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Cardiovascular Hybrid Drugs: New Benzazepinone Derivatives as Bradycardic Agents Endowed with Selective β_1-Non-competitive Antagonism

Alessandra Bisi,[a,*] Angela Rampa,[a] Roberta Budriesi,[a] Silvia Gobbi,[a] Federica Belluti,[a] Pierfranco Ioan,[a] Ermanno Valoti,[b] Alberto Chiarini[a] and Piero Valenti[a]

[a]*Department of Pharmaceutical Sciences, University of Bologna, via Belmeloro 6, 40126 Bologna, Italy*
[b]*Institute of Medicinal Chemistry, University of Milano, viale Abruzzi 42, 20131 Milano, Italy*

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Abstract—The synthesis and pharmacological profile of some hybrid compounds bearing both the benzazepinone moiety present in Zatebradine and typical β-blocker aryloxypropanolamine groups are described. The new compounds proved to be endowed with negative chronotropic and inotropic activity and are weak vasorelaxant agents. The cardiodepressant action is probably due to selective β_1-noncompetitive reversible antagonism. Both enantiomers of the most active compound **5c** were synthesized and they showed a different cardiovascular profile, that is (+)-(*R*)-enantiomer displays affinity for cardiac β_1-adrenoceptors, while (−)-(*S*)-enantiomer shows specificity for vessel smooth muscle.

Introduction

The pharmacological treatment of diseases having complex heterogeneous pathogenesis could represent a problem, as a single drug is not always able to adequately control the illness and the combinations of drugs with different pharmaco-therapeutic profile may be needed. The principle of combination drug therapy can be achieved by either using concomitant administration of two or more single active drugs or by drugs in which the single active agents are combined in one molecule, i.e., hybrid molecules. The design of hybrid molecules as potential cardiovascular drugs is based on calcium, sodium or potassium channel blockers and on β_1-adrenoreceptor antagonists.

β-Adrenoceptor antagonists represent a class of drugs widely used clinically for hypertension or angina pectoris in various countries.[1,2] With the exception of a few phenylethanolamines virtually all the clinically useful β-adrenoceptor antagonists contain a phenoxypropanolamine moiety, typically with isopropyl or *tert*-butyl as an *N*-substituent, linked to an aromatic or heterocyclic ring system.

Although the basic aryloxypropanolamine nucleus should remain intact for significant β-adrenoceptor antagonist activity, a wide variety of aromatic ring or nitrogen substituents can be tolerated. This has allowed for the design of therapeutic agents that combine β-adrenoceptor antagonist activity with several other useful pharmacological actions including α-adrenoceptor blockade,[3] calcium channel blockade,[4] direct vasodilatation,[5] inhibition of angiotensin converting enzyme[6] and class III antiarrhythmic activity.[7]

Zatebradine (**1**) (Fig. 1) was described in the mid 80's as the representative of a novel pharmacological class termed 'specific bradycardic agents'.[8,9] Since this molecule was found to reduce heart rate without concomitant

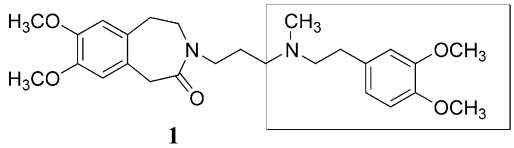

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Figure 1. Structure of zatebradine (**1**).

*Corresponding author. Tel.: +39-051-2099710; fax: +39-051-2099734; e-mail: albisi@alma.unibo.it

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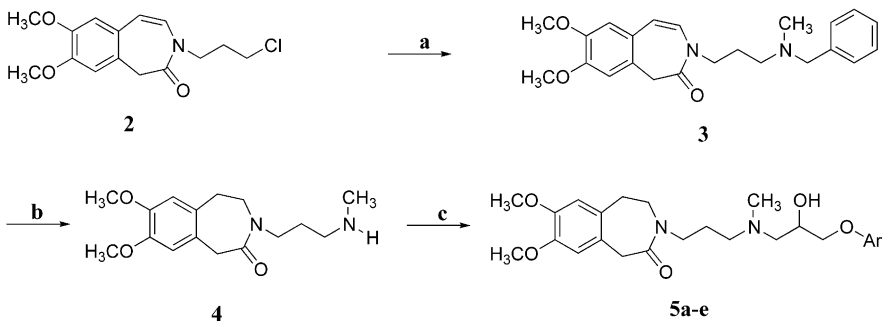


Scheme 1. Reagents: (a) *N*-methylbenzylamine; (b) H_2, Pd/C, CH_3COOH; (c) selected epoxide.

negative inotropic or hypotensive effects,[10] its mechanism of action has been deeply investigated in these years[11,12] and now its activity is believed to be due to the selective block of the I_f current.[13]

With regard to the bradycardic activity, the benzazepinone ring, the three carbon chain and the basic nitrogen atom are very sensitive to structural modifications. On the other hand, the arylalkyl moiety exhibits considerable potential for structural variations.[14,15] It seemed reasonable to assume[16] that this moiety could influence heart rate, blood pressure and the duration of biological activity. Indeed, compounds bearing peculiar fragments of bradycardic drugs acting with different mechanisms might show enhanced therapeutic efficacy.

Furthermore, as Smith and co-workers demonstrated that amidic substituents, which are powerful hydrogen bond acceptors, produced very potent and cardioselective drugs,[17,18] it is foreseeable that the benzazepinone moiety might increase the cardioselectivity of the selected β-blocking drugs by an increased receptor binding with its amidic group.

In this paper we report the synthesis and the pharmacological profile of a number of hybrid compounds in which the arylalkyl moiety of **1** is replaced with peculiar fragments of β-blocking agents, as shown in Figure 2.

The results obtained show that the new hybrid molecules **5a–e**, **8**, (−)-(*S*)-**5c** and (+)-(*R*)-**5c** are endowed with negative chronotropic and inotropic activity. (−)-(*S*)-**5c** is also weak vasorelaxant agent. The cardiodepressant action is probably due to selective β_1-non competitive reversible antagonism.

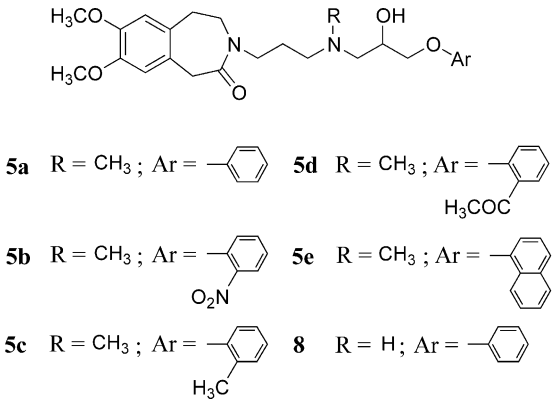


Figure 2. Structure of compounds.

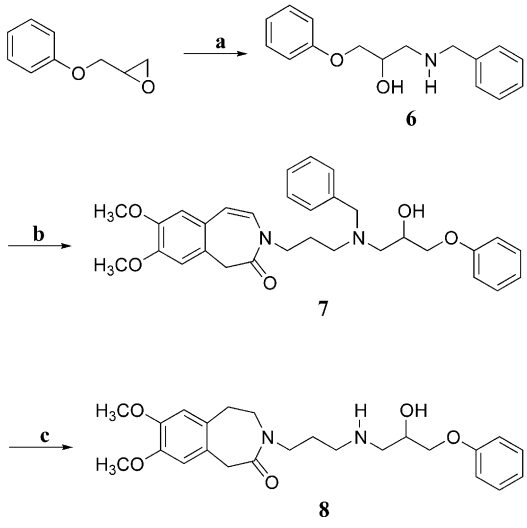
Chemistry

The synthesis of compounds **5a–e** was accomplished following Scheme 1.

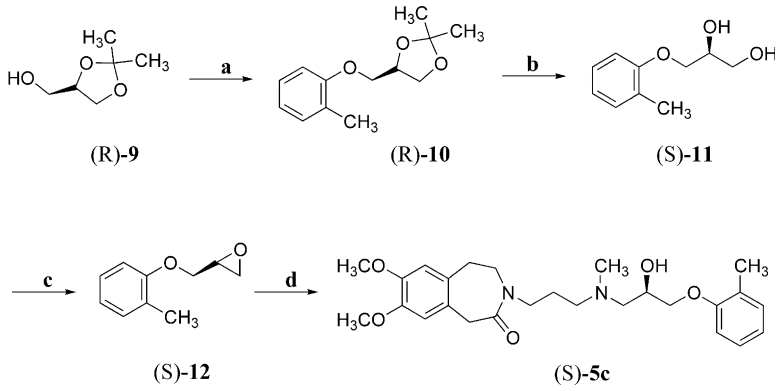
The key intermediate, 3-(3-chloropropyl)-7,8-dimethoxy-2,3-dihydro-1*H*-3-benzazepin-2-one **2** was prepared by standard procedures.[14] Condensation with *N*-methylbenzylamine afforded compound **3**[15] and subsequent hydrogenation gave compound **4**,[16] which was condensed with the selected epoxide to give the final products **5a–e**.

Synthesis of compound **8** was described in Scheme 2. The selected epoxide was condensed with benzylamine and the intermediate **6** was treated with **2** to give the benzyl derivative **7**. Hydrogenation using Pd/C as catalyst afforded compound **8**.

Synthesis of the pure enantiomers of **5c** was performed as shown in Scheme 3. The enantiomers of (*R*)- and (*S*)-isopropylidene-glycerol [(*S*)-**9** and (*R*)-**9**)], easily obtained by resolution of racemic glycerol,[19] were used as the key chiral building blocks in the enantioselective



Scheme 2. Reagents: (a) benzylamine; (b) 3-(3-chloropropyl)-7,8-dimethoxy-2,3-dihydro-1*H*-3-benzazepin-2-one (**2**), triethylamine; (c) H_2, Pd /C.



Scheme 3. Reagents: (a) *o*-cresol, TPP, DEAD, THF 0 °C; (b) HCl 1 N, 80 °C; (c) TPP, DEAD, benzene, 80 °C; (d) 7,8-dimethoxy-3-[3-(methylamino)propyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (**4**), 2-propanol, 82 °C.

synthesis of the title compounds. The first step (compounds **10**) involved reaction between *o*-cresol and the requisite enantiomer of compound **9** accomplished by using triphenylphosphine and diethylazodicarboxylate.

Successive acidic hydrolysis of the cyclic ketal (*R*)-**10** and (*S*)-**10** provided (*S*)-**11** and (*R*)-**11** respectively, having an higher than 98% enantiomeric excesses on the basis of the HPLC analysis performed with a chiral stationary phase Chiralcel OD. This diols were converted into the epoxides (*S*)-**12** and (*R*)-**12** by reaction with diethyl azodicarboxylate and triphenylphosphine. The opening of the (*S*)-**12** with the amine **4** gives the compounds **5c** having an *S* configuration at C-2. Analogously, the corresponding (*R*)-**5c** was obtained from (*R*)-**12**.

Pharmacology

The pharmacological profile of compounds was tested on guinea-pig isolated left and right atria to evaluate their inotropic and chronotropic effects, respectively, and on K^+-depolarized guinea-pig aortic strips to assess calcium antagonist activity. At first all compounds were checked at increasing doses to evaluate the percent decrease on developed tension on isolated left atrium driven at 1 Hz (negative inotropic activity), the percent decrease in atrial rate on spontaneously beating right atrium (negative chronotropic activity) and the percent inhibition of calcium-induced contraction on K^+-depolarized aortic strips (vasorelaxant activity). Data were analyzed by Student's *t*-test. The potency of drugs defined as EC_{50}, EC_{30} and IC_{50} was evaluated from log concentration–response curves (Probit analysis by Litchfield and Wilcoxon, $n = 6$–8) in the appropriate pharmacological preparations.

β_1- And β_2-adrenergic activities were determined on the atria and trachea of guinea-pig, respectively. The non-competitive β_1-antagonist potency was expressed as pIC_{50} value. All data are presented as mean ± SEM.[20]

Results and Discussion

As the benzazepinone ring in Zatebradine (**1**) is known to be of fundamental importance for bradycardic activity, we thought it would be of interest to replace the arylalkyl moiety of **1** with different 3-aryloxy-2-hydroxypropyl fragments of β-blocking drugs to obtain hybrid molecules which could possibly influence different cardiovascular parameters and consequently control heterogeneous pathologies.

The cardiovascular profile of the synthesized compounds was evaluated in isolated guinea-pig atria and aorta, namely spontaneously beating right atria, left atria driven at 1 Hz and K^+-depolarized aortic strips. The results obtained from myocardial and vascular preparations are summarized in Table 1.

It can be observed that all new hybrid compounds showed bradycardic activity as well as the reference compound **1**. However, the introduction of 3-aryloxy-2-propanol moiety does not change the order of potency, and this could be explained by the presence of the benzazepinone ring in all new compounds. As far as negative inotropic activity is concerned, all hybrid molecules elicited a fair potency with respect to **1**. At a first glance, the presence of an *ortho* substituent on the phenyl ring in our series of compounds could be of relevant importance for inotropism. In particular, it must be noted that the maximum negative inotropic potency seems to be due to the presence of electron donating groups, though weak such as a methyl (compound **5c**). On the contrary, a decrease in the negative inotropic efficacy seems to be related in a proportional manner to the increase of the electron withdrawing effect of the substituent, e.g., $-NO_2$ in **5b** and $-COCH_3$ in **5d**, which showed comparable negative inotropic profile. However, it is interesting to note that these latter compounds are more active than the phenyl unsubstituted derivative **5a**. The insertion of a naphthyl substituent (**5e**), further lowered the negative inotropic potency. Moreover, the removal of *ortho* phenyl substituent, as in compound **5a** or, in addition, the presence of a secondary amine, as in compound **8** both produced a sharp drop in negative inotropic properties. Among all compounds only **5c** showed a weak vasodilatory activity, probably due to calcium antagonist action as it was able to inhibit the potassium induced contraction of guinea pig aorta.

Table 1. Cardiovascular activity of compounds

N	Cardiovascular activity					
	Negative inotropy		Negative chronotropy		Vasorelaxant activity	
	Ia%[a] (±SEM)	EC_{50} (μM)[b] (95% cl)	Ia%[c] (±SEM)	EC_{30} (μM)[b] (95% cl)	Ia%[d] (±SEM)	IC_{50} (μM)[b] (95% cl)
1	45±3.1		70±2.8[e]	0.11 (0.08–0.13)	5±0.4	
5a	87±3.1	39 (30–49)	76±2.5[f]	0.12 (0.08–0.21)	25±1.5	
5b	89±2.4	1.20 (0.9–1.5)	80±6	0.56 (0.45–0.68)	40±4.5	
5c	73±0.6[e]	0.14 (0.10–0.19)	78±1.8[f]	0.23 (0.20–0.28)	52±2.0	36 (29.2–41.8)
(+)-(R)-**5c**	92±1.7[g]	0.59 (0.51–0.69)	74±2.2[e]	0.20 (0.17–0.24)	42±3.6	
(−)-(S)-**5c**	32±1.1[g]		73±0.4[e]	0.28 (0.22–0.36)	58±1.8	20 (16–25)
5d	88±2.5	1.00 (0.8–1.3)	90±6.1	0.37 (0.29–0.45)	36±1.1	
5e	91±4.2	3.90 (2.6–5.3)	86±4.7	0.40 (0.28–0.53)	42±2.5	
8	68±3.5	18.6 (16.7–21.2)	84±1.9	0.80 (0.71–0.92)	23±1.4[f]	

[a]Intrinsic activity: decrease in the developed tension in isolated guinea-pig left atrium at 10^{-4} M, expressed as percent changes from the control (n = 5–6). The left atria were driven at 1 Hz. The 10^{-4} M concentration gave the maximum effect for most compounds.
[b]Calculated from log concentration–response curves (Probit analysis by Litchfield and Wilcoxon with n = 6–7). When the maximum effect was <50%, the EC_{50} ino., EC_{30} chrono., IC_{50} values were not calculated.
[c]Intrinsic activity: decrease in the atrial rate on guinea-pig spontaneously beating isolated right atrium at 5×10^{-5} M, expressed as percent changes from the control (n = 7–8). The pretreatment heart rate ranged from 165 to 190 beats/min. 5×10^{-5} M gave the maximum effect for most compounds.
[d]Intrinsic activity: percent inhibition of calcium-induced contraction on K^+-depolarized guinea-pig aortic strip at 10^{-4} M (n = 5–6). The 10^{-4} M concentration gave the maximum effect for most compounds.
[e]At 5×10^{-6} M.
[f]At 10^{-5} M.
[g]At 5×10^{-5} M.

The β_1-adrenergic antagonist profile was evaluated on different isolated tissues from guinea-pig, namely right atria (β_1) and tracheal chains (β_2). The results are assembled in Table 2 and graphically shown in Figure 3.

Concerning β-adrenergic antagonist activity compound **1** is inactive, as expected, while all hybrid compounds do show reversible β_1 selective non competitive antagonism and are devoid of any type of interaction with β_2 receptors (see Table 2). Indeed right atrial isoprenaline response is fully recovered after 30 min washing of tissues inhibited by compounds at 1 μM concentration (Table 3).

The replacement of dimethoxyphenylalkyl moiety of **1** with an *o*-methylariloxypropanol group (**5c**) resulted in a significant improvement of the reversible non competitive antagonism at β_1-adrenergic receptors. In addition, it should be noted that in this series the presence of an *ortho* electron-donating group on the phenyl ring seems to be important for optimum β_1-affinity as compound **5c** proved to be the best reversible non competitive antagonist. On the other hand, the introduction of electron withdrawing groups in the phenyl ring decreased β_1-adrenergic activity in an inversely proportional manner with respect to the electron withdrawing effect, since compound **5b** bearing an *ortho* $-NO_2$ substituent proved less active than compound **5d** bearing *ortho* $-COCH_3$ group. Furthermore the presence of a secondary nitrogen in the backbone of the molecule significantly reduced β_1-affinity (**8**).

Since the importance of stereochemistry for interaction of adrenergic drugs with β-receptors is well recognized, we performed a stereoselective synthesis and pharmacological evaluation of the enantiomers of compound

Table 2. β-Adrenergic antagonistic activity of compounds

N	pIC_{50}[a]	
	β_1	β_2
	Isolated guinea-pig right atria	Isolated guinea-pig tracheal strips
1	NA	NA
5a	6.82±0.01	NA
5b	5.56±0.06	NA
5c	7.12±0.05	NA
(+)-(*R*)-**5c**	7.39±0.03	NA
(−)-(*S*)-**5c**	6.45±0.04	NA
5d	6.09±0.01	NA
5e	6.77±0.01	NA
8	5.28±0.08	NA

[a]pIC_{50} is the negative logarithm of the molar concentration that reduces the response to (−)-isoproterenol by 50%. NA = not active.

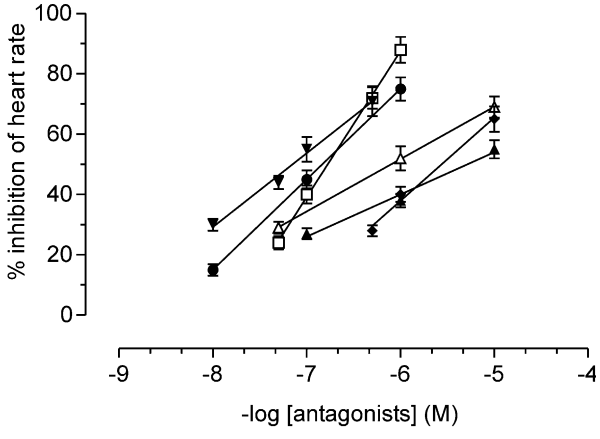


Figure 3. Guinea-pig right atria; non-competitive antagonism among (−)-isoprenaline, **5a** (●), **5b** (◆), **5c** (▼), **5d** (△), **5e** (□) and **8** (▲) at cardiac β_1-adrenoceptor. The results are expressed as the mean ± SEM of experiments performed in triplicate.

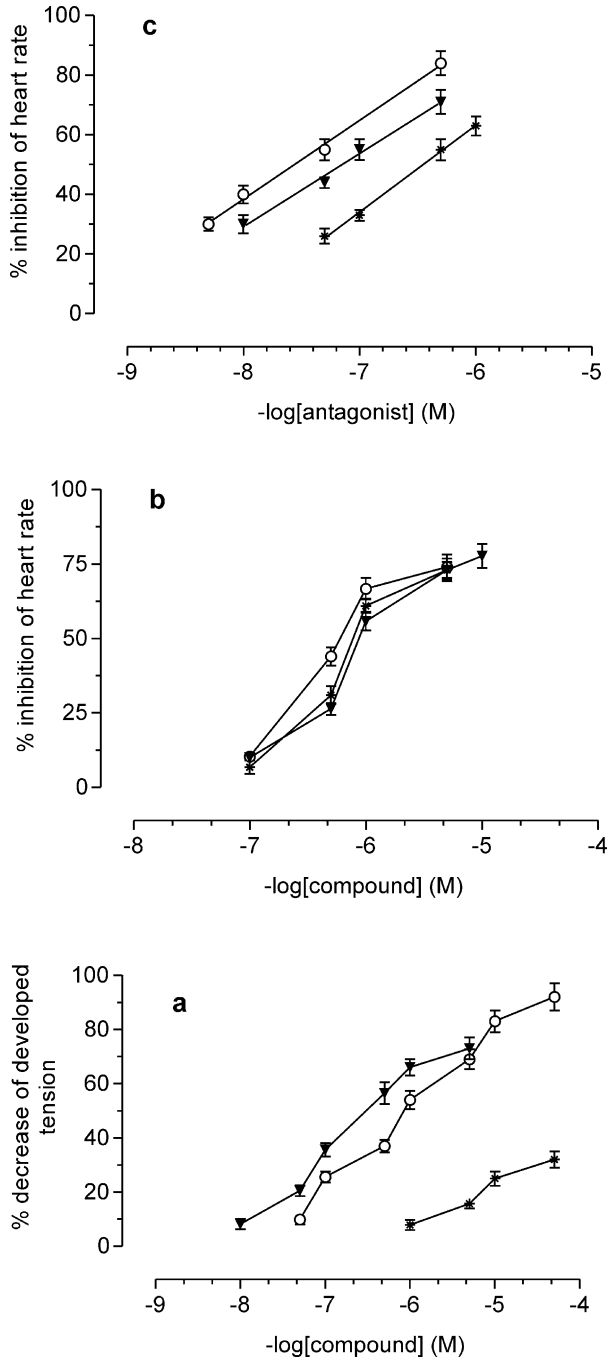


Figure 4. (a) Negative inotropic activity (left atria driven at 1 Hz) of **5c** (▼), (+)-(*R*)-**5c** (○) and (−)-(*S*)-**5c** (∗). (b) Negative inotropic activity (spontaneously beating right atria) of **5c** (▼), (+)-(*R*)-**5c** (○) and (−)-(*S*)-**5c** (∗). (c) Non-competitive antagonism among (−)-isoprenaline, **5c** (▼), (+)-(*R*)-**5c** (○) and (−)-(*S*)-**5c** (∗) at cardiac β_1-adrenoceptor in guinea-pig right atrium. Values shown are mean ± SEM ($n = 3$–5).

5c, which displayed the most interesting cardiovascular profile. The results, shown in Table 1 and graphically shown in Figure 4, indicate that the stereochemistry of the aryloxypropanolamine unit has a great influence on the cardiovascular activity, namely on cardiac parameters such as inotropism, chronotropism, on smooth muscle relaxation and on β_1 adrenergic affinity.

In particular, (+)-(*R*)-configuration in compound (+)-(*R*)-**5c**, showed both better cardiac efficacy and higher affinity and selectivity at β_1, with respect to β_2 adrenoceptors (see Table 2), which suggests that the two receptor subtypes have different stereochemical requirements. Furthermore, it should be noted that compound (+)-(*R*)-**5c** displayed a reversible non competitive antagonism comparable to bopindolol's which is, at our best knowledge, the most potent slowly reversible β_1 non competitive antagonist so far described.[21] A noteworthy feature of compound (−)-(*S*)-**5c** is that the (−)-(*S*)-enantiomer elicited a remarkable vasorelaxant activity ($IC_{50} = 20\ \mu M$) if compared to that of the other hybrid compounds. Probably, this action on vasculature is due to a calcium antagonist effect as (−)-(*S*)-**5c** inhibited potassium (80 mM)-contracted aortic smooth muscle. However, (−)-(*S*)-enantiomer maintained a negative chronotropic potency comparable to that of (+)-(*R*)-enantiomer.

Table 3. Inhibitory effect of compounds on (−)-isoproterenol-induced increase of right atrium frequency before and after washout

N	% β_1-Blockade[a]	
	30 Min incubation	30 Min incubation + 30 Min washing
1	NA	
5a	75±4.1	3±0.6
5b	40±2.8	0
5c	78±5.6	3±0.1
(+)-(*R*)-**5c**	94±3.8	0
(−)-(*S*)-**5c**	48±3.7	0
5d	52±3.6	1.5±0.1
5e	88±4.2	4±0.3
8	47±3.3	2±0.2

[a]Compounds were tested at 1 μM concentration and the result are expressed as the percentage decrease of maximum response to (−)-isoproterenol (M ± SEM) after 30 min incubation and after 30 min incubation followed by washing for 30 min. NA = not active.

In conclusion, keeping the pharmacophoric requirements of Zatebradine, on the basis of the above considerations it may be inferred that in these new hybrid molecules the presence of aryloxypropanol moiety produces high affinity and selectivity for β_1-adrenoreceptors. Moreover, the investigation on the enantiomers of the most potent hybrid compound **5c** has revealed a different cardiovascular profile, i.e., (+)-(*R*)-enantiomer displays affinity for cardiac β_1-adrenoreceptors, while (−)-(*S*)-enantiomer shows specificity for vessel smooth muscle, though to a lower extent. The trend noted in this series of hybrid molecules might help in developing useful structure–activity relationships to differentiate and to understand structural elements which give selectivity for β_1 versus β_2-adrenoreceptors.

Experimental

Chemistry

General methods. All melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. ^{1}H NMR were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$ solutions, with Me_4Si as the internal standard. Signal multiplicity was

designed according to the following abbreviations: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, br s = broad singlet, br t = broad triplet.

Mass spectra were recorded on a V.G. 7070 E spectrometer. Elemental analyses were within 0.4% of theoretical value unless otherwise indicated. The names of compound were obtained using AUTONOM, PC software for nomenclature in organic chemistry from Beilstein-Institut and Springer. Optical rotations were determined by a Perkin–Elmer 241 Polarimeter at 25 °C. Purification was performed by flash chromatography using silica gel (particle size 40–63 μm, Merck). HPLC analyses were performed on a Chiralcel-OD column from Daicel using a Waters 510 pump and a Pye unicam PU 4025 detector ($\lambda = 254$ nm).

The (*S*)- and (*R*)-isopropylidene-glycerol, (*S*)-**2** and (*R*)-**2**, were prepared by chemical resolution of the racemate as described in the literature.[19]

3-3-[Benzyl(methyl)amino]propyl-7,8-dimethoxy-2,3-dihydro-1*H*-3-benzazepin-2-one (3). *N*-Methylbenzylamine (13 mL, 100.0 mmol) were dropped into a solution of 15.07 g (51.0 mmol) of **2** in 200 mL of toluene, and the reaction mixture was heated under reflux for 8 h. The solution was then washed with water and evaporated to dryness. The compound was an oil (12.2 g, 65%), used for the subsequent step without further purification. Mp (HCl salt, methanol/diethylether) 148–151 °C.

7,8-Dimethoxy-3-[3-(methylamino)propyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (4). A solution of 12.2 g of compound **3** (32.2 mmol) in acetic acid was hydrogenated at 5 atm with Pd/C as catalyst. After filtration, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2, washed with a saturated solution of K_2CO_3, dried (Na_2SO_4) and evaporated to dryness to yield 6.11 g of **4** (65%), mp 72–75 °C (toluene). ^{1}H NMR ($CDCl_3$) δ 6.6 (s, 1H), 6.55 (s, 1H), 3.8 (s, 3H), 3.75 (s, 3H), 3.65 (t, 2H), 3.5 (m, 2H), 3.05 (m, 2H), 2.8-2.5 (m, 4H), 2.4 (s, 3H), 1.8 (m, 2H).

3-3-[(2-Hydroxy-3-phenoxypropyl)(methyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (5a). A solution of 1 g of compound **4** (3.42 mmol) and 0.51 g (3.42 mmol) of 2-(phenoxymethyl)oxirane[22] in 50 mL of ethanol was heated under reflux 12 h. The solvent was evaporated to dryness and the product was purified as hydrochloride salt: mp 183–185 °C (methanol/diethylether).Yield 1.06 g (70%). ^{1}H NMR (DMSO-d_6) δ 7.4–6.9 (m, 5H), 6.7 (s, 1H), 6.6 (s, 1H), 4.2–4.15 (m, 3H), 4.12 (s, 3H), 4.10 (s, 3H), 3.9 (t, 2H), 3.55 (m, 2H), 3.45 (m, 2H), 3.40 (m, 2H), 2.9–2.75 (m, 4H), 2.48 (s, 3H), 1.9 (m, 2H). MS: *m*/*z* (rel. abundance) 442 (M^+, 13.3), 305 (100), 262 (79.6). Anal. ($C_{25}H_{34}N_2O_5$.HCl): C, H, N.

3-3-[2-Hydroxy-3-(2-nitrophenoxy)propyl](methyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (5b). Using the previous procedure, compound **5b** (1.07 g, 65%) was obtained from **4** (1 g, 3.42 mmol) and 2-[(2-nitrophenoxy)methyl)]oxirane.[23] mp (HCl salt) 160–163 °C (methanol/diethylether). ^{1}H NMR ($CDCl_3$) δ 7.9–7.1 (m, 4H), 6.6 (s, 1H), 6.55 (s, 1H), 4.1–3.9 (m, 3H), 3.8 (s, 3H), 3.75 (s, 3H), 3.7 (t, 2H), 3.5 (m, 2H), 3.1 (m, 2H), 2.8–2.5 (m, 4H), 2.4 (s, 3H), 2.35 (s, 2H), 1.8 (m, 2H). MS: *m*/*z* (rel. abundance) 487 (M^+, 4.2), 305 (62.1), 262 (72.7) 139 (100). Anal. ($C_{25}H_{33}N_3O_7$.HCl): C, H, N.

3-3-[2-Hydroxy-3-(2-methylphenoxy)propyl](methyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (5c). Using the previous procedure, compound 5c (0.98 g, 63%) was obtained from 4 (1 g, 3.42 mmol) and 2-[(2-methylphenoxy)methyl]oxirane.[24] mp (HCl salt) 137–140 °C (methanol/diethylether). ^{1}H NMR ($CDCl_3$) δ 7.25–6.80 (m, 4H), 6.6 (s, 1H), 6.55 (s, 1H), 4.1–3.9 (m, 3H), 3.8 (s, 3H), 3.75 (s, 3H), 3.7 (t, 2H), 3.5 (m, 2H), 3.1 (m, 2H), 2.65–2.4 (m, 4H), 2.3 (s, 3H), 2.2 (s, 3H) 2.15 (s, 2H), 1.8 (m, 2H). MS: *m*/*z* (rel. abundance) 456 (M^+, 20), 305 (100), 262 (88.6). Anal. ($C_{26}H_{36}N_2O_5$.HCl): C, H, N.

3-3-[3-(2-Acetylphenoxy)-2-hydroxypropyl](methyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (5d). Using the previous procedure, compound **5d** (1.11 g, 67%) was obtained from **4** (1 g, 3.42 mmol) and 1-[2-(2-oxiranylmethoxy)phenyl]-1-ethanone.[25] mp (HCl salt) 182–185 °C (methanol/diethylether). ^{1}H NMR ($CDCl_3$) δ 7.8–6.9 (m, 4H), 6.6 (s, 1H), 6.55 (s, 1H), 4.1–3.9 (m, 3H), 3.8 (s, 3H), 3.75 (s, 3H), 3.7 (t, 2H), 3.5 (m, 2H), 3.1 (m, 2H), 2.7 (s, 3H), 2.65–2.4 (m, 4H), 2.3 (s, 3H), 2.15 (m, 2H), 1.8 (m, 2H). MS: *m*/*z* (rel. abundance) 484 (M^+, 33.3), 206 (77.7), 58 (100). Anal. ($C_{27}H_{36}N_2O_6$.HCl): C, H, N.

3-3-[2-Hydroxy-3-(1-naphthyloxy)propyl](methyl)amino]-propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (5e). Using the previous procedure, compound **5e** (1.11 g, 66%) was obtained from **4** (1 g, 3.42 mmol) and 2-[1(naphtyloxy)methyl]oxirane.[26] Mp (HCl salt) 187–189 °C (methanol/diethylether). ^{1}H NMR (DMSO-d_6) δ 7.9–6.7 (m, 7H), 6.7 (s, 1H), 6.55 (s, 1H), 4.22–4.15 (m, 3H), 4.12 (s, 3H), 4.10 (s, 3H), 3.9 (t, 2H), 3.55 (m, 2H), 3.45 (m, 2H), 3.41 (m, 2H), 2.9–2.75 (m, 4H), 2.48 (s, 3H), 1.95 (m, 2H). MS: *m*/*z* (rel. abundance) 492 (M^+, 16.7), 305 (100), 262 (72.9). Anal. ($C_{29}H_{36}N_2O_5$.HCl): C, H, N.

1-(Benzylamino)-3-phenoxy-2-propanol (6). 2-(Phenoxymethyl)oxirane (9 g, 59.9 mmol) and 7.9 mL of benzylamine (59.9 mmol) in 200 mL of toluene were heated under reflux for 16 h after cooling, the solution was washed with water, dried and evaporated to dryness, to yield 10.7 g (70%) of **6** as an oil, which was used for the subsequent step without further purification. Mp (HCl salt) 176–180 °C.

3-3-[Benzyl(2-hydroxy-3-phenoxypropyl)amino]propyl-7,8-dimethoxy-2,3-dihydro-1*H*-3-benzazepin-2-one (7). A solution of 4.7 g (15.9 mmol) of compound **2**, 4.1 g (15.9 mmol) of **6** and 2.3 mL of triethylamine in 50 mL of toluene was refluxed 8 h. After cooling, the solution was washed with water, dried and evaporated to dryness, affording 2.6 g (32%) of **7**. Mp (HCl salt) 182–185 °C. ^{1}H NMR ($CDCl_3$) δ 8.1–7.1 (m, 10H), 6.6

(s, 1H), 6.55 (s, 1H), 6.4 (s, 2H), 4.1–3.9 (m, 3H), 3.8 (s, 3H), 3.75 (s, 3H), 3.7 (t, 2H), 3.5 (s, 3H), 2.8-2.5 (m, 4H), 2.35 (s, 2H), 1.8 (m, 2H).

3-3-[(2-Hydroxy-3-phenoxypropyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (8). Compound **7,** 2.6 g (5.09 mmol) was dissolved in 30 mL of acetic acid and hydrogenated at 5 atm using Pd/C as catalyst. After filtration, the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2, washed with a saturated solution of K_2CO_3, dried and evaporated to dryness, to give 1.74 g (80%) of **8** which was purified by flash chromatography (eluant: toluene/acetone 3/2). Mp 145–148 °C (HCl salt). ^{1}H NMR (DMSO-d_6) δ 8.2 (broad, 1H, NH), 7.4–6.9 (m, 5H), 6.7 (s, 1H), 6.6 (s, 1H), 4.2–4.15 (m, 3H), 4.12 (s, 3H), 4.10 (s, 3H), 3.9 (t, 2H), 3.55 (m, 2H), 3.45 (m, 2H), 3.40 (m, 2H), 2.9–2.75 (m, 4H), 1.9 (m, 2H). Anal. ($C_{24}H_{32}N_2O_5$.HCl): C, H, N.

(2*R*)-3-Tolyloxy-1,2-propanediol acetonide [(*R*)-10]. To a cold (0 °C) stirred solution of *o*-cresol (10 g, 92.5 mmol), (2*R*)-glycerol-1,2 acetonide [(*R*)-**9**] (11 g, 83.2 mmol) and triphenylphosphine (32.6 g 124.5 mmol) in THF (80 mL) was added dropwise diethylazodicarboxylate (19.6 mL, 124.5 mmol). The mixture was stirred for 2 h and then concentrated under reduced pressure. The resulting residue was poured into diethylether to precipitate the phosphine oxide, which was removed by filtration. The filtrate was concentrated under reduced pressure to afford an oil which was purified by flash chromatography (cyclohexane/EtOAc 9:1) to yield the compound (*R*)-**10** (9.70 g, 52% yield). $[\alpha]_D = -20.9$ ($c = 0.5$, ethanol). ^{1}H NMR ($CDCl_3$) δ 7.25–7.10 (m, 2H), 6.98–6.80 (m, 2H), 4.54 (m, 1H), 4.29–3.93 (m, 4H), 2.30 (s, 3H), 1.53 (s, 3H), 1.48 (s, 3H).

(2*S*)-3-Tolyloxy-1,2-propanediol acetonide [(*S*)-10]. Prepared from *o*-cresol and (2*S*)-glycerol-1,2 acetonide [(*S*)-**9**] as described for (*R*)-**10**: $[\alpha]_D = +21.5$ ($c = 0.5$, ethanol).^{1}H NMR identical to (*R*)-**10**.

(2*S*)-3-Tolyloxy-1,2-propanediol [(*S*)-11]. (*R*)-**10** (9.5 g, 42.7 mmol) in 75 mL of 10% HCl was heated at 80 °C for 3 h. The mixture was cooled and, after evaporation of the acetone, extracted with CH_2Cl_2 overnight. The extract was dried (Na_2SO_4) and concentrated. The resulting solid was crystallized from toluene to yield (*S*)-**11** (5.9 g, 76% yield). Mp 93 °C; $[\alpha]_D = -0.6$ ($c = 0.5$, ethanol) (e.e. > 98% on the basis of the HPLC analysis performed with a chiral stationary phase Chiralcel OD). ^{1}H NMR (DMSO-d_6) 7.22–7.09 (m, 2H), 7.00–6.79 (m, 2H), 4.94 (d, 1H, *OH*), 6.68 (t, 1H, *OH*) 4.07–3.63 (m, 3H), 3.58–3.47 (m, 2H), 2.22 (s, 3H, Ar–CH_3).

(2*R*)-3-Tolyloxy-1,2-propanediol [(*R*)-11]. Prepared from (*S*)-**10** as described for (*S*)-**11**: mp 92 °C; $[\alpha]_D = +0.5$ ($c = 0.5$, ethanol) (e.e. > 98% on the basis of the HPLC analysis on a chiral stationary phase Chiralcel OD). ^{1}H NMR identical to (*S*)-**11**.

(2*S*)-3-Tolyloxy-1,2-epoxypropane [(*S*)-12]. A mixture of (*S*)-**11** (3 g, 16.5 mmol), triphenylphosphine (6.48 g, 24.7 mmol) and diethylazodicarboxylate (3.88 mL, 24.7 mmol) in benzene (50 mL) was refluxed for 12 h. After evaporation of the solvent, ether was added to precipitate the phosphine oxide, which was removed by filtration. The filtrate was concentrated and the residue purified by flash chromatography (cyclohexane/EtOAc 9:1) yielding 1.5 g of (*S*)-**12** as a colourless oil. $[\alpha]_D = +14.8$ ($c = 0.5$, ethanol). ^{1}H NMR ($CDCl_3$) δ 7.23–7.07 (m, 2H), 6.97–6.76 (m, 2H), 4.23 (dd, 1H), 3.97 (dd, 1H), 3.38 (m, 1H), 2.92 (br t, 1H), 2.80 (dd, 1H), 2.27 (s, 3H).

(2*R*)-3-Tolyloxy-1,2-epoxypropane [(*R*)-12]. Prepared from (*R*)-**11**] as described for (*S*)-**12**: $[\alpha]_D = -15.0$ ($c = 0.5$, ethanol). ^{1}H NMR identical to (*S*)-**12**.

3-3-[(2*S*)-2-Hydroxy-3-(2-methylphenoxy)propyl](methyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one [(*S*)-5c]. A mixture of (*S*)-**12** (562 mg, 3.42 mmol) and amine **4** (1 g, 3.42 mmol) in 2-propanol (25 mL) was refluxed for 24 h. After evaporation of the solvent, the residue was treated with DCM (20 mL) and 1 N NaOH (10 mL). The aqueous phase was separated and extracted with DCM (2 × 15 mL). The combined extracts were dried and concentrated to give a residue which was chromatographed on silica gel. Elution with DCM/MeOH/TEA (95/4.5/0.5) yielded 1.05 g of **5c**-(*S*) as oil. $[\alpha]_D = -19.7$ ($c = 0.5$, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.18–7.10 (m, 2H), 6.90–6.80 (m, 2H), 6.60 (s, 1H), 6.56 (s, 1H), 4.09–3.87 (m, 3H), 3.83 (s, 6H), 3.75 (s, 2H), 3.72 (br t, 2H), 3.52 (br t, 2H), 3.05 (br t, 2H), 2.66–2.24 (m, 5H), 2.30 (s, 3H), 2.23 (s, 3H), 1.77 (m, 2H).

3-3-[(2*R*)-2-Hydroxy-3-(2-methylphenoxy)propyl](methyl)amino]propyl-7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one [(*R*)-5c]. Prepared from (*R*)-**12** and **4** as described for (*S*)-**5c**: $[\alpha]_D = +20.3$ ($c = 0.5$, $CHCl_3$). ^{1}H NMR identical to (*S*)-**5c**.

Functional studies

Guinea-pig atrial preparations. Guinea-pigs (300–400 g female) were sacrificed by cervical dislocation. After thoracotomy the heart was immediately removed and washed by perfusion through the aorta with oxygenated Tyrode solution of the following composition (mM): 136.9 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 0.4 $NaH_2PO_4xH_2O$, 11.9 $NaHCO_3$ and 5.5 glucose. The physiological salt solution (PSS) was buffered at pH 7.4 by saturation with 95% O_2–5% CO_2 gas, and the temperature was maintained at 35 °C. Isolated guinea-pig heart preparations were used, spontaneously beating right atria and left atria driven at 1 Hz. For each preparation, the entire left and right atria were dissected from the ventricles, cleaned of excess tissue, and hung vertically in a 15 mL organ bath containing the PSS continuously bubbled with 95% O_2–5% CO_2 gas at 35 °C, pH 7.4. The contractile activity was recorded isometrically by means of a force transducer (FT 0.3, Grass Instruments, Quincy, MA) using Power Lab software (Basile, Italy). The left atria were stimulated by rectangular pulses of 0.6–0.8 ms duration and about 50% threshold voltage through two platinum contact

electrodes in the lower holding clamp (Grass S88 stimulator). The right atrium was in spontaneous activity. After the tissue was beating for several min, a length-tension curve was determined, and the muscle length was maintained at which elicited 90% of maximum contractile force observed at the optimal length. A stabilization period of 45–60 min was allowed before the atria were used to test compounds. During the equilibration period, the bathing solution was changed every 15 min and the threshold voltage was ascertained for the left atria. Atrial muscle preparations were used to examine the inotropic and chronotropic activity of the compounds (0.1, 0.5, 1, 5, 10, 50 and 100 μM), first dissolved in DMSO and then diluted with PSS. According to this procedure, the concentration of DMSO in the bath solution never exceeded 0.3%, a concentration that did not produce appreciable inotropic and chronotropic effects. During the construction of cumulative dose-response curves, the next higher concentration of the compounds was added only after the preparation reached a steady state.

Guinea-pig aortic strips. The thoracic aorta was removed and placed in Tyrode solution of the following composition (mM): 118 NaCl, 4.75 KCl, 2.54 $CaCl_2$, 1.20 $MgSO_4$, 1.19 KH_2PO_4, 25 $NaHCO_3$ and 11 glucose equilibrated with 95% O_2–5% CO_2 gas at pH 7.4. The vessel was cleaned of extraneous connective tissue. Two helicoidal strips (10 × 1 mm) were cut from each aorta beginning from the end most proximal to the heart. Vascular strips were then tied with surgical thread (6-0) and suspended in a jacketed tissue bath (15 mL) containing aerated physiological salt solution (PSS) at 35 °C. Strips were secured at one end to a force displacement (FT 0.3, Grass) transducer for monitoring changes in isometric contraction. Aortic strips were subjected to a resting force of 1 g and washed every 20 min with fresh PSS for 1 h after the equilibration period; guinea-pig aortic strips have been contracted by washing in PSS containing 80 mM KCl (equimolar substitution of K^+ for Na^+). After the contraction reached a plateau (about 45 min) the compounds (0.1, 0.5, 1, 5, 10, 50 and 100 μM) were added cumulatively to the bath allowing for any relaxation to obtain an equilibrated level of force. Addition of the drug vehicle had no appreciable effect on K^+-induced contraction (DMSO for all compounds).

β-Adrenergic activities

Right atria. These were suspended in PSS (see above) at the proper temperature (35 °C) and a pH 7.4 and aerated with 95% O_2–5% CO_2 gas. Benextramine (10^{-6} M) was added to bath solution to block α-adrenoceptors. The tissue was mounted under a 0.5 g of tension in a 15 mL organ bath. Following a 45 min period during the PSS was changed every 15 min, a cumulative dose–response curve for the agonist INA was performed. In preliminary experiments, reproducibility of the dose–response curve obtained by the first to the third trials in the absence of β-antagonists was confirmed. Following incubation with the antagonist for 30 min, a new dose–response curve to the agonist was obtained either in the presence or after removal of the antagonist.

The trachea. This was dissected transversally and transferred to a dish containing Krebs solution, and cut transversally between the segments of cartilage. Six of the tracheal rings were tied together and mounted under a tension of 1 g at 37 °C in a 15 mL organ bath containing Krebs–Ringer solution of the following composition (mM): 95 NaCl, 4.7 KCl, 2.50 $CaCl_2$, 1.00 $MgSO_4$, 1.17 KH_2PO_4, 25 $NaHCO_3$ and 10.6 glucose equilibrated with 95% O_2–5% CO_2 gas at pH 7.4. The tissues were allowed to stabilize for 90 min. Isometric force was recorded from the preparations by a force-displacement transducer. A constant level of tone was induced by the addiction of carbachol chloride (5×10^{-7} M) to the bath, and, after 15 min, a control concentration-response curve for isoprenaline was obtained. The tissue was washed thoroughly, and 30 min later a single concentration of antagonist was added to the bath and allowed for 30 min. During the last 15 min. of antagonistic incubation, carbachol chloride (5×10^{-7} M) was added to the bath and the cumulative concentration-response curve for isoprenaline was again determined. All responses to different concentrations of isoprenaline were expressed as percentage of the maximal relaxation recorded for the control curve.

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References and Notes

1. Hieble, J. P.; Bondinell, W. E.; Ruffolo, R. R. *J. Med. Chem.* **1995**, *38*, 3415.
2. Ruffolo, R. R.; Bondinell, W. E.; Hieble, J. P. *J. Med. Chem.* **1995**, *38*, 3681.
3. Uchida, W.; Shibasaki, K.; Asano, M.; Takenaka, T. *J. Cardiovasc. Pharmacol.* **1993**, *21*, 701.
4. Shibasaki, K.; Uchida, W.; Shirai, Y.; Inagaki, O.; Asano, M.; Takenaka, T. *Arch. Int. Pharmacodyn.* **1994**, *328*, 213.
5. Hieble, J. P. In *β-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology;* Ruffolo, R. R., Jr. Ed.; Karger: Basel, 1991; Vol. 7, p 105.
6. Allan, G.; Cambridge, D.; Hardy, G. W.; Follenfant, M. J. *Br. J. Pharmacol.* **1987**, *90*, 609.
7. Argentieri, T. M.; Troy, H. H.; Carrol, M. S.; Doroshuk, C. M.; Sullivan, M. E. *J. Cardiovasc. Pharmacol.* **1993**, *21*, 647.
8. Kobinger, W.; Lillie, C. *Eur. J. Pharmacol.* **1984**, *104*, 9.
9. Kobinger, W.; Lillie, C. *Eur. Heart J.* **1987**, *8* (suppl. L), 7.
10. Franke, H.; Su, C. A.; Schumacher, K.; Seiberling, M. *Eur. Heart J.* **1987**, *8* (suppl. L), 91.
11. Glasser, S. P.; Michie, D. D.; Thadani, U.; Baiker, W. M. *Am. J. Cardiol.* **1997**, *79*, 1401.
12. Kruger, C.; Landerer, V.; Zugck, C.; Ehmke, H.; Kubler, W.; Haass, M. *Cardiovasc. Res.* **2000**, *45*, 900.
13. Bom, A.; Booth, S.; Bruin, J.; Clark, J.; Miller, S.; Wathey, B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2351.
14. Reiffen, M.; Eberlein, W.; Muller, P.; Psiorz, M.; Noll, K.; Heider, J.; Lillie, C.; Kobinger, W.; Luger, P. *J. Med. Chem.* **1990**, *33*, 1496.

15. Budriesi, R.; Rampa, A.; Bisi, A.; Fabbri, G.; Chiarini, A.; Valenti, P. *Arzneim.-Forsch./Drug Res*. **1995**, *45*, 234.
16. Bomhard, A.; Reiffen, M.; Heider, J.; Psiorz, M.; Lillie, C. *J. Med. Chem*. **1991**, *34*, 942.
17. (a) Large, M. S.; Smith, H. *J. Med. Chem*. **1982**, *25*, 1286. (b) Large, M. S.; Smith, H. *J. Med. Chem*. **1982**, *25*, 1417.
18. Large, M. S.; Smith, H. *J. Med. Chem*. **1983**, *26*, 352.
19. Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *Tetrahedron Asymmetry* **1994**, *5*, 5.
20. Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, 2nd ed.; Springer-Verlag: New York, 1992.
21. Hosohata, Y.; Hattori, K.; Shen, Y.; Okuyama, M.; Kaneko, H.; Ohnuki, T.; Suzuki, J.; Nagatomo, T. *Pharmacol*. **1998**, *57*, 180.
22. *Chem. Ber*. **1891**, *24*, 2146.
23. Brenans, P. *Bull. Soc. Chim. Fr*. **1913**, *4*, 13 533.
24. Boyd, V.; Knowlton, M. *J. Chem. Soc*. **1909**, *95*, 1805.
25. Beasley, Y. M.; Petrow, V.; Stephenson, O. *J. Pharm. Pharmacol*. **1958**, *10*, 47.
26. Marle, M. *J. Chem. Soc*. **1912**, *101*, 309.