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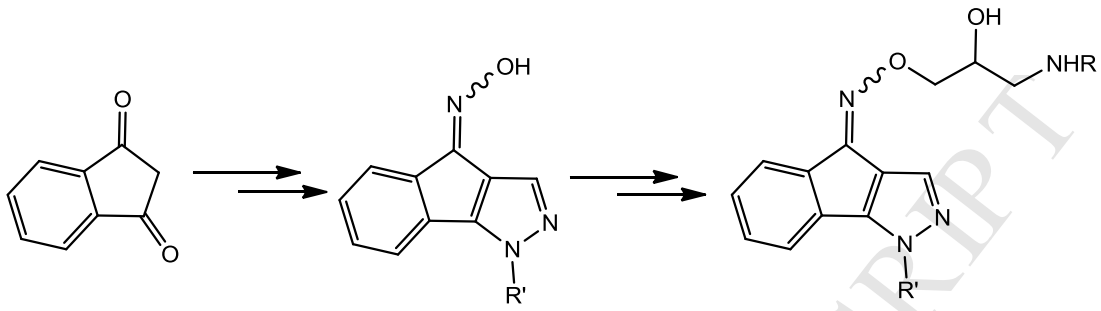
Graphical abstract



Highlights

► A new heterocyclic system indeno[1,2-*c*]pyrazole oxime ethers was reported. ► Some derivatives of this heterocyclic system have been prepared. ► A selected derivative was screened for its β_1-adrenergic blocking activity. ► The oxime ether (**7b**) reduces cardiac contractility and selectively antagonizes β_1-AR.

Indenopyrazole Oxime Ethers: Synthesis and β_1-Adrenergic Blocking Activity

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Abstract

This paper reports the synthesis and cardiac activity of new β-blockers derived from *(Z/E)*-indeno[1,2-*c*]pyrazol-4(1*H*)-one oximes (**5a,b**). The latter compounds were allowed to react with epichlorhydrin, followed by reacting the oxiranyl derivatives formed (**6a,b**) with some aliphatic amines to give the target compounds *(Z/E)*-1-phenyl-1*H*-indeno[1,2-*c*]pyrazol-4-one *O*-((2-hydroxy-3-(substituted

amino)propyl)oxime (**7a-c**) and *(Z/E)*-1-methyl-1*H*-indeno[1,2-*c*]pyrazol-4-one *O*-((2-hydroxy-3-(substituted amino)propyl)oxime (**8a-c**). These final products **7a-c** and **8a-c** were evaluated for their ability to modulate the cardiac performance of a prototype mammalian heart. The results showed that, out of these molecules tested, **7b** elicits a more potent depressant effect on contractility and relaxation, and competitively antagonizes β_1-adrenergic receptors.

Keywords. β-Adrenergic blocking agents, β-adrenoceptors, indenopyrazoles, indenopyrazole oximes, hydroxypropanolamines.

1. Introduction

The β-blockers are an important class of drugs used in the treatment of several cardiovascular diseases such as: hypertension, angina pectoris, heart attack and certain arrhythmias [1]. Indeed, their action is characterized by a mechanism of competitive antagonism on β_1-receptor, localized on the heart, on the arteriolar smooth muscle and on the β-cells of the juxtaglomerular apparatus, thus chronotropic, inotropic and dromotropic effect is obtained [2]. Drugs, generally, used in therapy and/or commercially available as β-blockers are compounds with a well-defined structure **I**. They are all chiral molecules (optically active or racemic) in which three functions can be identified: an aryl group linked through -OCH_2- (or another group) to a moiety containing both an alcohol and an amino function bearing an *i*-propyl, *tert*-butyl or a more bulky residue [3] (Figure 1).

Thus, β-adrenoceptor-blocking agents generally belong to the arylethanolamine **II** and aryloxypropanolamine classes. Many of the most potent β-blockers used for the treatment of hypertension, belong to the latter class such as propranolol **III** [4,5].

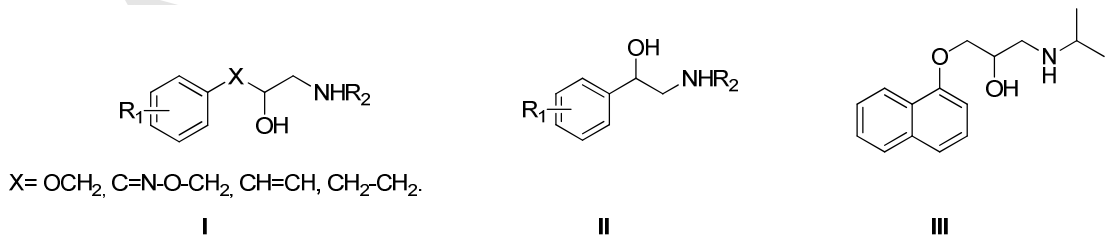


Figure 1: Structures of β-blockers.

Several β-blockers are now available; however some of them show certain side effects. These side effects are mainly due to the bronchoconstriction action due to the β_2-receptor localized mainly on the bronchial smooth muscle. Moreover, the stimulation of the β_3-receptors, mainly present on the adipose tissue lead to a consequent block and alteration of the activity of the enzyme lipase interfering with the triglyceride synthesis. Therefore the synthesis of new specific β_1-antagonists is still needed.

Previous studies [6,7,10-22] demonstrated that the imino group of β-blockers side chain bearing the required hydroxyalkyamino side chain attached to an oximino group, did not abolish the β-adrenoceptor activity of these β-blocking agents. In the same time some of these β-blockers showed β_1- and others showed β_2-selective antagonism [6-10].

Several publications have described the discovery and characterization of the biological, microbiological and pharmacological activities of novel oxime ethers as β-adrenoceptor ligands [17, 23]. In the light of the potent β-adrenoceptor blocking activity of fluorenone oxime ether (IPS 339) **IV** [22], we wish herein to designe a synthesis in order to rapidly pharmacomodulate this structure using the commercially available indanedione (Figure 2) as a starting material to obtain the target heterocycles **(7a-c** and **8a-c)**. The biological activities of these novel heterocycles, analogs of IPS 339 **IV,** were evaluated for their β_1-adrenergic blocking activity, in *ex vivo* Langendorf rat heart preparation.

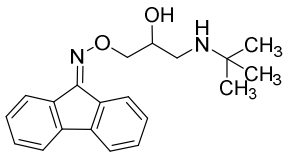


Figure 2: Structure of IPS 339 (**IV**).

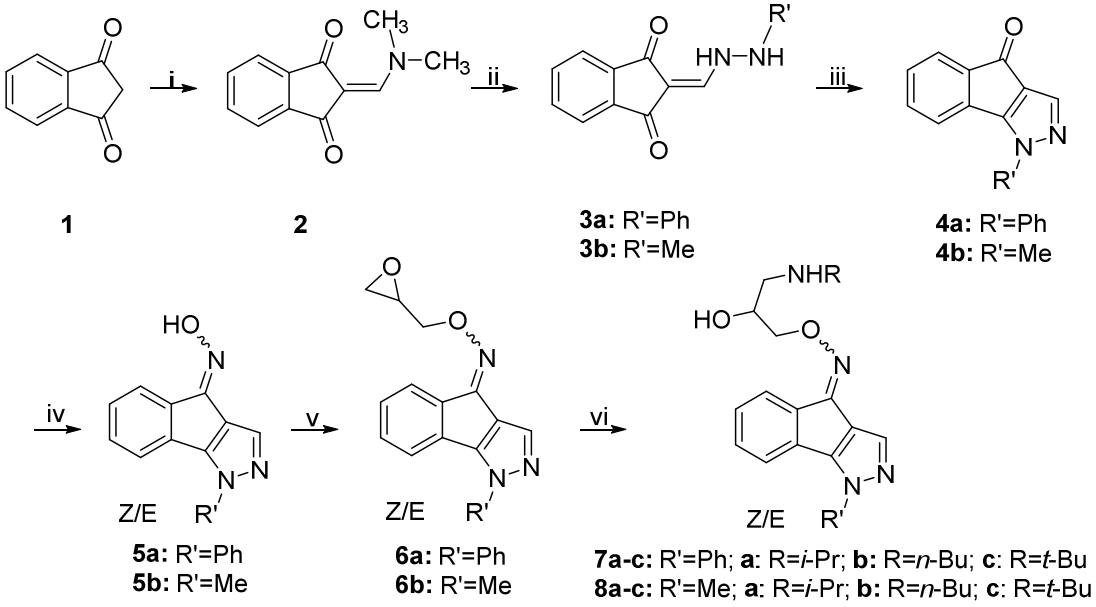
2. Results and discussion

2.1 Chemistry

The synthesis of some unknown oxime ethers **7a-c** and **8a-c** derived from indeno[1,2-*c*]pyrazole could be envisaged by substituting one of the benzene rings of the fluorene moiety of **IV** by a pyrazole ring. Thus, we have adopted the procedure reported by

Schenone *et al.* [24] to prepare the indeno[1,2-*c*]pyrazoles **3a-b** using indane-1,3-dione as a starting material (Scheme 1). When this dione was reacted with *N*,*N*-dimethylformamide dimethyl acetal (DMF/DMA) the 2-(*N*,*N*-dimethylaminomethylene)indane-1,3-dione **(2)** was obtained. This was reacted with phenylhydrazine or methylhydrazine in butanol in the presence of acetic acid to give 2-*(N'*-phenylhydrazinomethylene)indane-1,3-dione **(3a)** and 2-*(N'*-methylhydrazinomethylene)indane-1,3-dione **(3b)** respectively which were cyclised, upon heating under reflux in anhydrous toluene and in the presence of *p*-toluenesulfonic acid, giving 1-phenyl-1*H*-indeno[1,2-*c*]pyrazol-4-one **(4a)**, with 42% yield, and 1-methyl-1*H*-indeno[1,2-*c*]pyrazol-4-one **(4b)**, with 35% yield.

The reaction of the latter compound with hydroxylamine hydrochloride in pyridine gave the corresponding oximes **5a-b** (isomeric mixture *E/Z*). These oximes were then converted into the corresponding ethers **7a-c** and **8a-c** in a two-step reaction following the classical route as indicated in Scheme 1. Thus the oximes **5a-b** were reacted with epichlorohydrin to give the oxiranyl derivatives **6a-b** which were allowed to react with the appropriate amines; *i*-propylamine, *n*-butylamine and *tert*-butylamine to obtain **7a-c** and **8a-c**.



Reagents and conditions: (i) *N*,*N*-dimethylformamide dimethyl acetal (DMF/DMA), toluene, reflux, 24h; (ii) Phenyl hydrazine or methyl hydrazine, butan-1-ol, AcOH, overnight, 0 °C; (iii) TsOH, toluene, reflux, 24h; (iv) $NH_2OH.HCl$, pyridine, rt, 3h; (v) Epichlorhydrin, acetone/water, K_2CO_3, reflux, 6 days. (vi) RNH_2, anhydrous toluene.

Scheme 1. Synthetic pathway of compounds **7a-c** and **8a-c**.

2.2 Biology

The present study shows that six newly synthesized molecules, namely **7a-c** and **8a-c**, structurally similar to the known beta-blocker fluorenone oxime ether **IV** (IPS 339), are able to modulate the mammalian cardiac performance with different order of potency. On the isolated and Langendorff perfused rat heart, **7b** determined a dose-dependent reduction of basal myocardial contractility (inotropism) and relaxation (lusitropism). This effect, which is more potent than that elicited by **7a** and **7c**, is obtained without changing heart rate (HR) and coronary pressure (CP). In addition, **7b** elicited competitive antagonism against β_1-adrenergic receptors. Because of its higher influence on the cardiac performance, only **7b** was included in the study on the mechanism of action. **8a** and **8b** did not induce significant effects on cardiac performance except for a dose-dependent positive inotropism induced by **8c**.

*2.2.1 Effect of **7a-c** and **8a-c** on basal cardiac performance*

β-Blockers are known by their basal inotropic action on the mammalian heart. An example is the classic negative inotropism and lusitropism induced by propranolol, a typical non-selective β-blocker [25]. In our study we found that **7b** reduces myocardial contraction and relaxation, suggesting this molecule to be functionally similar to β-blockers. Analysis of the IC_{50} values on left ventricular pressure (LVP) revealed that the inhibitory concentration of **7b** is $5x10^{-10}$ M. This is of relevance since many common β-blockers, such as propranolol, nadolol, metapropol show an IC_{50} of $12x10^{-9}$ M [26]. Accordingly, we suggest that **7b** is able to elicit inhibitory effects at a concentration lower than that of other β-blockers. Contrarily, **7a**, **7c**, **8a** and **8b** did not affect basal inotropism and lusitropism except for the positive inotropism induced by **8c.** This could be attributed to the different structural characteristics of the six molecules at the level of the substituent on the nitrogen atom of the amine portion, important structural region for the β-blocker activity, and also of the N_1 of the pyrazole ring. In particular, compounds **7a, 8a** and **7b, 8b** contain an *i*-propyl- and *n*-butyl group respectively on the aminic portion, while **7c, 8c** have a *tert*-butyl chain.

2.2.1.1 Basal conditions

Langendorff perfused heart- Cardiac parameters, obtained after 20 min equilibration, are indicated in Materials and Methods. Endurance and stability of the preparations, analyzed by measuring the performance variables every 10 min, showed that each heart was stable up to 180 min.

*2.2.1.2 **7a-c** and **8a-c** stimulated preparations*

Preliminary experiments (data not shown) obtained by repetitive exposure of each heart to one concentration of **7a**, **7b**, **7c, 8a, 8b** or **8c** (1 nM) revealed the absence of desensitization.

The biological potency of these putative novel beta-blockers was evaluated by analyzing the hemodynamic performance of rat hearts *ex vivo* perfused according to Langendorff. We found that application of **7a** from 1 pM to 10 nM resulted in a negative inotropic effect revealed by the reduction of LVP and +(LVdP/dT)max which reached a maximum (20%) at the highest doses tested. **7a** did not modify HR and CP. **7b**, which is ineffective on HR and CP, induced a strong reduction of inotropic parameters (LVP and +(LVdP/dT)max) at all concentrations tested (1 pM-10 nM), reaching a maximun of reduction of 40% at 10 nM. The IC_{50} of **7b**-dependent negative inotropism was 5 x 10^{-10} M. In contrast to the other molecules, **7c** did not significantly affect inotropism, while it induced an insignificant increment of HR and CP (Figure 3-5). Of note, **8a** and **8b** elicited limited and insignificant effects on cardiac performance. **8a** increased inotropism (LVP and (LVdP/dt)max) only at concentration of 10^{-9} M; it enhanced HR at 10^{-10} M and 10^{-9} M with a non significant vasoconstriction. **8b** was able to decrease only LVP at 10^{-9}M and increase HR at 10^{-10} M and 10^{-9} M. **8c** induced dose-dependent positive inotropism at all concentration tested without changes in HR and CP (Figure 6-8).

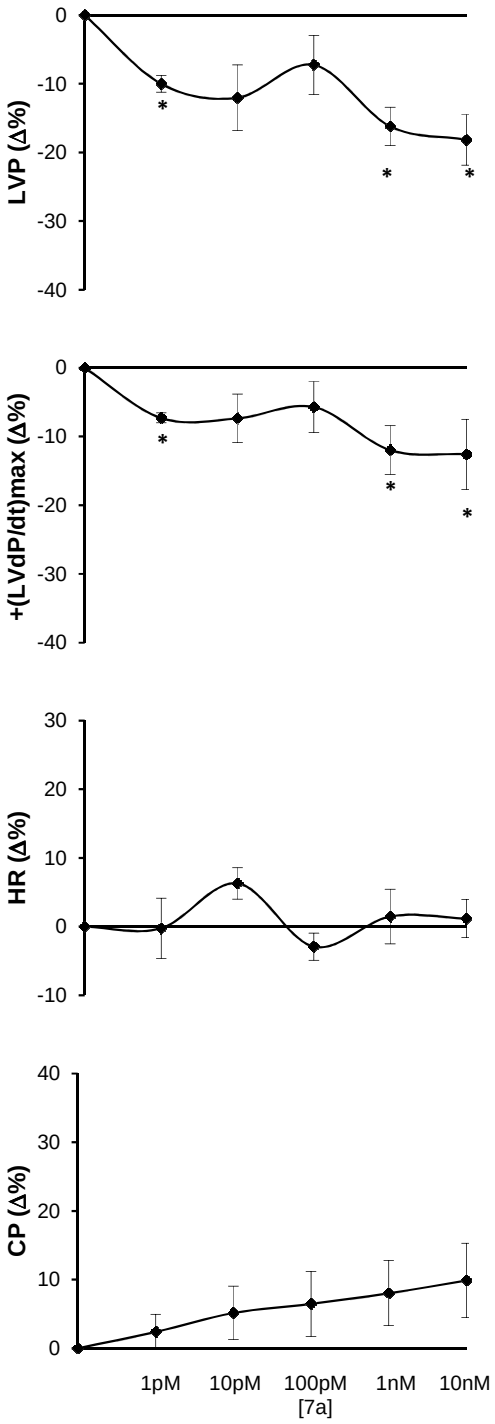


Figure 3. Dose-dependent response curves of **7a** (1 pM-10 nM) on LVP, +(LVdP/dT)max, CP and HR, on Langendorff perfused rat heart preparations. Percentage changes were evaluated as means ± SEM of 7 experiments. Significance of difference from control values was evaluated by one-way ANOVA; $P = 0 < 0.05$.

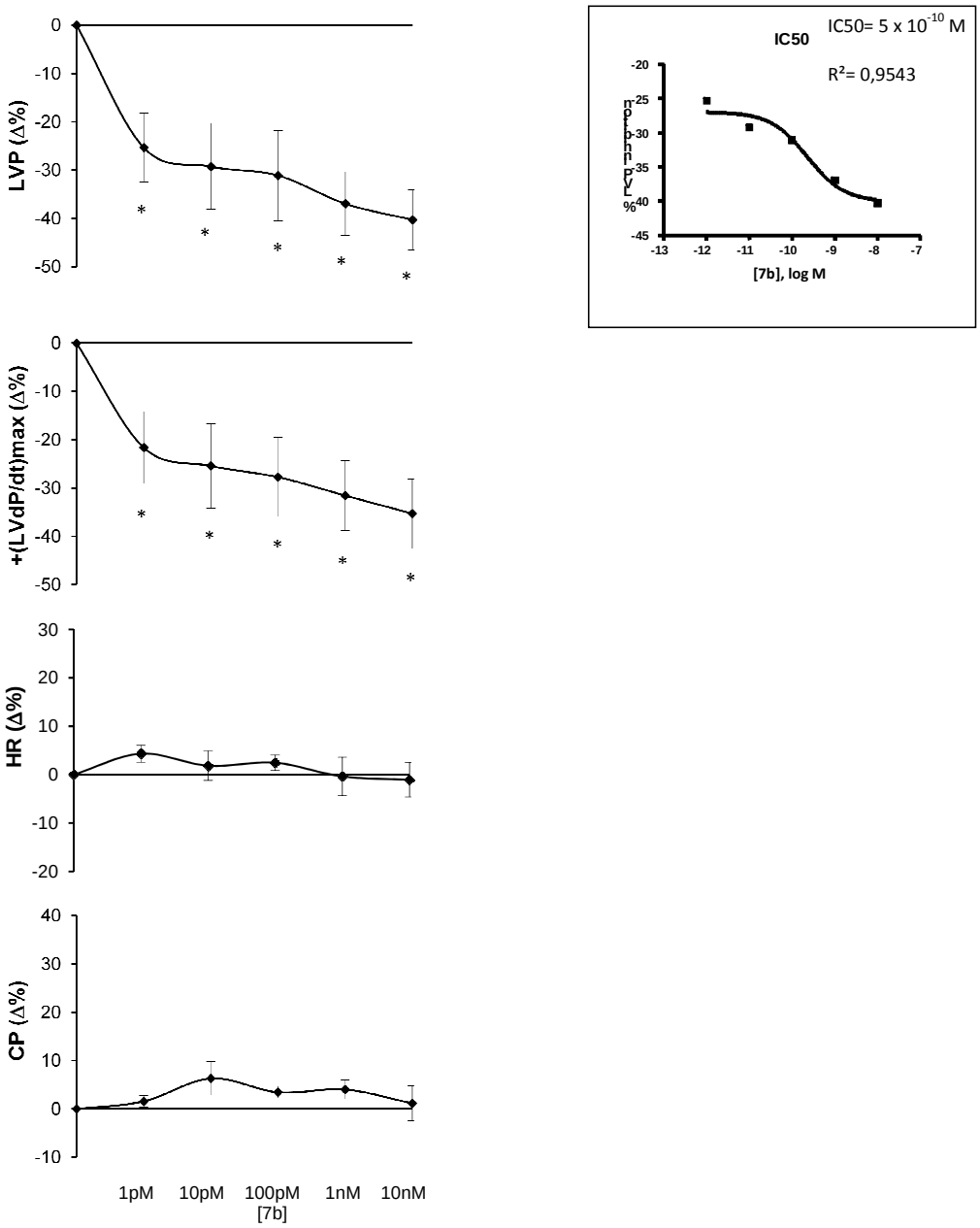


Figure 4. Dose-dependent response curves of **7b** (1 pM-10 nM) on LVP, +(LVdP/dT)max, CP and HR, on Langendorff perfused rat heart preparations. Percentage changes were evaluated as means ± SEM of 7 experiments. Significance of difference from control values was evaluated by one-way ANOVA; *P= 0<0.*05.

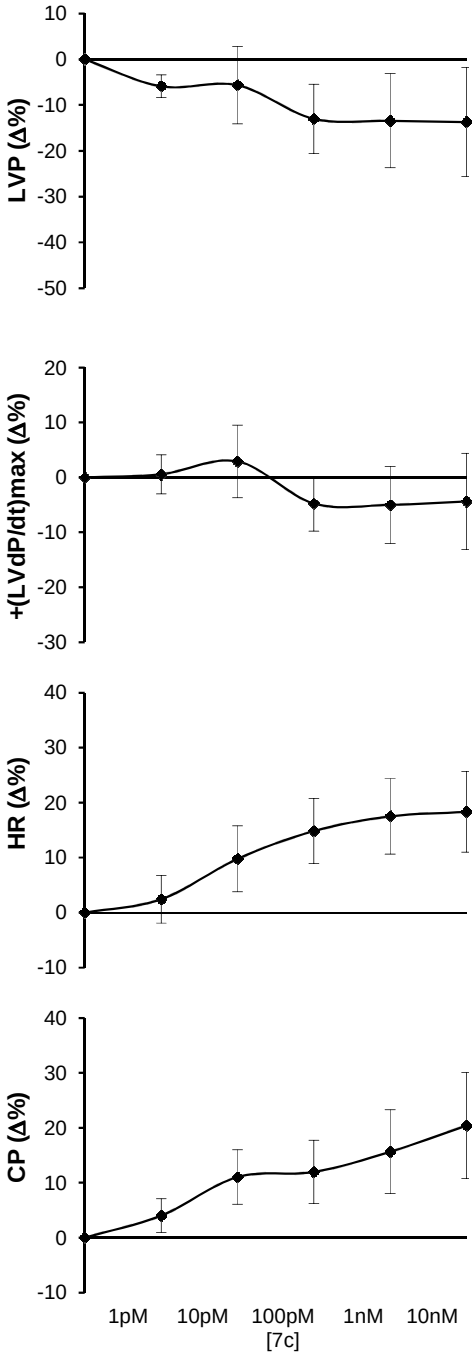


Figure 5. Dose-dependent response curves of **7c** (1 pM-10 nM) on LVP, +(LVdP/dT)max, CP and HR, on Langendorff perfused rat heart preparations. Percentage changes were evaluated as means ± SEM of 7 experiments. Significance of difference from control values was evaluated by one-way ANOVA; $P = 0 < 0.05$.

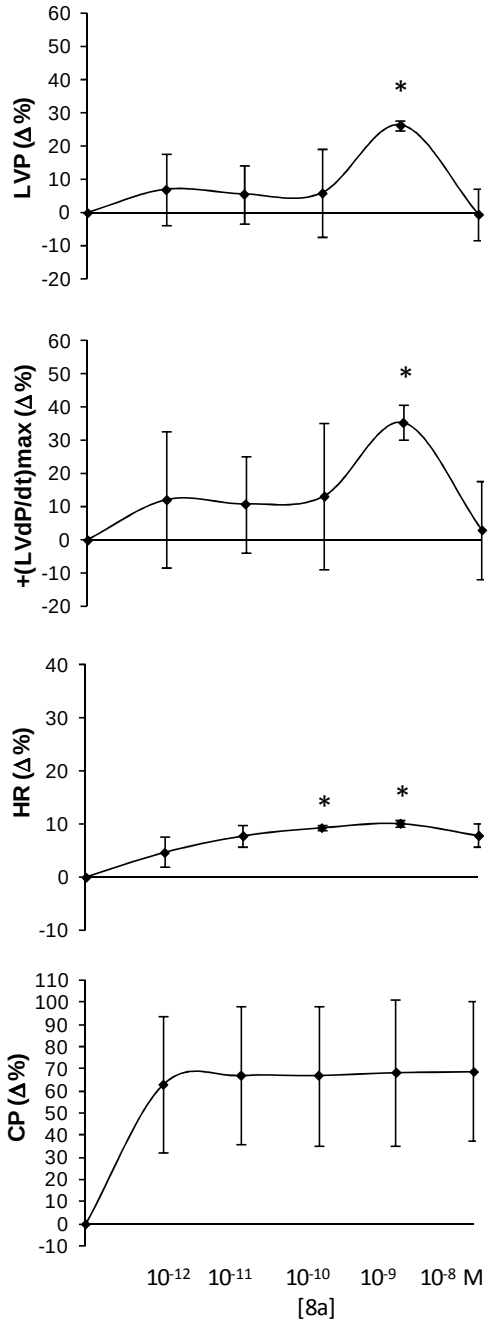


Figure 6. Dose-dependent response curves of **8a** (10^{-12} M – 10^{-8} M) on LVP, +(LVdP/dT)max, HR and CP, on Langendorff perfused rat heart preparations. Percentage changes were evaluated as means ± SEM of 7 experiments. Significance of difference from control values was evaluated by one-way ANOVA; *P= 0<0.*05.

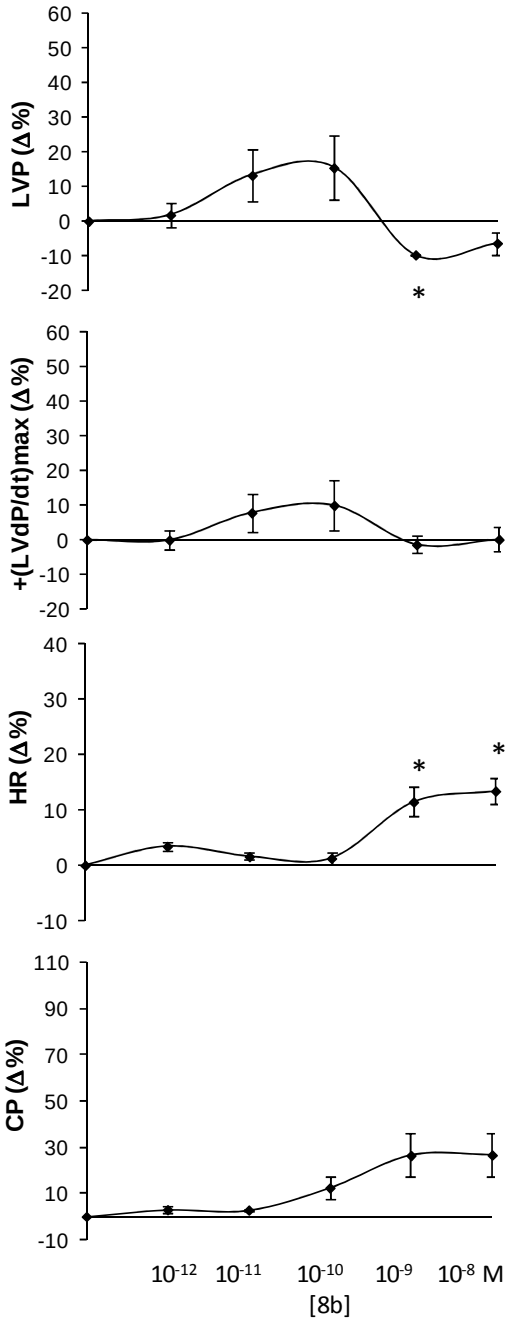


Figure 7. Dose-dependent response curves of **8b** (10^{-12} M – 10^{-8} M) on LVP, +(LVdP/dT)max, HR and CP, on Langendorff perfused rat heart preparations. Percentage changes were evaluated as means ± SEM of 7 experiments. Significance of difference from control values was evaluated by one-way ANOVA; *P= 0<0.*05.

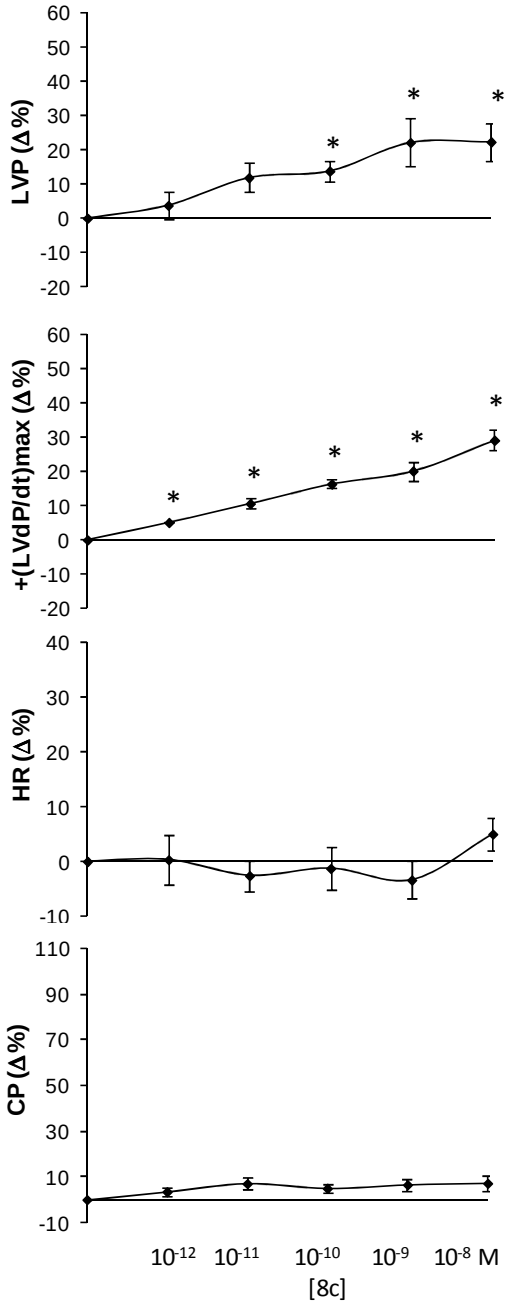


Figure 8. Dose-dependent response curves of **8c** (10^{-12} M – 10^{-8} M) on LVP, +(LVdP/dT)max, HR and CP, on Langendorff perfused rat heart preparations. Percentage changes were evaluated as means ± SEM of 7 experiments. Significance of difference from control values was evaluated by one-way ANOVA; *P= 0<0.*05.

*2.2.2 Anti-adrenergic action of **7b***

It is reported that propranolol elicits competitive antagonism against adrenergic stimulation and this occurs with an EC_{50} of 30 nM [27]. Our experiments on the rat heart revealed that, like propranolol, **7b** exerted competitive antagonism in the presence of an isoproterenol-dependent adrenergic stimulation, being able to counteract both positive inotropism and lusitropism. Notably, this **7b**-induced competitive antagonism is obtained at a concentration of 0.5 nM, lower than that reported for propranolol (30 nM; [27]). This confirms the β-blocker-like property of this new synthesized molecule which appears to behave as a classic anti-adrenergic drug, but at higher potency. This may be of notable pharmacological interest since it is recognized that the lower is the active dose of a molecule, the lower can be the possibility of side effects [28].

Of paramount importance in relation to both basic research and clinical application, is the catecholamine-dependent regulation of cardiac function which occurs through activation of β_1 and β_2 types of β-adrenergic receptors [29]. β_1-Receptors activates Gs proteins, with consequent stimulation of adenylate cyclase, increase of intracellular cAMP and protein kinase-A (PKA) activation. This induces phosphorylation of phospholamban, troponin I and sarcoplasmic reticulum Ca^{2+}/ATPasi (SERCA), all these effects contributing to the positive inotropic and the lusitropic actions which characterize β_1-receptors activity [29]. On the other hand, cardiac β_2 receptors, alternatively coupled to Gs and Gi proteins, are associated to positive and negative inotropic effects, respectively [30]. In recent years the heart was found to express another class of adrenergic receptors, namely β_3 [31]. Stimulation of these receptors leads to negative inotropism and lusitropism [32,33], and counteracts the effects elicited by β_1 and β_2 activation [34]. This cardiodepression depends on G_i, $G_{i/o}$ protein, nitric oxide (NO) generation via the endothelial isoform of Nitric Oxide Synthase (eNOS), the subsequent increase of intracellular cGMP [31,34] and activation of protein kinase G (PKG) [33]. In this study we observed that, β_3-receptors are inhibited by a specific antagonist (SR59230), **7b** efficacy in counteracting the effects of isoproterenol-induced adrenergic stimulation is still detectable. This observation may contribute to characterize **7b** as a β_1-receptor antagonist, excluding this molecule to function as a partial β_3-receptor agonist. β_1-receptor selectivity was

further confirmed in the presence of non-selective α-adrenergic antagonism by phentolamine.

*2.2.2.1 Anti-adrenergic action of **7b**-Langendorff perfused heart*

To verify the possible anti-adrenergic action of **7b**, heart preparations were perfused with KHs containing increasing concentrations of Isoproterenol (Iso: 0.1 nM to 1 µM) either alone or in combination with **7b**. Iso stimulation induced a significant increase of RPP from 5 nM to 1 µM (Figure 9). The subsequent analysis of the percentage of variations of RPP provided the EC_{50} values in the presence of either increasing concentrations of Iso alone or of Iso plus **7b** (5×10^{-10} M). Results showed that **7b** exerts a competitive antagonism on adrenergic stimulation inducing a dose-dependent reduction of Iso intrinsic activity. EC_{50} values (in logM) and the intrinsic activity of Iso alone and of Iso in the presence of **7b** are shown in the legend of Figure 9.

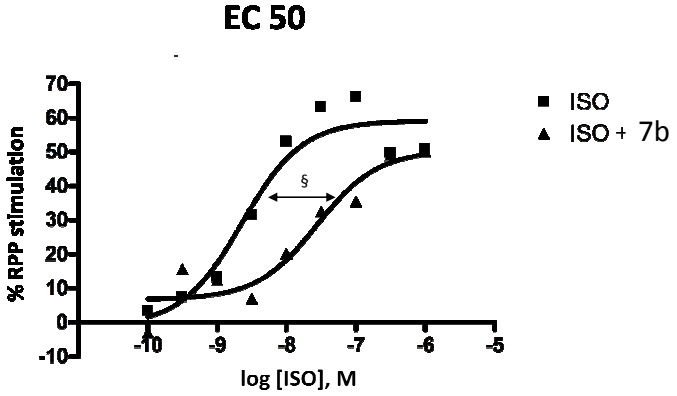


Figure 9. The sigmoid concentration-response curves of ISO-mediated stimulation on RPP of ISO (from 10^{-10} to 10^{-6} M) alone and ISO (from 10^{-10} to 10^{-6} M) plus a single concentration of **7b** (5×10^{-10} M) on the isolated and perfused Langendorff rat heart preparation. Contraction is expressed as a percentage of RPP [baseline= 0%, peak constriction by Iso and Iso plus **7b**=100 %]. The EC50 values (in logM) of Iso alone was -8.64 ± 0.23 (r^2= 0.93) and of Iso plus **7b** (5×10^{-10} M) was -7.6 ± 0.26 (r^2= 0.90). Comparison between groups (n=6 for each group) (ANOVA, Duncan's test); § = $p < 0.05$.

*2.2.2.2. Beta 2-AR, Beta3-AR and Alpha-AR receptors involvement in the anti-adrenergic antagonism of **7b**-Langendorff perfused heart*

To verify the selectivity of β-adrenergic receptor antagonism of **7b**, hearts were perfused with a single concentration of ISO (5 nM) plus **7b** (5 nM) plus β2-adrenergic antagonist (ICI118,551: 100 nM) or Alpha and β3-adrenergic antagonists (phentolamine: 100 nM; SR59230: 100 nM, respectively). As expected, ISO alone induced positive inotropism and lusitropism. These effects were abolished by co-administration of ISO plus **7b**. Contrarily, co-administration of ISO plus **7b** plus phentolamine and SR59230 or plus ICI118,551 did not affect the anti-adrenergic effect of **7b** (Figure 10a,b).

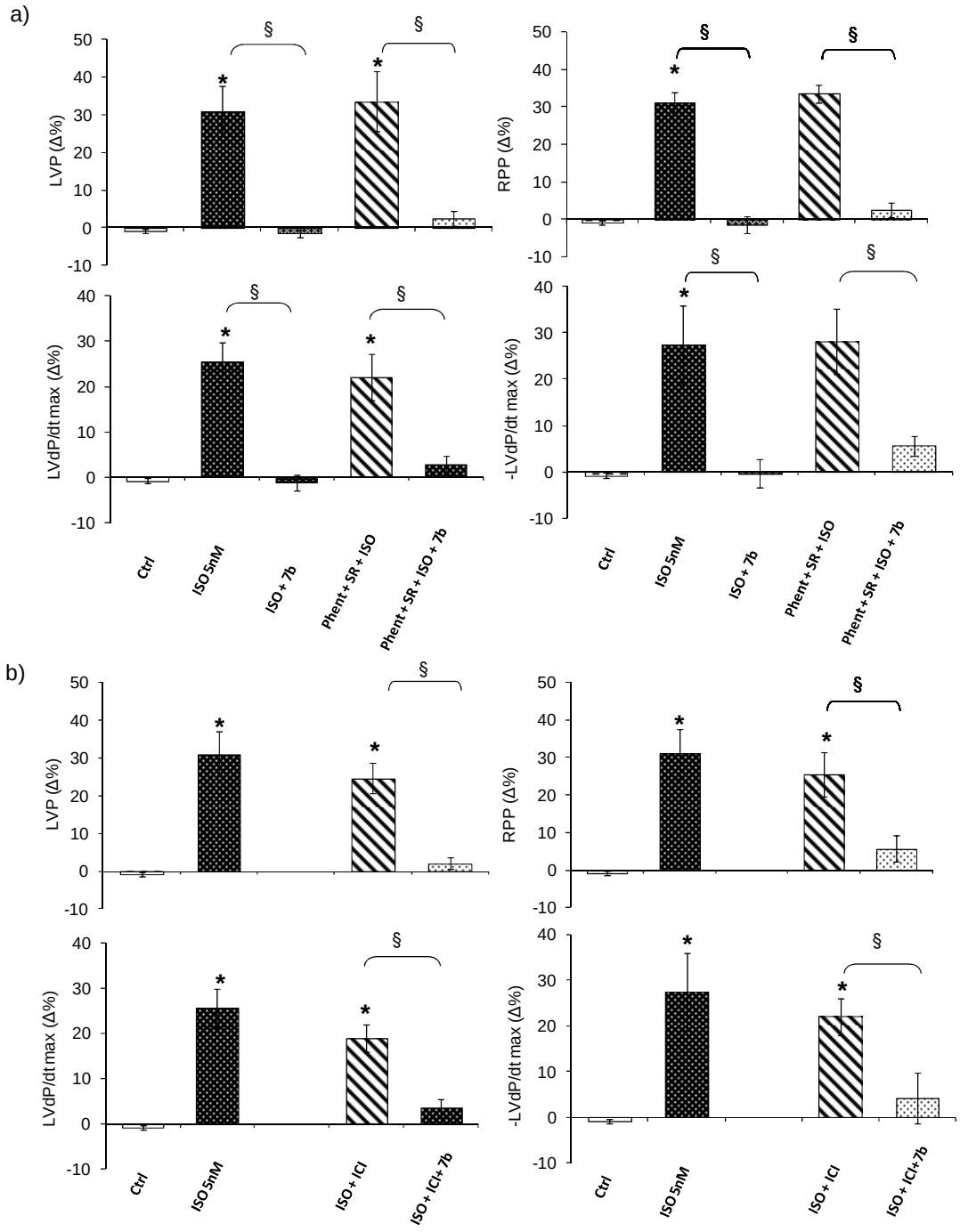


Figure 10. Effects of ISO (5 nM) before and after treatment with **7b** ($5x10^{-10}$ M), or phentolamine (100 nM) plus SR59230 (100 nM), or phentolamine plus SR59230 plus **7b** or ICI118,551 (100 nM), or ICI118,551 plus 7b on LVP (left ventricular pressure), RPP (rate pressure product), LVdP/dtmax and -LVdP/dtmax on the rat isolated and Langendorff perfused heart. Percentage changes were evaluated as means ± SEM of 5 experiments for each group. Significant difference from control values; * = $p < 0.05$. Comparison between groups; § = $p < 0,05$.

3. Conclusion

New β-adrenergic blockers **7a-c** and **8a-c** derived from the key intermediates 1-phenylindeno[1,2-*c*]pyrazol-4(1*H*)-one oxime (**5a**) and 1-methylindeno[1,2-*c*]pyrazol-4(1*H*)-one oxime (**5b**) respectively have been synthesized and evaluated for their ability to modulate the cardiac performance of a prototype mammalian heart, showing different degrees of potency. The results obtained showed that **7b** is the most potent derivative in eliciting a dose-dependent reduction of basal myocardial contractility and relaxation without affecting heart rate and coronary pressure. This is of physiological relevance since excessive and uncontrolled changes in coronary pressure and frequency may be detrimental for cardiac homeostasis. Based on our results, **7b** proved to be the best candidate to elicit β-blocker function for both its potency and effectiveness in counteracting β_1-adrenergic stimulation in a competitive manner. In contrast to classical effects induced by β-adrenergic blockers, **8c** showed positive inotropism suggesting this molecule as a partial β-adrenergic agonist rather than antagonist. However, this aspect requires further insight.

4. Experimental section

4.1 Synthesis and characterization

Commercial reagents were purchased from Aldrich, Acros Organics and Alfa Aesar and used without additional purification. Melting points were determined on a Kofler melting point apparatus. IR spectra were taken with a Perkin Elmer BX FT-IR. Mass spectra were taken on a JEOL JMS GCMate spectrometer at ionising potential of 70 eV (EI) or were performed using a spectrometer LC-MS Waters alliance 2695 (ESI^+). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL Lambda 400 spectrometer 400 MHz (400 MHz for ^{1}H, 100 MHz for the ^{13}C) or on a Bruker 300 MHz spectrometer (300 MHz for ^{1}H, 75 MHz for the ^{13}C). ^{1}H and ^{13}C NMR chemical shifts (δ*)* were reported in parts per million (ppm) and were referenced to the solvent peak; $CDCl_3$ (7.26 ppm for ^{1}H and 76.90 ppm for ^{13}C) and $(CD_3)_2CO$-d_6 (2.05 ppm for ^{1}H and 29.48 ppm for ^{13}C). Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constants (*J*) are reported in Hertz (Hz). Thin layer chromatography (TLC) was performed on silica gel 60F-264 (Merck).

2-(*N*,*N*-Dimethylaminomethylene)indane-1,3-dione (**2**) was prepared as described in the literature [24,35].

*4.1.1 General procedure for the preparation of 2-(N'–phenyl(methyl)hydrazinomethylene) indane-1,3-diones (**3a,b**)*

Phenylhydrazine or methylhydrazine (7.14 mmol) in 1-butanol (4 mL) was slowly added with stirring to a solution of 2-(*N*,*N*-dimethylaminomethylene)indane-1,3-dione **2** (1.37 g, 6.80 mmol) in 1-butanol (10 mL) and acetic acid (0.5 ml). The resulting solution was stirred at 0-5 °C for overnight. The resulting mixture was extracted with dichloromethane (2 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified on a silica gel column chromatography in petroleum ether/ethyl acetate (1/1) to give **3a,b.**

*4.1.1.1 2-(N'–phenylhydrazinomethylene)indane-1,3-dione (**3a**).*

Brown solid, mp 110 °C, (yield 44 %). IR (KBr, cm^{-1}): ν = 3452; 2936; 2358,03; 1646; 1621; 1560; 1462; 1340; 1288; 1232; 1166; 1023; 864; 751; 670. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm = 4.02-4.08 (m, 1H, N*H*-NH-Ar); 6.26 (br, 1H, NH-N*H*-C_6H_5), 6.87-6.91 (m, 2H, *Ar*); 7.03-7.07 (m, 1H, *Ar*); 7.31-7.35 (m, 2H, *Ar*); 7.67-7.70 (m, 2H, *Ar*); 7.79-7.82 (m, 1H, *Ar*); 7.83-7.85 (m, 1H, *Ar*); 7.97 (s, 1H, C=C*H*-NH). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 104.45; 113.82; 121.71; 122.27; 122.78; 129.67; 133.64; 133.77; 139.83; 140.37; 146.16; 153.67; 189.66; 193.71. MS (ESI^+): 265 (M^++1). HRMS (ESI^+) m/z calcd for $C_{16}H_{12}N_2O_2$: 265.0977; found 265.0983.

*4.1.1.2 2-(N'–methylhydrazinomethylene)indane-1,3-dione (**3b**).*

Orange solid, mp 210 °C, (yield 30 %). IR (KBr, cm^{-1}): ν = 3442; 2925; 2369; 1631; 1570; 1503; 1437; 1370; 1160; 1104; 1038; 859; 711; 593; 511. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm = 3.52 (s, 3H, CH_3); 5.29 (s, 2H, 2N*H*); 7.32 (s, 1H, C*H*-NH); 7.57-7.64 (m, 2H, *Ar*); 7.70-7.73 (m, 2H, *Ar*). ^{13}C-NMR (DMSO-d_6, 75 MHz): δ 38.65; 99.60; 128.50; 130.40; 132.75; 158.00; 191.98. MS (ESI^+): 203 (M^++1). HRMS (ESI^+) m/z calcd for $C_{11}H_{10}N_2O_2$: 202.2145; found 202.2150.

*4.1.2 General procedure for the preparation of 1-phenyl-1H-indeno[1,2-c]pyrazol-4-ones (**4a,b**).*

A mixture of hydrazinomethylene-indane-1,3-dione (**3a,b**) (3.0 mmol) and *p*-toluensulfonic acid (0.015 g, 8.7 x 10^{-2} mmol) in anhydrous toluene (10 mL) was refluxed in a Dean-Stark apparatus for 24 h. The resulting reaction mixture was cooled, and extracted with dichloromethane (2 x 50 mL). The combined organic layer was washed with an aqueous solution of NaOH (1 N, 100 mL) then with brine and finally dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified on silica gel using dichloromethane/ethyl acetate/petroleum ether (1/8/1) as an eluent to give **4a,b**.

*4.1.2.1 1-Phenyl-1H-indeno[1,2-c]pyrazol-4-one (**4a**).*

Brown solid, mp 230 °C, (yield 42 %). IR (KBr, cm^{-1}): ν = 3442; 2977; 2930; 2859; 2363; 1657; 1631; 1503; 1472; 1380; 1309; 1176; 1135; 1048; 976; 956; 762; 711; 674. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm = 7.17-7.19 (m, 1H, *Ar*), 7.29-7.33 (m, 2H, *Ar*), 7.49-7.53 (m, 1H, *Ar*), 7.56-7.60 (m, 3H, *Ar*), 7.68-7.72 (m, 3H, *Ar*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 119.62; 123.44; 124.78; 129.05; 129.62; 130.11; 133.00; 136.26; 138.78; 140.79; 158.00; 184.00. MS (ESI^+): 247 (M^++1). HRMS (ESI^+) m/z calcd for $C_{16}H_{10}N_2O$: 247.0871; found: 247.0883.

*4.1.2.2 1-Methyl-1H-indeno[1,2-c]pyrazol-4-one (**4b**).*

Orange solid, mp 140 °C, (yield 35 %). IR (KBr, cm^{-1}): ν = 3442; 2936; 1713; 1611; 1554; 1468; 1268; 1196; 1064; 966; 879; 639; 639. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm = 3.75 (s, 3H, C*H*$_3$); 6.96-7.45 (m, 4H, *Ar*); 7.59-7.61 (m, 1H, *Ar*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 37.84; 118.70; 121.72; 123.03; 124.79; 129.78; 132.75; 135.04; 140.97; 183.20; 157.50. MS (ESI^+): 185 (M^++1). HRMS (ESI^+) m/z calcd for $C_{11}H_8N_2O$: 185.0715; found: 185.0714.

*4.1.3 General procedure for the preparation of (Z/E)-1-phenyl(methyl)-1H-indeno[1,2-c] pyrazol-4-one oximes (**5a,b**).*

To a solution of indenopyrazole derivatives **4a,b** (1.26 mmol) in pyridine (5 mL) hydroxylamine hydrochloride (0.096 g, 1.39 mmol) was added and the resulting mixture was stirred at rt for 3 h. The reaction mixture was then poured onto an ice-water mixture 0-5 °C and stirred for 2 h. followed by extraction twice with dichloromethane (2 x 50 mL) and the combined organic layer was washed with a solution of HCl (1N, 150 mL) and then with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **5a,b**.

*4.1.3.1 (Z/E)-1-Phenyl-1H-indeno[1,2-c]pyrazol-4-one oxime (**5a**).*

Orange crystals, mp 212 °C, (yield 97 %). IR (KBr, cm^{-1}): ν = 3442; 2977; 2930; 2859; 2363; 1703; 1636; 1457; 1370; 1207; 1156; 1115; 1040; 961; 723; 685. ^{1}H-NMR ($(CD_3)_2CO$), 400 MHz): δ ppm = 7.31-7.34 (m, 3H, *Ar*); 7.44-7.46 (m, 2H, *Ar*); 7.53-7.57 (m, 2H, *Ar*); 7.69-7.82 (m, 3H, *Ar*); 11.25 (s, 1H, C=N-O*H*). ^{13}C-NMR ($(CD_3)_2CO$), 100 MHz): δ 120.65; 123.17; 124.06; 128.83; 129.13; 130.11; 130.44; 130.88; 137.75; 140.55; 141.33; 146.98. MS (ESI^+): 262 (M^++1). HRMS (ESI^+) m/z calcd for $C_{16}H_{11}N_3O$: 262.0980; found: 262.0974.

*4.1.3.2 (Z/E)-1-Methyl-1H-indeno[1,2-c]pyrazol-4-one oxime (**5b**).*

Yellow solid, mp 210 °C, (yield 60 %). IR (KBr, cm^{-1}): ν = 3422; 3217; 2854; 2363; 1646; 1564; 1469; 1263; 1166; 1068; 752; 711. ^{1}H-NMR ($(CD_3)_2CO$, 300 MHz): δ ppm = 3.98 (s, 3H, C*H*$_3$), 7.10-7.34 (m, 2H, *Ar*), 7.43-7.67 (m, 3H, *Ar*), 8.50 (br, 1H, O*H*). ^{13}C-NMR (DMSO-d_6, 75 MHz): δ 37.56; 119.71; 122.01; 127.61; 129.21; 134.59; 148.00. MS (ESI^+): 200 (M^++1). HRMS (ESI^+) m/z calcd for $C_{11}H_9N_3O$: 199.2139; found: 199.2133.

*4.1.4 General procedure for the preparation of (Z/E)-1-phenyl(methyl)-1H-indeno[1,2-c] pyrazol-4-one O-(oxiranylmethyl)oximes (**6a,b**).*

To a solution of **5a** or **5b** (1.26 mmol) in acetone (10 mL), K_2CO_3 (0.35 g, 2.52 mmol), epichlorhydrin (0.13 g, 1.38 mmol) and H_2O (1 mL) were added. The reaction mixture was heated under reflux for 6 days [22,36]. The solvent was evaporated under reduced pressure and the residue was extracted with dichloromethane (3 x 50 mL).

The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude products **6a,b** were used as such for the next reaction.

*4.1.4.1 (Z/E)-1-Phenyl-1H-indeno[1,2-c]pyrazol-4-one O-(oxiranylmethyl)oxime (**6a**).* Orange solid, mp 210 °C, (yield 98 %). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm = 2.73-2.75 (m, 1H, C*H*-H-O), 2.89-2.91 (m, 1H, CH-*H*-O); 3.38-3.42 (m, 1H, CH_2-C*H*-O); 4.31-4.35 (m, 1H, CH-*H*-O-N=), 4.53-4.57 (m, 1H, C*H*-H-O-N); 7.32-7.33 (m, 1H, *Ar*); 7.42-7.46 (m, 1H, *Ar*); 7.52-7.56 (m, 3H, *Ar*); 7.74-7.84 (m, 4H, *Ar*); 7.92 (s, 1H, *Ar*). MS (ESI^+): 318 (M^++1).

*4.1.4.2 (Z/E)-1-Methyl-1H-indeno[1,2-c]pyrazol-4-one O-(oxiranylmethyl)oxime (**6b**).* Brown oil, (yield 97 %). ^{1}H-NMR ($(CD_3)_2CO$, 300 MHz): δ ppm = 3.50-4.15 (5H, -C*H*$_3$, -C*H*$_2$-); 4.30-4.50 (m, 2H, -C*H*$_2$); 5.20-5.32 (m, 1H, -C*H*-O-); 7.10-7.38 (m, 3H, *Ar*); 7.48-7.70 (m, 2H, *Ar*).

*4.1.5 General procedure for the preparation of (Z/E)-1-phenyl(methyl)-1H-indeno[1,2-c] pyrazol-4-one O-((2-hydroxy-3-(substituted amino)propyl)oximes (**7a-c**) and (**8a-c**).*

To a solution of **6a** or **6b** (0.1 mmol) in anhydrous toluene (10 mL) was added *i*-propylamine, *n*-butylamine, or *tert*-butylamine (5 mL). The reaction mixture was heated at 80 °C in a sealed tube for 7 days [36,37]. The reaction mixture was cooled, evaporated under reduced pressure, and the solid obtained was purified on silica gel using dichloromethane/methanol (9:1) as an eluent to give **7a-c** (for **6a**) and **8a-c** (for **6b**).

*4.1.5.1 (Z/E)-1-Phenyl-1H-indeno[1,2-c]pyrazol-4-one O-((2-hydroxy-3-(isopropylamino)propyl)oxime (**7a**).*

Orange solid, mp 145 °C, (yield 10 %). IR (KBr, cm^{-1}): ν = 3466; 3433; 2082; 1635; 1531; 1461; 1384; 1261; 1101; 1055; 972; 947; 867; 762; 643; 694; 725. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm = 1.22-1.52 (m, 6H, 2C*H*$_3$); 3.04-3.30 (m, 3H, C*H*$_2$-NH; C*H*); 4.17-4.18 (m, 1H, C*H*-OH); 4.31-4.50 (m, 2H, C*H*$_2$-OH); 5.27 (s, 1H, N*H*); 5.42 (s, 1H, O*H*); 7.19-7.24 (m, 1H, *Ar*); 7.27-7.29 (m, 1H, *Ar*); 7.43-7.45 (m, 1H, *Ar*);

7.50-7.54 (m, 2H, *Ar*); 7.65-7.70 (m, 4H, *Ar*); 7.87 (s, 1H, *Ar*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 14.38; 19.62; 32.15; 37.04; 50.77; 68.51; 119.37; 119.82; 123.07; 123.25; 128.20; 128.37; 128.77; 129.47; 129.53; 130.25; 130.87; 137.60; 139.29; 140.15; 146.63; 149.08. MS (ESI^+): 377 (M^++1). HRMS (ESI^+) m/z calcd for $C_{22}H_{24}N_4O_2$: 377.1978; found: 377.1968.

*4.1.5.2 (Z/E)-1-Phenyl-1H-indeno[1,2-c]pyrazol-4-one O-((3-n-butylamino)-2-hydroxypropyl)oxime (**7b**).*

Brown solid, mp 215 °C, (yield 25 %). IR (KBr, cm^{-1}): ν = 3943; 3861; 3802; 3585; 3521; 3497; 3400; 3230; 3178; 2934; 2859; 1637; 606. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ ppm = 0.70-0.90 (m, 3H, C*H*$_3$); 1.10-1.40 (m, 4H, 2C*H*$_2$); 1.80-2.10 (m, 2H, C*H*$_2$-NH); 1.50 (br, 1H, O*H*) 2.68-3.10 (m, 2H, C*H*$_2$-NH-); 3.40-3.50 (m, 3H, C*H*-OH, C*H*$_2$-O-N); 7.35-7.55 (m, 5H, *Ar*); 7.60-7.70 (m, 4H, *Ar*); 8.12 (s, 1H, *Ar*); 9.70-9.75 (m, 1H, N*H*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 14.45; 22.34; 32.46; 37.16; 50.89; 69.78; 120.43; 120.67; 123.11; 123.35; 128.34; 128.45; 128.87; 129.54; 129.67; 130.34; 130.98; 137.87; 139.76; 140.54; 146.32; 150.08. MS (ESI^+): 391 (M^++1). HRMS (EI) m/z calcd for $C_{23}H_{26}N_4O_2$: 390.2148; found: 390.2157.

*4.1.5.3 (Z/E)-1-Phenyl-1H-indeno[1,2-c]pyrazol-4-one O-(3-tert-butylamino)-2-hydroxypropyl)oxime (**7c**).*

Brown solid, mp 112 °C, (yield 20 %). IR (KBr, cm^{-1}): ν = 3465; 3434; 2926; 2069; 1634; 1529; 1495; 1445; 1383; 1260; 1094; 1030; 969; 759; 697; 580. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ ppm = 1.15 (s, 9H, (C*H*$_3$)$_3$); 2.20 (br, 1H, O*H*); 3.10-3.80 (m, 3H, C*H*$_2$-NH, C*H*-OH); 4.30-4.70 (m, 2H, C*H*$_2$-O-N); 7.00-7.70 (m, 10H, *Ar*); 7.95-8.00 (m, 1H, N*H*).^{13}C-NMR ($CDCl_3$, 100 MHz): δ 15.56; 19.78; 32.45; 37.12; 60.72; 70.59; 119.97; 120.32; 123.12; 123.65; 128.43; 128.56; 129.01; 129.47; 129.65; 130.65; 130.98; 138.60; 139.65; 141.15; 146.78; 149.08. MS (ESI^+): 391 (M^++1). HRMS (EI) m/z calcd for $C_{23}H_{26}N_4O_2$: 390.2148; found: 390.2158.

*4.1.5.4 (Z/E)-1-Methyl-1H-indeno[1,2-c]pyrazol-4-one O-((2-hydroxy-3-(isopropylamino)propyl)oxime (**8a**).*

White solid, mp > 210 °C, (yield 35 %). IR (KBr, cm^{-1}): ν = 3457; 2930; 2839; 2337; 1754; 1642; 1550; 1396; 1237; 1038; 603; 547. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ ppm = 1.20-1.25 (m, 6H, 2C*H*$_3$); 1.80-2.10 (m, 3H, C*H*$_3$); 2.50-2.90 (m, 2H, 2C*H*); 3.00 (br, 1H, N*H*); 5.20 (s, 1H, O*H*); 3.50-5.10 (m, 4H, 2CH_2); 7.20-7.35 (m, 2H, *Ar*); 7.50-7.70 (m, 2H, *Ar*); 8.00 (s, 1H, *Ar*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 14.43; 19.78; 32.32; 37.14; 37.80; 50.87; 69.23; 118.34; 122.83; 123.10; 129.80; 132.98; 136.65; 139.45; 150.03. MS (ESI^+): 315 (M^++1). HRMS (EI) m/z calcd for $C_{17}H_{22}N_4O_2$: 314.3905; found: 314.3914.

.

*4.1.5.5 (Z/E)-1-Methyl-1H-indeno[1,2-c]pyrazol-4-one O-((3-n-butylamino)-2-hydroxypropyl)oxime (**8b**).*

Orange solid, mp 208 °C, (yield 25 %). IR (KBr, cm^{-1}): ν = 3463; 2945; 1737; 1215; 1158; 1034; 748; 666; 601. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.70-0.90 (m, 3H, C*H*$_3$); 1.00-1.20 (m, 4H, 2C*H*$_2$); 1.90-2.00 (m, 3H, C*H*$_3$); 2.10-2.30 (m, 2H, C*H*$_2$-NH); 2.90-3.90 (m, 6H, O*H;* C*H*$_2$-NH, C*H*-OH, C*H*$_2$-O-N); 7.10-7.35 (m, 5H, *Ar*); 9.60 (s, 1H, N*H*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 14.54; 20.54; 32.34; 37.14; 38.83; 51.21; 67.86; 119.35; 122.76; 123.21; 129.67; 133.11; 136.76; 140.12; 151.12. HRMS (EI) m/z calcd for $C_{18}H_{24}N_4O_2$: 328.4176; found: 328.4182.

*4.1.5.6 (Z/E)-1-Methyl-1H-indeno[1,2-c]pyrazol-4-one O-(3-tert-butylamino)-2-hydroxypropyl)oxime (**8c**).*

Brown solid, mp 210 °C, (yield 28 %). IR (KBr, cm^{-1}): ν = 3460; 2927; 1735; 1230; 1032; 797; 724; 601. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.50 (s, 9H, 3C*H*$_3$); 2.10 (s, 3H, C*H*$_3$); 3.40-3.60 (m, 1H, C*H*); 3.80-4.10 (m, 4H, 2C*H*$_2$); 4.60 (br, 1H, N*H*); 5.00 (s, 1H, O*H*); 6.90 (s, 1H, *Ar*); 7.50-7.90 (m, 3H, *Ar*); 8.00 (s, 1H, *Ar*). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 15.21; 21.53; 32.67; 38.11; 39.65; 51.22; 67.98; 120.11; 122.32; 123.87; 130.12; 133.32; 136.87; 140.23; 150.21. HRMS (EI) m/z calcd for $C_{18}H_{24}N_4O_2$: 328.4176; found: 328.4183.

4.2 Biological assay

4.2.1 *Animals*

Male Wistar rats (HARLAN, Italy), weighing 180-250 g were used. Animal care, sacrifice and experiments were done in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Isolated and Langendorff perfused heart preparation Rats were anesthetized by intraperitoneal injection of ethyl carbamate (2 g/kg rat, ip) and the rapidly excised hearts were immediately transferred in ice-cold buffered Krebs-Henseleit solution (KHs) for immediate cannulation through the aorta with the use of a glass cannula. Then, perfusion started at a constant flow-rate (12 ml/min). To avoid fluid accumulation, the apex of the left ventricle (LV) was pierced. A water-filled latex balloon, connected to a pressure transducer (BLPR; WRI, Inc., Sarasota, FL), was inserted through the mitral valve into the LV, which allowed the recording of isovolumic contractions and continuous mechanical parameters. Another pressure transducer located just above the aorta was used to record coronary pressure (CP). The perfusion solution consisted of a modified non-recirculating KHs containing (in millimoles) NaCl 113, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2, glucose 11, mannitol 1.1, Na-pyruvate 5 (pH 7.4; 37 °C; 95% O_2; 5% CO_2). Hemodynamic parameters were assessed using a PowerLab data acquisition system and analyzed using Chart software (both purchased by ADInstruments, Oxford, United Kingdom).

4.2.2 *Basal conditions*

Cardiac performance was evaluated for inotropism by analyzing the left ventricular developed pressure (LVP; in mm Hg: an index of contractile activity) and the maximal value of the first LVP derivative (mm Hg per second: an index of the maximal rate of LV contraction). Lusitropism was determined by calculating the maximal rate of LVP decline [-(LVdP/dT)max; mmHg/sec] and T/−t ratio between the maximal rate of LV contraction (+(LVdP/dT)max) and the maximal rate of LV relaxation [−(LVdP/dT)max]. After 20 minutes of stabilization, the following basal recordings were measured: LVP=89±3 mmHg, heart rate=280±7 beats/min, RPP=2.5±0.1 104 mmHg beats/min, CP=63±3mmHg, +(LVdP/dT)max=2492±129 (mmHg/sec), T/-t=0.08±0.01 (sec), -(LVdP/dT)max = 1663±70 (mmHg/sec), HTR=0.05±0.01 (sec) and T/-t or +(LVdP/dT)max/ LVdP/dT)max=1.49±1.84

(mmHg/sec). Endurance and stability of the preparation, analyzed by measuring performance variables every 10 min, showed that the heart preparation is stable for up to 180 min on the perfusion apparatus.

4.2.3 Protocols

*4.2.3.1 **7a-c** and **8a-c** stimulated preparations*

Repetitive exposure of each heart to a single concentration (1 nM) of each of the six derivatives **7a-c** or **8a-c** revealed absence of desensitization (data not shown). Thus, concentration-response curves were generated by perfusing cardiac preparations with KHs supplemented with increasing concentrations of **7a-c** or **8a-c** (from 1 pM to 10 nM) for 10 min.

4.2.3.2 Isoproterenol stimulated preparations

To obtain preliminary information on the antagonistic action of **7b** (5×10^{-10} M) toward the Iso-dependent stimulation, dose-response curves were generated by perfusing heart preparations with KHs enriched with increasing concentrations of Iso (0.1 nM to 1 µM) alone. These curves were then compared to those obtained by exposing other cardiac preparations to the same perfusion medium containing increasing concentrations of Iso (0.1 nM to 1 µM) plus a single concentration of either **7b** (5×10^{-10} M).

4.2.3.3 Beta2-AR, Beta3-AR and Alpha-AR receptors involvement

To evaluate the involvement of Beta2-AR, Beta3-AR and Alpha-AR receptors in the mechanism of anti-adrenergic action of **7b**, the hearts were perfused with ISO alone (5 nM) for 5 min and then washed-out with KHs. After returning to control conditions, each heart was perfused with ISO (5 nM) containing **7b** (5×10^{-10} M) and then washed-out with KHs. After returning to control conditions, each heart was perfused with ISO (5 nM) containing **7b** (5×10^{-10} M), plus phentolamine (100 nM), a selective alpha adrenergic antagonist, plus SR59230 (100 nM), a selective beta3

adrenergic antagonist or ICI118,551 (100 nM), a selective beta2-adrenergic antagonist.

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Highlights

► A new heterocyclic system indeno[1,2-*c*]pyrazole oxime ethers was reported. ► Some derivatives of this heterocyclic system have been prepared. ► A selected derivative was screened for its β_1-adrenergic blocking activity. ► The oxime ether (**7b**) reduces cardiac contractility and selectively antagonizes β_1-AR.