed with a better inotropism profile than compound 7d, bearing a less hydrophobic group.

On the contrary, derivatives characterized by a dehydrosparteine nucleus (6b, 6c, 6e, and 6f) differently arranged within the sigma-1 receptor binding site, due to the more rigid and planar core. Consequently, they displayed a reversed docking mode with respect to PD144418, moving the phenyl ring and the dehydrosparteine cycle near the phenyl ring and the basic moiety of the reference compounds.

Concerning sparteine itself, it proved to partially overlap the related nucleus of the analog 7b, exhibiting a quite comparable positioning. In particular, it moved much more in proximity of E172, displaying the required salt-bridge, while any contact with D126 is lacking. Conceivably, the absence of a phenyl substitution impairs the possibility to interact with the aforementioned residues A98, L182, and Y206.

On all these bases, the relevant sparteine derivatives may indeed act as efficient ligands for sigma-1 receptor.

Conclusions
Ten sparteine derivatives (2-(4-substituted-phenyl)-2-dehydrospar- teines and 2-(4-substituted-phenyl)sparteines) were tested for inotropic and chronotropic activity on reserpinized guinea pig atria. Four of them (6b, 6e, 7b, and 7f) exhibited significant inotropic activity that, at the highest concentrations tested, was followed by negative inotropism or toxicity. Vice versa, compound 7e (2-(4-tol- yl)sparteine) exhibited a steep dose-depending positive inotropic activity up to the highest concentration (300 μM). At 10 μM, its activity was comparable with that of milrinone, but at 100 μM, the activity of 7e was more than twice that of milrinone. Thus, in comparison with digoxin and milrinone, 7e resulted less potent, but the most active.

The inotropic activity of compound 7b was confirmed on electrically driven left atria from untreated guinea pig and was shown that its activity was not related to adrenergic stimulation or to inhibition of phosphodiesterase. A similar behavior was observed in the past for two, somewhat analogous, aryalkyl derivatives of cytisine. Therefore, it is advanced the hypothesis that all the relevant compounds 6–9, characterized by the combination of aromatic moieties with polycyclic alkaloidal frameworks, may share the same mechanism of action. On the base of some structural similarities between compounds 6–9 and sigma-1 receptor ligands, endowed with activity on cardiac muscle, it is supposed that the presently observed inotropic activity might be related to interaction with this receptor. Docking studies have, indeed, shown an excellent fitting of compounds 7b and 7e on the sigma-1 receptor binding site.

Moreover, compound 7b was shown to possess potent diuretic and saluretic activities, which might result very valuable in association to the positive inotropism to relieve symptoms of CHF.

**Figure 7.** Docking mode of 7b and of 7e within the X-ray crystallographic data of the human sigma-1 receptor and of the ligand PD144418 (C atom; brown). The most important residues are labeled.
Concluding, the 2-arylsparteine pharmacophore appears worthy of further investigation in order to develop novel agents for the treatment of CHF.

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The authors declare that they have no conflict of interest.

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References
1. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J 2008;29:2388–442.
2. Feldman AM. Classification of positive inotropic agents. J Am Coll Cardiol 1993;22:1223–7.
3. Francis GS, Bartos JA, Adaya S. Inotropes. J Am Coll Cardiol 2014;63:2069–78.
4. Tariq S, Aronow WS. Use of inotropic agents in treatment of systolic heart failure. Int J Mol Sci 2015;16:29060–8.
5. Endoh M, Hori M. Basic pharmacology and clinical application of new positive inotropic agents. Drugs Today 1993;29:29–54.
6. Tham YK, Bernardo BC, Ooi JY, et al. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. Arch Toxicol 2015;89:1401–38.
7. Matsumori A. The role of NF-kappaB in the pathogenesis of heart failure and endotoxemia. Drugs Future 2004;29:733–9.
8. Shin DD, Brandimarte F, De Luca L, et al. Review of current and investigational pharmacologic agents for acute heart failure syndromes. Am J Cardiol 2007;99:4A–23A.
9. Suffredini S, Mugelli A, Cerbai E. If channels as a therapeutic target in heart disease. Future Cardiol 2007;3:657–66.
10. Tamargo J, Duarte J, Caballero R, Delpón E. New therapeutic targets for the development of positive inotropic agents. Discov Med 2011;12:381–92.
11. (a) Forster N, Hoefke W. A comparison of the effects of sparteine, quinidine and nicotine on isolated myocardium of cats and rats. Naunyn-Schmiedeberg’s Arch. Expt Pathol Pharmacol 1960;239:383–92. (b) von Philipsborn G, Wilhelm E, Homburger H. Effect of sparteine in the isolated atrial myocardium of guinea-pigs. Naunyn-Schmiedeberg’s Arch Pharmacol 1973;277:281–90. (c) Raschack M. Actions of sparteine and sparteine derivatives on the heart and circulation. Arzneim Forsch 1974;24:753–9. (d) Engelmann K, Raake W, Petter A. The importance of hydrophobic groups for the antiarrhythmic results of alkylated sparteine. Arzneim Forsch 1974;24:759–61.
12. Schmidt HD, Padeken D, Beck L. Cardiovascular effect of sparteine in anaesthetized dogs with and without blockade of cardiac autonomic nerves. Arznei Forsch 1986;36:1481–4.
13. Kimura M, Kimura I, Chui L-H, Okuda S. Positive inotropic action and conformation difference of lupin alkaloids in isolated cardiac muscle of guinea pig and bullfrog. Phytother Res 1989;3:101–5.
14. Zetler G, Strubelt O. Antifibrillatory, cardiovascular and toxic effects of sparteine, butylsparteine and pentylsparteine. Arzneimittelforschung 1980;30:1497–502.
15. (a) Schoen U, Kehrbach W, Hachmeister B, et al. Ger. Offen. DE3522475-A1-19870102. Chem Abstr 1987;106:156761s. (b) Schoen U, Kehrbach W, Hachmeister B, et al. Ger. Offen. DE3643402-A1-19980630. Chem Abstr 1998;110:63753h.
16. (a) Winterfeld K, Hoffmann E. Über das Verhalten des Lupanins bei der Grignardierung (Zugleich XII. Mitteilung über die Alkaloida der Lupinen). Arch Pharm 1937;275:5–27. (b) Winterfeld K, Hoffmann E. Zur Kenntnis des Anisoyl-Sparteines (Zugleich XIV. Mitteilung über die Alkaloida der Lupinen). Arch Pharm 1937;275:526–32.
17. Jack W. Untersuchung einiger Sparteinabkömmlinge auf die fimmerwidrige Wirkung am Froscherzen. Arch Exptl Pathol Pharmacol 1942;200:528–35.
18. (a) Sparatore F, Boido V, Preziosi P, et al. Synthesis and pharmacodynamic properties of various lupinane derivatives. Farmaco, Ed. Sci 1969;24:587–621. (b) Boido V, Boido A, Boido Canu C, Sparatore F. Quinolozidinylalkylamines with antiisdpertensive activity. Farmaco, Ed. Sci 1979;34:673–87.
19. (a) Canu Boido C, Sparatore F. Synthesis and preliminary pharmacological evaluation of some cytisine derivatives. Farmaco 1999;54:438–51. (b) Canu Boido C, Tasso B, Boido V, Sparatore F. Cytisine derivatives as ligands for neuronal nicotine receptors and with various pharmacological activities. Farmaco 2003;58:265–77.
20. (a) Sparatore A, Sparatore F. Preparation and pharmacological activities of 10-homolupinanyloyl-2-R-phenthoazines. Farmaco 1994;49:5–17. (b) Sparatore A, Sparatore F. Preparation and pharmacological activities of homolupinanyloyl anilides. Farmaco 1995;50:153–66.
21. Vazza I, Budriesi R, Terranova E, et al. Novel quinolizidinyl derivatives as antiarrhythmic agents. J Med Chem 2007;50:334–43.
22. Tasso B, Budriesi R, Vazza I, et al. Novel quinolizidinyl derivatives as antiarrhythmic agents: 2. Further investigation. J Med Chem 2010;53:4668–77.
23. Boczon W. Further studies on the stereochemistry of sparteine, its isomers and derivatives. XV. Synthesis, structure and spectroscopic properties of 2-methyl-2-dehydrosparteine and 2-(p-tolyl)-2-dehydrosparteine (free bases) and their diprotonated salts. Bull Pol Acad Sci Chem 1988;36:21–36.
24. Boczon W, Koziol B. Further studies on the stereochemistry of sparteine, its isomers and derivatives. XXIV. 2-(p-Tolyl)sparteine and its monoperchlorate salt. J Mol Struct 1997;403:171–81.
25. MOE: Chemical Computing Group Inc. Montreal. H3A 2R7 Canada. http://www.chemcomp.com.
26. (a) Fossa P, Cichero E. In silico evaluation of human small heat shock protein HSP27: homology modeling, mutation analyses and docking studies. Bioorg Med Chem 2015;23:3215–20. (b) Franchini S, Manasia E, Sorbi C, et al. Synthesis, biological evaluation and molecular modeling of 1-oxa-4-thiaspiro-1,4-dithiaspiro[4.5]decane.
derivatives as potent and selective 5-HT1A receptor agonists. Eur J Med Chem 2016;125:435–52. (c) Deiana V, Gómez-Canas M, Pazos MR, et al. Tricyclic pyrazoles. Part 8. Synthesis, biological evaluation and modelling of tricyclic pyrazole carboxamides as potential CB2 receptor ligands with antagonist/inverse agonist properties. Eur J Med Chem 2016;112:66–80.

27. Ligon EWm. Jr., Action of lupine alkaloids on the motility of the isolated rabbit uterus. J Pharmacol 1941;73:151–8.

28. (a) Sparatore A, Novelli F, Sparatore F. Quinolizidine derivatives as ligands for sigma receptors. Med Chem Res 2002;11:1–11. (b) Sparatore A, Novelli F, Sparatore F. 1-(Arylalkyl)quinolizidine derivatives and thio-isosteric analogs as ligands for sigma receptors. Helv Chim Acta 2004;87:580–91.

29. (a) De Costa BR, Radesca L, Di Paolo L, Bowen WD. Synthesis, characterization, and biological evaluation of a novel class of N-(arylethyl)-N-alkyl-2-(1-pyrrolidinyl)ethylamines: structural requirements and binding affinity at the sigma receptor. J Med Chem 1992;35:38–47. (b) Glennon RA. Pharmacophore identification for sigma-1 (sigma1) receptor binding: application of the “deconstruction-reconstruction-elaboration” approach. Mini Rev Med Chem 2005;5:927–40. (c) Matsumoto RR, Bowen WD, Su TP. Sigma receptors. Chemistry, cell biology and clinical implications. New York: Springer; 2007. ISBN 978-0-387-36514-5.

30. Monassier L, Bousquet P. Sigma receptors: from discovery to highlights of their implications in the cardiovascular system. Fundam Clin Pharmacol 2002;16:1–8.

31. Zhang H, Cuevas J. Sigma receptor activation blocks potassium channels and depresses neuroexcitability in rat intracardiac neurons. J Pharmacol Exp Ther 2005;313:1387–96.

32. Bhuian MS, Fukunaga K. Targeting sigma-1 receptor signaling by endogenous ligands for cardioprotection. Expert Opin Ther Targets 2011;15:145–55.

33. Novakova M. Effects of sigma receptor ligand BD737 in rat isolated hearts. Scr Med (Brno) 2007;80:255–62.

34. Novakova M, Bruderova V, Sulova Z, et al. Modulation of expression of the sigma receptors in the heart of rat and mouse in normal and pathological conditions. Gen Physiol Biophys 2007;26:110–17.

35. (a) Laurini E, Marson D, Dal Col V, et al. Another brick in the wall. Validation of the σ1 receptor 3D model by computer-assisted design, synthesis, and activity of new σ1 ligands. Mol Pharm 2012;9:3107–26. (b) Franchini S, Battisti UM, Prandi A, et al. Scouting new sigma receptor ligands: synthesis, pharmacological evaluation and molecular modeling of 1,3-dioxolane-based structures and derivatives. Eur J Med Chem 2016;112:1–19.

36. Meyer C, Schepmann D, Yanagisawa S, et al. Pd-catalyzed direct C-H bond functionalization of spirocyclic σ1 ligands: generation of a pharmacophore model and analysis of the reverse binding mode by docking into a 3D homology model of the σ1 receptor. J Med Chem 2012;55:8047–65.

37. Brune S, Schepmann D, Klempnauer KH, et al. The sigma enigma: in vitro/in silico site-directed mutagenesis studies unveil σ1 receptor ligand binding. Biochemistry 2014;53:2993–3003.

38. Schmidt HR, Zheng S, Gurpinar E, et al. Crystal structure of the human σ1 receptor. Nature 2016;532:527–30.