Novel Quinolizidinyl Derivatives as Antiarrhythmic Agents

Iana Vazzana,[†] Roberta Budriesi,[‡] Emanuela Terranova,[†] Pierfranco Ioan,[‡] Maria Paola Ugenti,[‡] Bruno Tasso,[†] Alberto Chiarini,[‡] and Fabio Sparatore*,[†]

Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, 16132 Genova, and Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy

Received July 26, 2006

Eighteen analogues of lidocaine, mexiletine, and procainamide were synthesized, replacing their aminoalkyl chains with the rigid and cumbersome quinolizidine nucleus. The target compounds were tested for antiarrhythmic, inotropic, and chronotropic effects on isolated guinea pig (gp) heart tissues and to assess calcium antagonist activity. Most compounds exhibited from moderate to high antiarrhythmic activity, and compounds **7**, **9**, and **19** were more active and potent than quinidine and lidocaine, while producing only modest inotropic, chronotropic, and vasorelaxant effects. These compounds were studied on spontaneously beating Langendorff-perfused gp heart. While quinidine and amiodarone produced a dose-dependent prolongation of all the ECG intervals, compounds **7**, **9**, and **19**, even at concentrations 10−20 times higher than EC_{50} for the antiarrhythmic activity, only moderately prolonged the PR and QT intervals, leaving unchanged the QRS complex. Ether **7** deserves further investigations due to its interesting cardiovascular profile.

Introduction

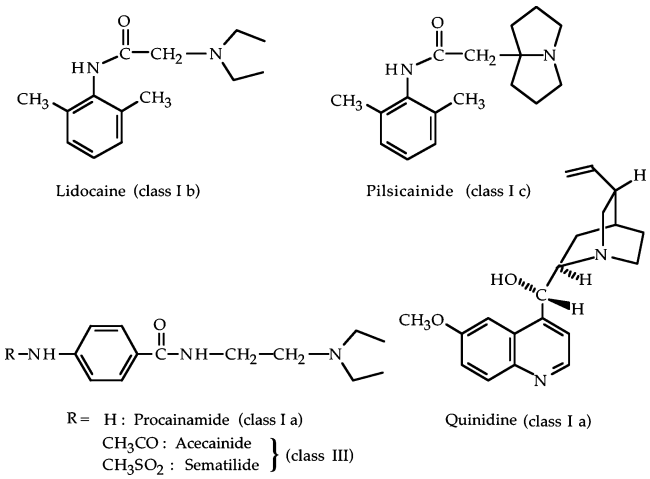
Arrhythmia is an abnormality of cardiac rhythm affecting about 4% of the population over 60 and about 9% over 80.[1] Arrhythmias are commonly induced or accompanied by heart disease. However, arrhythmias may occur in patients with no clinically apparent heart disease but with other diseases and under certain drug therapies. Although the therapeutic treatment of the underling disorders is placed in the foreground, arrhythmias represent an actual danger to the patients.[2] However, acute, as well as long-term, treatment of arrhythmias is often limited by side effects such as cardiac depression caused by drugs.[3]

Antiarrhythmic drugs are able to suppress dysrhythmic cardiac activity whatever their modes of action. Several attempts of classification according to clinical criteria were made in the 1970s, but none of them were generally accepted.[4−6] Currently, the most common and extensively described characterization of antiarrhythmic drugs is based on their fundamental electrophysiological effects. As a matter of fact, antiarrhythmic drugs display their activity through several different mechanisms and, on the basis of their electrophysiological properties, have been arranged by Vaughan Williams in four classes.[7−10] The first class of sodium channel blockers was further divided into three subclasses depending on the length of the depolarized phase.

It is worth noting that in each class compounds with quite different molecular structures are present. However, compounds exhibiting only minimal or moderate differences in their structures may belong to distinct classes or subclasses. Thus, lidocaine and pilsicainide belong to the Ib and Ic classes, respectively, while the simple introduction of a substituent on the amino group of procainamide (class Ia) produces acecainide and sematilide, both collocated in class III (Chart 1). On the other hand, amiodarone, rightly included in class III for its repolarization lengthening activity, also exhibits class Ib and, to minor extents, class II and IV antiarrhythmic effects.

Therefore, medicinal chemists should feel encouraged to search for better antiarrhythmic drugs.[11] After so many years from its introduction in therapy, lidocaine (class Ib) still continues to suggest the design of new analogues to overcome its drawbacks (cardiac depression and CNS disturbances), expecially in long-term treatment of arrhythmias, where amiodarone and other agents with multiple mechanisms of action are finding wider acceptance.

Chart 1. Structure of Some Antiarrhythmic Agents



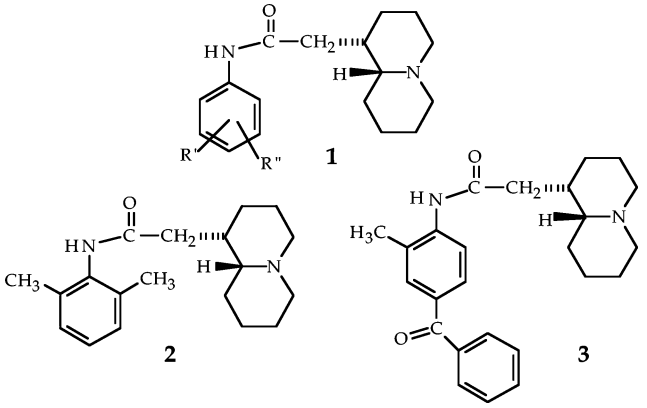
On this ground, a first set of novel lidocaine-like antiarrhythmic agents, corresponding to the general formula **1**, were described by Sparatore and Sparatore in 1995 (Chart 2).[12] These compounds are characterized by the presence of a bulky, highly lipophilic, and strongly basic quinolizidine moiety. Indeed the replacement of the various flexible dialkylaminoalkyl chains, which characterize many drugs, with such a rigid and cumbersome moiety could either compromise the interaction with the biological structure responsible for the activity or give place to a very selective one.

In fact, some of the mentioned compounds, especially **2** and **3**, resulted endowed with strong antiarrhythmic activity in in vitro and in vivo assays. Despite its structural analogy with lidocaine and pilsicainide, compound **2** was devoid of local

* To whom correspondence should be addressed. Phone: +39 0103538359. Fax: +39 0103538358. E-mail: sparator@unige.it.
[†] Università di Genova.
[‡] Università di Bologna.

10.1021/jm060878m CCC: $37.00
Published on Web 12/24/2006

Chart 2. Structures of Antiarrhythmic Homolupinanoylanilides



anesthetic activity and hence of sodium channel blocking activity. Compound **2** also lacked β-adrenergic and calcium channel blocking activities. On the other hand, compound **3** exhibited some local anesthetic and calcium channel blocking activities and was moderately able to displace [^{3}H]batrachotoxin from the sodium channel of rat brain preparations ($IC_{50} = \sim 3.7$ μM); therefore, the strong antiarrhythmic activity must result from these combined mechanisms.

At present, the interest in compounds possessing a multiple mode of action is steadily increasing, and the overlapping of the useful characteristics of class III with those of class Ib, which could balance the proarrhythmic risk inherent to the former, is particularly pursued.[10,13]

Over the past few years, Matyus et al.[14] described a set of (2,6-dimethylphenoxy)alkylamines, embodying the structural features of mexiletine (class Ib) and amiodarone (class III), and which, indeed, exhibited excellent antiarrhythmic effects through the simultaneous block of sodium and potassium channels (Chart 3).

Pursuing our research on antiarrhythmic agents, we now describe three series of new quinolizidine derivatives embodying some of the mentioned structural elements (Chart 4).

The first group (**4**−**6**) represents the extension of the previously described[12] *N*-homolupinanoylanilines **1**, with the introduction of nitro, mesylamino, and benzoyl residues on the benzene ring which are present in the phenoxyalkylamines of Matyus et al.[14]

A second and larger group of compounds is formed by the lupinyl (1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl (**7**−**11**) and *epi*-lupinyl (1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl (**12**−**16**) ethers of 2,6-dimethyl 4-substituted phenols, which more closely recall the compounds of Matyus et al.

The third group is formed by the analogues of procainamide, acecainide, and sematilide, whose diethylaminoethyl chain is replaced by the lupinyl moiety, to which the 4-nitrobenzoyl derivative is added. Compounds **17** and **18** were previously described by Sparatore many years ago,[15] but the antiarrhythmic activity was not studied at that time; our interest in this kind of compound was restored by the observed good antiarrhythmic activity of *N*-lupinyl-4-amino-5-chloro-2-methoxybenzamide, which was prepared by Iusco et al. as an analogue of metoclopramide.[16]

Each of the three groups of compounds shares a peculiar aromatic substructure with lidocaine (ArNH−CO−CH_2−), mexiletine and amiodarone (Ar−O−CH_2−), and procainamide (Ar−CONH−CH_2−), respectively, but all of them share the "bulkiness" of the respective strongly basic moiety with quinidine (quinolizidine versus quinuclidine nucleus).

Chemistry

Compounds **2**, **4**, and **6** were prepared by reacting the homolupinanoyl chloride hydrochloride with the suitable aromatic amines in $CHCl_3$ solution and in the presence of *N*,*N*-diisopropylethylamine. The homolupinanoyl chloride hydrochloride [(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)ethanoyl chloride] was prepared as described by Sparatore et al.[17,18] The noncommercially available 2,6-dimethyl-4-nitroaniline was prepared according to Wepster indications,[19] while 4-amino-3,5-dimethylbenzophenone was obtained by adapting the indications given by Staskun[20] for other aminobenzophenones. The compound was similarly prepared by Artini et al.,[21] but no details were given, and the mp was quite different. Compound **4** was reduced catalytically, and the resulting amine was treated with mesyl chloride to give **5** (Scheme 1).

Ethers **7**, **8**, and **11** and **12**, **13**, and **16** were prepared by reacting chlorolupinane[22] (1-(chloromethyl)octahydro-(1*R*,9a*R*)-2*H*-quinolizine) and *epi*-chlorolupinane[23] (1-(chloromethyl)-octahydro-(1*S*,9a*R*)-2*H*-quinolizine), respectively, with suitable phenols in ethanolic sodium hydroxide solution (Scheme 2).

Chart 3. Structures of Some Antiarrhythmic Phenoxyalkylamines

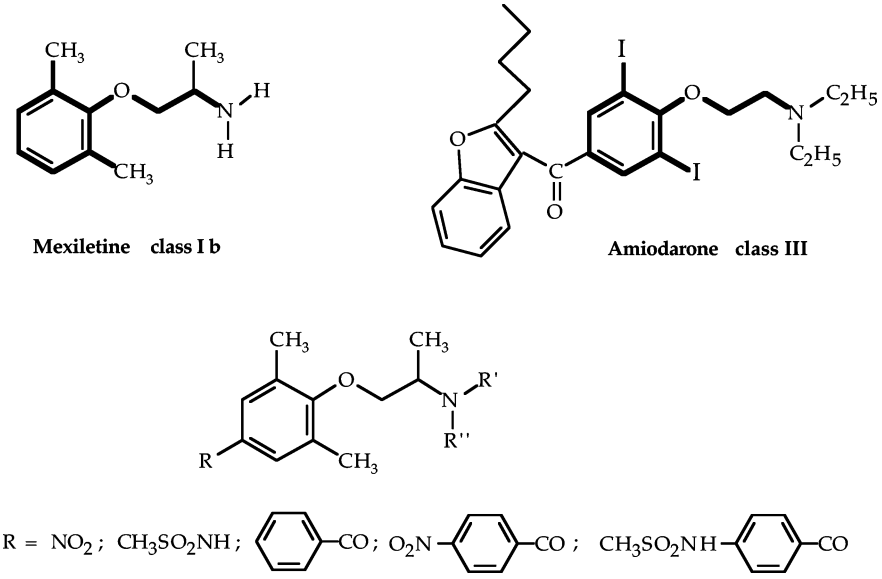
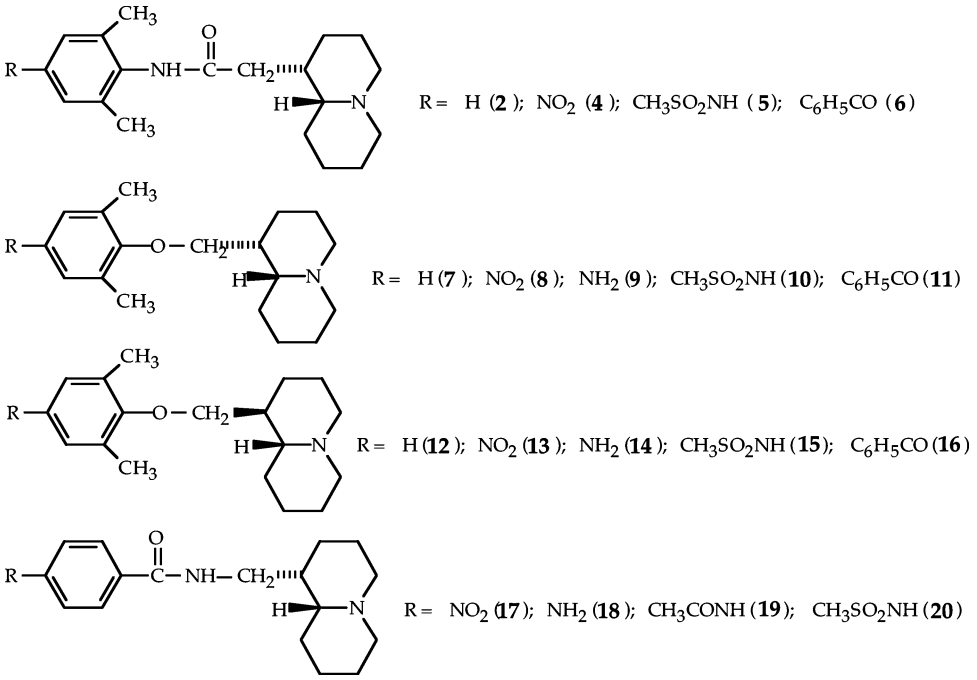
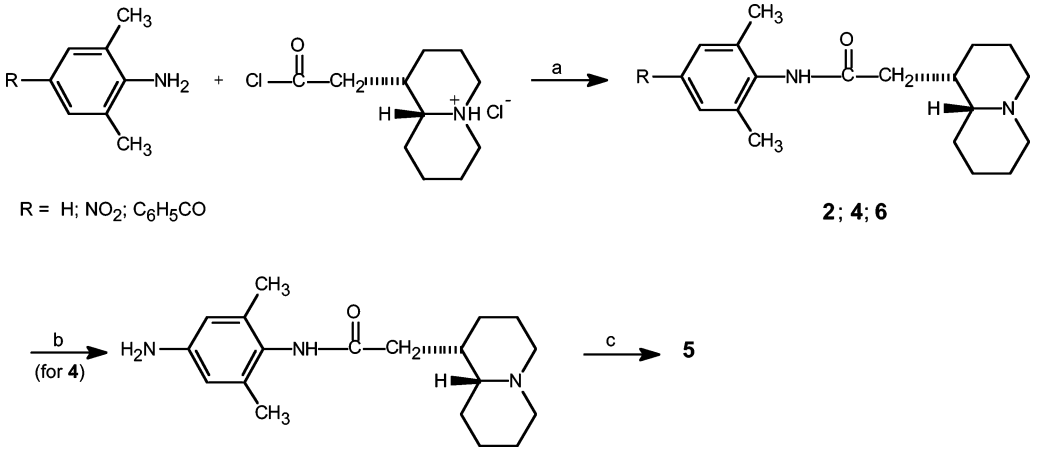


Chart 4. Structures of Novel Quinolizidine-Derived Antiarrhythmic Agents

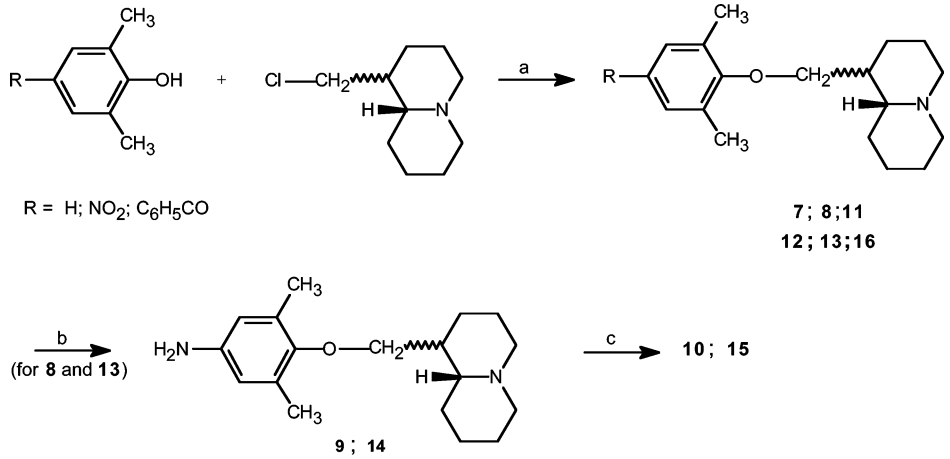


Scheme 1[a]



[a] Reagents and conditions: (a) *i*-Pr_2EtN, $CHCl_3$, Δ; (b) H_2/Pd−C, EtOH, rt; (c) MsCl, $CHCl_3$, rt.

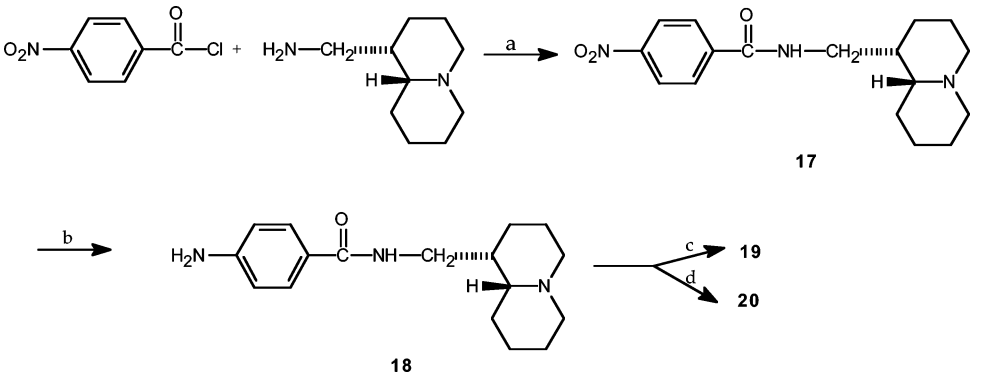
Scheme 2[a]



[a] Reagents and conditions: (a) NaOH, EtOH, Δ; (b) H_2/Pd−C, EtOH, rt; (c) MsCl, Pyr, rt.

The required 3,5-dimethyl-4-hydroxybenzophenone was obtained by Ruminski[24] by decarboxylation of 2-(3,5-dimethyl-4-hydroxybenzoyl)benzoic acid, followed by several purification steps. To avoid this complex preparation, 2,6-dimethylphenol was condensed with benzoic acid in poly(phosphoric acid) at 180 °C; this method, though still requiring repeated purification,

Scheme 3[a]



[a] Reagents and conditions: (a) Et_3N, $PhCH_3$, Δ; (b) H_2/Pd−C, EtOH, rt; (c) Ac_2O, H_2O/CH_3COOH, Δ; (d) MsCl, $CHCl_3$, rt.

appears somewhat advantageous. The nitro derivatives **8** and **13** were reduced catalytically, and amines **9** and **14** were reacted with mesyl chloride to give **10** and **15**, respectively (Scheme 2).

Compound **17** was prepared, as already described,[15] by reacting 4-nitrobenzoyl chloride with lupinylamine[22] ((1-aminomethyl)octahydro-(1*S*,9a*R*)-2*H*-quinolizine). Catalytic reduction of **17** gave amino compound **18**, which with acetic anhydride and mesyl chloride gave **19** and **20**, respectively (Scheme 3).

All structures were supported by elemental analyses and spectral data.

Pharmacology

The pharmacological profile of all compounds was tested for antiarrhythmic activity on isolated guinea pig left atria driven at 1 Hz, 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.

The Langendorff-perfused guinea pig heart was used to assay the whole cardiac activity of compounds **7**, **9**, and **19** and of the reference quinidine. Compounds were checked at increasing doses to evaluate changes in heart rate (chronotropic activity) and in electrocardiogram (ECG) signals: PR, QRS, QT.

Data were analyzed using Student's *t* test and are presented as the mean (M) ± SEM.[25] Since the drugs were added in a cumulative manner, only the significance ($P < 0.05$) between the control and the experimental values at each concentration is indicated by an asterisk.

The potency of drugs defined as EC_{50}, EC_{30}, and IC_{50} was evaluated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[26] or using GraphPad Prism software[25,27]) in the appropriate pharmacological preparations.

Results and Discussion

Compounds **2** and **4**−**20** reported in Chart 4, and amiodarone, lidocaine, procainamide, and quinidine as reference drugs, were evaluated in vitro for antiarrhythmic activity on isolated guinea pig left atria driven at 1 Hz and for influence on the cardiovascular parameters inotropy and chronotropy and on vascular smooth muscle. The results of these assays are collected in Tables 1 and 2.

Simultaneously for the most interesting compounds **7**, **9**, and **19**, the cardiovascular profile was extended to the Langendorff-perfused guinea pig heart to detect the influence on ECG parameters. The data are reported in Table 3 compared to those of the reference compound quinidine.

Table 1. Antiarrhythmic Activity of Compounds

compd	max % increase of threshold of ac-arrhythmia after pretreatment with compds[a] (M ± SEM)	EC_{50}[b] (μM)	95% conf lim (μM)
amiodarone	10 ± 0.5* [c]		
lidocaine	34 ± 2.6*		
procainamide	11 ± 0.4*		
quinidine	69 ± 0.4*	10.26	8.44−12.46
2	78 ± 2.5*	5.71	4.38−7.46
4	inactive[c]		
5	inactive[d]		
6	55 ± 3.0*	4.81	4.38−5.27
7	176 ± 10.2* [d]	0.46	0.30−0.69
8	27 ± 1.0*		
9	166 ± 5.6* [c]	0.97	0.78−1.33
10	60 ± 3.2* [e]	1.25	1.02−1.48
11	64 ± 1.5*	6.75	5.41−8.40
12	35 ± 2.1* [d]		
13	23 ± 1.7* [f]		
14	38 ± 1.6* [d]		
15	5 ± 0.2[c]		
16	104 ± 2.6*	19.87	15.46−25.54
17	18 ± 1.3* [c]		
18	28 ± 1.7* [d]		
19	92 ± 3.7*	0.47	0.29−0.76
20	34 ± 2.1* [c]		

[a] Increase of the threshold of ac-arrhythmia: increase in the current strength of a 50 Hz alternating current required to produce arrhythmia in guinea pig left atria driven at 1 Hz in the presence of each tested compound at 5×10^{-5} M. An asterisk indicates $P < 0.05$. [b] Calculated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[25] with $n = 6-8$). When the maximum effect was <50%, the EC_{50} values were not calculated. [c] At 10^{-4} M. [d] At 10^{-5} M. [e] At 5×10^{-6} M. [f] At 10^{-6} M.

Considering that Na^+ channel inhibitory antiarrhythmic drugs such as quinidine or lidocaine increase the threshold of ac-arrythmia, we used a simple experimental procedure which, in contrast to chemical methods, minimizes the damage to the myocardium (see the Experimental Section).[28,29]

Indeed, quinidine and lidocaine clearly increased the threshold of ac-arrhythmia, procainamide was moderately active, and amiodarone was almost inactive up to 100 μM (+10%). The new compounds show different effects on the threshold of ac-arrythmia. With only three exceptions (**4**, **5**, and **15**), all compounds exerted from moderate to high activity, and most of them at a concentration of 50 μM were more active than lidocaine and procainamide and comparable or superior to quinidine; in particular, ethers **7** and **9** exhibited 176% and 166% increased thresholds of ac-arrythmia versus 69% with quinidine. Moreover, six compounds exhibit the maximal activity (23−

Table 2. Influences of Tested Compounds on Cardiovascular Parameters

comp	% decrease (M ± SEM) negative inotropic activity[a]	% decrease (M ± SEM) negative chronotropic activity[b]	EC_{50} of inotropic negative activity EC_{50}[c] (μM)	95% conf lim (μM)	EC_{30} of chronotropic negative activity EC_{30}[c] (μM)	95% conf lim (μM)	vasorelaxant activity[d] (M ± SEM)
amiodarone	30 ± 2.6* [e]	72 ± 4.5*			5.57	4.93−6.02	3 ± 0.1
lidocaine	88 ± 3.0* [f]	29 ± 0.9* [g,h]	0.017	0.012−0.024			14 ± 0.9*
procainamide	92 ± 1.4* [i]	9 ± 0.6[h]	0.014	0.011−0.017			3 ± 0.2
quinidine	71 ± 3.6*	86 ± 0.5* [j]	3.38	2.69−4.25	3.99	3.81−4.06	30 ± 1.6*
2	95 ± 1.2*	8 ± 0.1[f,h]	0.62	0.42−0.94			5 ± 0.1
4	79 ± 0.5*	25 ± 1.4*	3.46	2.69−4.49			4 ± 0.3
5	86 ± 3.4* [e]	8 ± 0.2[h]	0.31	0.27−0.43			24 ± 1.5*
6	88 ± 2.6*	70 ± 1.7* [j]	0.76	0.51−1.12	3.98	3.15−4.42	31 ± 0.3*
7	25 ± 1.9* [k]	19 ± 0.8[l]					26 ± 1.4* [j]
8	91 ± 4.2* [e]	66 ± 2.4* [j]	8.39	7.88−8.93	23.66	21.15−24.05	16 ± 0.9*
9	83 ± 2.9* [g]	38 ± 2.7*	0.29	0.086−0.71			15 ± 1.0*
10	83 ± 3.7* [f]	12 ± 0.7	0.031	0.023−0.042			30 ± 2.1*
11	94 ± 0.3*	41 ± 3.4* [j]	0.16	0.10−0.26			17 ± 0.7*
12	68 ± 4.9* [m]	13 ± 0.6[l]	0.014	0.009−0.019			21 ± 1.5*
13	83 ± 3.4* [l]	25 ± 1.6* [g]	0.60	0.32−0.65			19 ± 0.9* [j]
14	91 ± 3.4* [l]	44 ± 2.5*	0.63	0.44−0.91			32 ± 2.1* [j]
15	64 ± 2.7* [f]	34 ± 0.7*	0.14	0.083−0.89			23 ± 2.1*
16	64 ± 1.5* [f]	75 ± 3.4* [j]	0.16	0.13−0.19	6.63	6.02−7.13	38 ± 0.9* [j]
17	74 ± 1.7* [f]	28 ± 2.1*	0.038	0.027−0.045			18 ± 0.9*
18	95 ± 3.8* [e]	13 ± 0.8[h]	0.46	0.32−0.71			3 ± 0.2*
19	86 ± 0.8* [l]	14 ± 1.4[j]	0.059	0.041−0.087			3 ± 0.2*
20	92 ± 3.6*	4 ± 0.2	0.25	0.15−0.38			1 ± 0.1*

[a] Activity: decrease in the developed tension in isolated guinea pig left atria at 5×10^{-5} M, expressed as the percentage change from the control ($n = 4-6$). The left atria were driven at 1 Hz. An asterisk indicates $P < 0.05$. [b] Activity: decrease in the atrial rate in guinea pig spontaneously beating isolated right atria at 10^{-4} M, expressed as the percentage change from the control ($n = 6-8$). The pretreatment heart rate ranged from 170 to 195 beats/min. An asterisk indicates $P < 0.05$. [c] Calculated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[25] with $n = 6-8$). When the maximum effect was <50%, the EC_{50} inotropic, EC_{30} chronotropic, and IC_{50} vasorelaxant values were not calculated. [d] Activity: percent inhibition of calcium-induced contraction on K^+-depolarized guinea pig aortic strips at 10^{-4} M. The 10^{-4} M concentration gave the maximum effect for most compounds. An asterisk indicates $P < 0.05$. [e] At 10^{-4} M. [f] At 10^{-6} M. [g] At 5×10^{-6} M. [h] Positive chronotropic effect. [i] At 5×10^{-7} M. [j] At 5×10^{-5} M. [k] At 5×10^{-8} M. [l] At 10^{-5} M. [m] At 10^{-7} M.

Table 3. Influence of **7**, **9**, and **19** on ECG Parameters Evaluated in the Spontaneously Beating Langendorff-Perfused Guinea Pig Heart in Comparison with That of Reference Compounds Quinidine and Amiodarone[e]

compd	param	basal	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
amiodarone	HR[a]	187 ± 11	187 ± 15	180 ± 13	171 ± 12	164 ± 9*	132 ± 8*
	PR[b]	55 ± 4	54 ± 3	55 ± 5	55 ± 2	56 ± 6	58 ± 7
	QRS[c]	20 ± 1.5	20 ± 1.7	20 ± 1.9	21 ± 2	22 ± 2.1	24 ± 1.8
	QT[d]	161 ± 8	161 ± 7	161 ± 11	169 ± 9	174 ± 10	182 ± 7*
quinidine	HR[a]	165 ± 13	160 ± 10	153 ± 11	140 ± 10	123 ± 12*	107 ± 9*
	PR[b]	52 ± 2	53 ± 4	54 ± 2	57 ± 1*	60 ± 3*	70 ± 4*
	QRS[c]	24 ± 1	25 ± 2	27 ± 1	31 ± 2*	35 ± 1*	39 ± 4*
	QT[d]	158 ± 5	158 ± 5	170 ± 8	182 ± 10*	200 ± 11*	229 ± 15*
7	HR[a]	218 ± 10	218 ± 6	215 ± 3	210 ± 4	206 ± 5*	203 ± 7*
	PR[b]	60 ± 3	60 ± 2	64 ± 4	66 ± 5	68 ± 3*	70 ± 6*
	QRS[c]	30 ± 1	30 ± 2	30 ± 3	28 ± 1	30 ± 4	31 ± 3
	QT[d]	155 ± 6	155 ± 4	160 ± 5	170 ± 7	200 ± 6*	210 ± 7*
9	HR[a]	200 ± 1	192 ± 3*	190 ± 2*	184 ± 3*	180 ± 5*	175 ± 3*
	PR[b]	58 ± 2	58 ± 3	60 ± 4	64 ± 3	66 ± 4*	70 ± 6*
	QRS[c]	33 ± 1	30 ± 2	33 ± 3	31 ± 1	32 ± 2	32 ± 3
	QT[d]	156 ± 2	160 ± 1	170 ± 5*	182 ± 4*	194 ± 3*	205 ± 9*
19	HR[a]	180 ± 6	180 ± 5	175 ± 3	170 ± 2	166 ± 3*	165 ± 2*
	PR[b]	61 ± 3	62 ± 2	65 ± 4	70 ± 5	72 ± 7*	75 ± 5*
	QRS[c]	32 ± 1	34 ± 2	33 ± 1	32 ± 3	34 ± 2	33 ± 4
	QT[d]	161 ± 3	170 ± 2	182 ± 5*	190 ± 4*	195 ± 6*	204 ± 1*

[a] HR = heart rate calculated from the RR interval on the ECG signal. [b] PR = atrioventricular conduction time (ms). [c] QRS = intraventricular conduction time (ms). [d] QT = duration of the ventricular action potential (ms). [e] Each value corresponds to the mean ± SEM. An asterisk indicates $P < 0.05$.

176%) at concentrations of 1, 5, and 10 μM, which are, respectively, 50, 10, and 5 times lower than the concentration at which lidocaine and quinidine exhibit their maximal activity (34% and 69%).

For compounds exhibiting an increase of the threshold of acarrhythmia higher than 50%, the EC_{50} values were calculated (Table 1). These data indicate that most compounds were not only more efficacious, but also more potent than reference drugs. Particularly, compounds **7**, **9**, and **19** ($EC_{50} = 0.46$ μM, $EC_{50} = 0.97$ μM, and $EC_{50} = 0.47$ μM, respectively) were 22.30, 10.58, and 21.83 times more potent than quinidine ($EC_{50} = 10.26$ μM).

The observation of antiarrhythmic activity in all the studied subsets of compounds indicates that the characterizing, rigid, and cumbersome quinolizidine ring is itself well tolerated by the biological structures involved in the regulation of heart activity and may be responsible for the higher activity of **2** and **18** with respect to lidocaine and procainamide, respectively. On the other hand, the effects of the substituents introduced in the para position of the aromatic moieties appear to be quite variable in the different subsets. This statement is supported in particular by the comparison of the antiarrhythmic activity of the lupinyl and *epi*-lupinyl ethers. Thus, the nitro group reduced the potency in the former (**8**) while it increased it in the latter (**13**); the

opposite effect was observed for the mesylamino, which increased the potency of the lupinyl ether **10**, but abolished the activity in the *epi*-lupinyl ether **15**.

Thus, these effects partially obscured the meaning of the steric disposition of the quinolizidine ring relative to the aromatic moiety in the two subsets of ethers **7**−**11** and **12**−**16**. It is well-known that stereoselective active sites are present on cardiac sodium channels,[30,31] which, for instance, preferentially bind the *R*-(−)-enantiomer of mexiletine.[32] Similarly, the *R*-enantiomers of the chiral sodium channel blockers mepivacaine, ropivacaine, and bupivacaine exhibit a consistently higher cardiotoxicity than the *S*-enantiomers.[33] An even larger and consistent difference of activity should have been expected for the epimeric group of compounds **7**−**11** and **12**−**16**.

In the *N*-(4-substituted benzoyl)lupinylamine group the antiarrhythmic potency was lowest for the mesyl derivative, while among the diethylaminoethylamino derivatives the sematilide was much more potent than acecainide and procainamide.[34] However, the lupinyl analogue of acecainide (**19**) was definitely more active than the analogues of procainamide and sematilide.

To better evaluate the pharmacological profile of the investigated antiarrhythmic agents, the comparison of their influence on additional cardiovascular parameters with that elicited by the four reference compounds (Table 2) was deemed necessary. Thus, compounds **2** and **4**−**20** were evaluated for (a) inotropic activity on isolated guinea pig driven left atria, (b) chronotropic activity on isolated guinea pig spontaneously beating right atria, and (c) vasorelaxant activity expressed as inhibition of calcium-induced contraction on K^+-depolarized guinea pig aorta strips.

All compounds, with the exception of **7**, decreased the developed tension on driven left atria (Table 2), and compounds **12**, **10**, **17**, and **19** were in that order the most potent, with EC_{50} in the range 0.014−0.059 μM, while lidocaine exhibited EC_{50} = 0.017 μM. Only two compounds (**4**, **8**) had EC_{50} higher than that of quinidine (3.38 μM); the remaining compounds exhibited EC_{50} in the range 0.14−0.76 μM. As already observed by Busch et al.,[35] amiodarone did not influence the contractile force. A weak negative inotropic activity was observed for compound **7** only at very low concentration (50 nM).

The negative chronotropic activity (Table 2) detected on spontaneously beating right atria was not particularly marked, being practically comparable to that of quinidine (EC_{30} = 3.99 μM) for two compounds (**6**, **16**), 5.93-fold less potent for compound **8**, and definitely minor for all the remaining compounds. In this in vitro model, amiodarone, like quinidine, decreased cardiac frequency (EC_{30} = 5.57 μM). It is also notable that compounds **2**, **5**, and **18** showed positive chronotropic activity as observed for lidocaine and procainamide.

Even more modest was the vasorelaxant activity (Table 2), as expressed by the inhibition of calcium-induced contraction on K^+-depolarized (80 mM) guinea pig aortic strips, with inhibition values in the range 15−38% at 50−100 μM, as was also observed for quinidine and lidocaine. Compounds **2**, **4**, and **18**−**20** were practically inactive, as were procainamide and amiodarone.

The most interesting antiarrhythmic compounds **7**, **9**, and **19** were studied on Langendorff retrogradely perfused guinea pig spontaneously beating heart[36] and compared with amiodarone and quinidine. While increasing concentrations of quinidine up to its EC_{50} for antiarrhythmic activity (10.26 μM) produced a substantial dose-dependent prolongation of all the ECG intervals and a 35% reduction of the heart rate, high concentrations of the novel compounds, even 10−22 times higher than their EC_{50} for antiarrhythmic activity, only moderately prolonged the PR and QT intervals, leaving the QRS complex unchanged and reducing the heart rate by only 7−12%. Amiodarone increased the QT interval by only 13% at 10 μM concentration, which is, however, 10 times lower than that able to produce a perceptible increase of the threshold of ac-arrhythmia (see Table 1). Moreover, amiodarone, like quinidine, at that concentration produced a strong (−29%) reduction of the heart rate. Therefore, compounds **7**, **9**, and **19** compared favorably also with amiodarone.

Conclusion

The studied quinolizidine derivatives represent, on the whole, an interesting novel group of antiarrhythmic agents, among which very high activity and potency may be found, as in compounds **7**, **9**, and **19**, which compared favorably with amiodarone, lidocaine, procainamide, and quinidine. The different structural features which characterize the investigated compounds seem to play an interwoven role, making it difficult to define structure−activity relationships.

Compound **7** exhibits an interesting cardiovascular profile, being endowed with significant antiarrhythmic activity and potency with only modest negative inotropic and chronotropic effects and vasorelaxant activity that may even be clinically profitable, while more pronounced effects could induce secondary arrhythmias through discharges from the controlled *focus*. Therefore, compound **7** deserves further investigations.

Experimental Section

Chemistry. Melting points were taken in open glass capillaries on a Büchi apparatus and were uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Gemini 200 spectrometer. Chemical shifts (δ) are reported in parts per million from the peak for internal Me_4Si. Values of the coupling constants (J) are reported in hertz. Lup = lupinyl (octahydroquinolizin-1-ylmethyl) residue, and Q = octahydroquinolizine ring. Thin-layer chromatography (TLC) was performed on silica gel plates [Merck (G F_{254})] and the spots were observed under UV light or were visualized with I_2 vapor. Column chromatography (CC) was performed by using silica gel 60 (Merck) or basic alumina (Across). Elemental analyses were performed on a Carlo Erba EA 1110 CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmaceutical Sciences of the University of Genova.

Intermediates. The required 2,6-dimethylaniline, 2,6-dimethylphenol, 2,6-dimethyl-4-nitrophenol, and 4-nitrobenzoyl chloride were purchased from Aldrich. 2,6-Dimethyl-4-nitroaniline was prepared according to the Wepster method.[19] The known benzophenone derivatives of Schemes 1 and 2 [(4-amino-3,5-dimethylphenyl)phenylmethanone and (3,5-dimethyl-4-hydroxyphenyl)phenylmethanone] were prepared as follows.

(4-Amino-3,5-dimethylphenyl)phenylmethanone. A mixture of 2,6-dimethylaniline (2.52 mL, 20 mmol), benzoic acid (5.2 g, 42.5 mmol), and poly(phosphoric acid) (PPA; 40 g) was heated at 180 °C and stirred for 30 min under nitrogen. The reaction mixture was diluted with 16 mL of water, heated again at 180 °C for 40 min, and then poured into 300 mL of water. The acidic suspension was extracted several times with CH_2Cl_2. The organic phase was washed with 1 N NaOH solution and then with water, dried over Na_2SO_4, and evaporated to dryness. The residue was taken up in dry ether, leaving behind some *N*-benzoyl-2,6-dimethylaniline (460 mg, mp 157−158 °C). The ether solution was evaporated, and the residue was crystallized from EtOH/H_2O (3:1) and further purified by CC (SiO_2, CH_2Cl_2) to obtain 1.04 g (23%) of the title compound. Mp: 136−137.5 °C (lit.[21] 85−86 °C). ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 6H, $2CH_3$); 4.10 (br s, 2H, NH_2, collapses with D_2O); 7.42−7.60 (m, 5H, aromatic protons); 7.70−7.80 (m, 2H, aromatic protons). Anal. ($C_{15}H_{15}NO$) C, H, N. Some unreacted 2,6-dimethylaniline was recovered from the acidic aqueous solution.

(3,5-Dimethyl-4-hydroxyphenyl)phenylmethanone. A mixture of 2,6-dimethylphenol (2.26 g, 20 mmol), benzoic acid (5.2 g, 42.5 mmol), and PPA (40 g) was heated at 180 °C and stirred for 30 min. The mixture was diluted with 16 mL of water, further heated at 165 °C for 40 min, and finally poured into 200 mL of water and extracted with CH_2Cl_2. The organic phase was extracted three times with 1 N NaOH solution and then with water; from the organic phase 2.76 g of an oil composed mainly of 2,6-dimethylphenyl benzoate was recovered. The basic solution was acidified and extracted with CH_2Cl_2; the dichloromethane solution was extracted repeatedly with 5% $NaHCO_3$ solution to remove unreacted benzoic acid and then with water, dried (Na_2SO_4), and evaporated to dryness to give 1.54 g of the required compound.

The crude 2,6-dimethylphenyl benzoate (2.76 g) was mixed with 20 g of PPA and heated at 180 °C for 20 min to promote the para transposition of the benzoyl group. Working up the mixture as above, a further crop of 0.51 g of the required compound was obtained. The two fractions were joined and chromatographed on silica gel eluting with CH_2Cl_2; 1.75 g (39% yield) of the title compound was obtained. Mp: 136−138 °C. ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 6H, $2CH_3$); 5.22 (s,1H, OH, collapses with D_2O); 7.42−7.68 (m, 5H, aromatic protons); 7.74−7.80 (m, 2H, aromatic protons). Anal. ($C_{15}H_{14}O_2$) C, H.

***N*-Homolupinanoylanilines 2, 4, and 6: General Method.** A mixture of the suitable amine and diisopropylethylamine (6.4 mol each) in 10 mL of $CHCl_3$ was added drop by drop to a solution of 1.61 g (6.4 mmol) of homolupinanoyl chloride hydrochloride[17,18] in 15 mL of ethanol-free $CHCl_3$ (passed on basic alumina). The mixture was refluxed for 5 h under a slow stream of dry N_2. The solvent was removed in vacuo and the residue taken up in water (100 mL). From the resulting turbid solution (pH 4−5) the unreacted weakly basic amine was extracted with $CHCl_3$, which however extracted also some of the hydrochloride of the desired compounds, expecially **6** (solution A). The aqueous solution was basified and extracted again with chloroform; after evaporation of the solvent the residue was rinsed with dry ether, leaving the main portion of the expected amide. Additional portions of the amides were recovered from solution A: the solvent was evaporated and the residue chromatographed repeatedly on basic alumina (1:40) eluting with chloroform.

Data for *N*-(2,6-Dimethylphenyl)-2-(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)acetamide (2). Mp: 187−187.5 °C (EtOH). Yield: 70%.[12] ^{1}H NMR ($CDCl_3$): δ 1.18−2.92 (m with superimposed s at 2.24, 18H of Lup + 6H, $2CH_3$); 6.85 (s, 1H, CONH, collapses with D_2O); 7.05−7.18 (m, 3H, aromatic protons). Anal. ($C_{19}H_{28}N_2O$) C, H, N.

Data for *N*-(2,6-Dimethyl-4-nitrophenyl)-2-(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)acetamide (4). Mp: 229−235 °C (absolute EtOH). Yield: 42%. ^{1}H NMR ($CDCl_3$): δ 1.24−2.92 (m with superimposed s at 2.33, 18H of Lup + 6H, $2CH_3$); 7.22 (s, 1H, CONH, collapses with D_2O); 7.97 (s, 2H, aromatic protons). Anal. ($C_{19}H_{27}N_3O_3$) C, H, N.

Data for {3,5-Dimethyl-4-[*N*-(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)acetyl]aminophenyl}phenylmethanone (6). Mp: 98−102 °C (benzene/pentane). Yield: 26%. ^{1}H NMR ($CDCl_3$): δ 1.20−2.95 (m, 18H of Lup + superimposed s at 2.29, 6H, $2CH_3$); 7.10 (s,1H, CONH, collapses with D_2O); 7.45−7.65 (m, 5H, aromatic protons); 7.78−7.90 (m, 2H, aromatic protons). Anal. ($C_{26}H_{32}N_2O_2$) C, H, N.

***N*-(4-Amino-2,6-dimethylphenyl)-2-(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)acetamide.** The nitro compound **4** (0.48 g, 1.4 mmol) and 10% Pd on activated carbon (0.1 g) were suspended in 40 mL of EtOH and hydrogenated at room temperature (rt) and atmospheric pressure. The catalyst was filtered and the solvent removed in vacuo to yield white crystals (0.44 g, quantitative yield). Mp: 147−148 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−2.94 (m, 18H of Lup + superimposed s at 2.14, 6H, $2CH_3$); 3.45 (br s, 2H, NH_2, collapses with D_2O); 6.41 (s, 2H, aromatic protons); 6.70 (s, 1H, CONH, collapses with D_2O). Anal. ($C_{19}H_{29}N_3O$) C, H, N.

***N*-(2,6-Dimethyl-4-mesylaminophenyl)-2-(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)acetamide (5).** The foregoing amino compound (0.4 g, 1.27 mmol) was dissolved in EtOH-free $CHCl_3$ (7 mL) and treated with mesyl chloride (0.1 mL, 1.29 mmol) diluted with $CHCl_3$ (5 mL). The mixture was stirred for 6 h at rt and then evaporated to dryness. The residue was chromatographed several times on basic alumina eluting with $CHCl_3$ containing increasing quantities of MeOH up to 1%. Finally, 0.130 g (26%) of **5** was obtained. Mp: 178−181 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−2.96 [m, 18H of Lup + 2 superimposed s at 2.18 (6H, $2CH_3$) and 2.95 (3H, CH_3SO_2)]; 5.95 (br s, 1H, N*H*SO_2CH_3, collapses with D_2O); 6.82 (s, 2H, aromatic protons); 7.05 (s, 1H, CONH, collapses with D_2O). Anal. ($C_{20}H_{31}N_3O_3S$) C, H, N, S.

Lupinyl (7, 8, 11) and *epi*-Lupinyl (12, 13, 16) Ethers: General Method. The suitable phenol (4.5−6 mmol) was dissolved in EtOH (2−2.5 mL) and treated with 6 N NaOH solution (0.75−1 mL) and then with 4.5−6 mmol of chlorolupinane[21] or *epi*-chlorolupinane.[22] The mixture was heated in an Aldrich pressure tube at 140 °C for 10−12 h with magnetic stirring. After cooling, the solvent was evaporated in vacuo, and the residue was taken up in water and strongly alkalinized. The mixture was extracted with ether and the organic phase dried (Na_2SO_4) and evaporated to dryness. In the case of lupinyl ethers the residue was crystallized from dry ether, while in the case of *epi*-lupinyl ethers the residue was distilled under reduced pressure (0.04 Torr) to eliminate the unreacted *epi*-chlorolupinane (bp 90−100 °C, air bath temperature) and then crystallized from dry ether, unless otherwise stated.

Data for (1*R*,9a*R*)-1-[(2,6-Dimethylphenoxy)methyl]octahydro-2*H*-quinolizine (7). Low melting point crystals. Yield: 15%. ^{1}H NMR ($CDCl_3$): δ 1.05−2.30 (m, 14H of Q, with superimposed s at 2.2, 6H, $2CH_3$); 2.70−2.85 (m, 2H, Hα near N of Q); 3.75−3.95 (m, 2H, CH_2O); 6.75−6.95 (m, 3H, aromatic protons). Anal. ($C_{18}H_{27}NO$) C, H, N. The following are data for the hydrochloride. Mp: 247−255 °C dec (EtOH + Et_2O). Anal. ($C_{18}H_{27}NO\cdot HCl$) C, H, N.

Data for (1*R*,9a*R*)-1-[(2,6-Dimethyl-4-nitrophenoxy)methyl]-octahydro-2*H*-quinolizine (8). Red-orange crystals. Mp: 88−89 °C. Yield: 48%. ^{1}H NMR ($CDCl_3$): δ 1.10−2.30 (m, 14H of Q); 2.36 (s, 6H, $2CH_3$); 2.78−2.92 (m, 2H, Hα near N of Q); 3.92−4.12 (m, 2H, CH_2O); 7.93 (s, 2H, aromatic protons). Anal. ($C_{18}H_{26}N_2O_3$) C, H, N.

Data for (1*R*,9a*R*)-{3,5-Dimethyl-4-[(octahydro-2*H*-quinolizin-1-yl)methoxy]phenyl}phenylmethanone (11). Orange crystals. Mp: 84−87 °C. Yield: 56%. ^{1}H NMR ($CDCl_3$): δ 1.10−2.30 (m, 14H of Q); 2.34 (s, 6H, $2CH_3$); 2.78−2.92 (m, 2H, Hα near N of Q); 3.92−4.12 (m, 2H, CH_2O); 7.24−7.65 (m, 5H, aromatic protons); 7.75−7.84 (m, 2H, aromatic protons). Anal. ($C_{25}H_{31}NO_2$) C, H, N.

Data for (1*S*,9a*R*)-1-[(2,6-Dimethylphenoxy)methyl]octahydro-2*H*-quinolizine (12). Low melting point solid. Yield: 16%. ^{1}H NMR ($CDCl_3$): δ 1.00−2.40 (m, 14H of Q, with superimposed s at 2.20, 6H, $2CH_3$); 2.60−2.85 (m, 2H, Hα near N of Q); 3.64 (d, J = 4.6 Hz, 2H, CH_2O); 6.70−6.95 (m, 3H, aromatic protons). Anal. ($C_{18}H_{27}NO$) C, H, N. The following are data for the hydrochloride. Mp: 235−240 °C dec (EtOH + Et_2O). Anal. ($C_{18}H_{27}NO\cdot HCl$) C, H, N.

Data for (1*S*,9a*R*)-1-[(2,6-Dimethyl-4-nitrophenoxy)methyl]-octahydro-2*H*-quinolizine (13). Yellow crystals. Mp: 72−73 °C (pentane). Yield: 25%. ^{1}H NMR ($CDCl_3$): δ 1.10−2.30 (m, 14H of Q); 2.36 (s, 6H, $2CH_3$); 2.78−2.92 (m, 2H, Hα near N of Q); 3.92−4.12 (m, 2H, CH_2O); 7.93 (s, 2H, aromatic protons). Anal. ($C_{18}H_{26}N_2O_3$) C, H, N.

Data for (1*S*,9a*R*)-{3,5-Dimethyl-4-[(octahydro-2*H*-quinolizin-1-yl)methoxy]phenyl}phenylmethanone (16). Oil. Yield: 22%. ^{1}H NMR ($CDCl_3$): δ 1.10−2.20 (m, 14H of Q); 2.33 (s, 6H, $2CH_3$); 2.82−2.95 (m, 2H, Hα near N of Q); 3.79 (d, J = 4.4 Hz, 2H, CH_2O); 7.40−7.64 (m, 5H, aromatic protons); 7.74−7.83 (m, 2H, aromatic protons). The following are data for the hydrochloride. Mp: 242−244 °C (EtOH). Anal. ($C_{25}H_{31}NO_2\cdot HCl$) C, H, N.

(1*R*,9a*R*)-1-[(4-Amino-2,6-dimethylphenoxy)methyl]-octahydro-2*H*-quinolizine (9). The nitro derivative **8** (0.91 g, 2.9 mmol) was dissolved in 50 mL of EtOH mixed with 10% Pd on activated carbon (90 mg) and hydrogenated at rt and atmospheric pressure.

The catalyst was filtered and the solvent removed in vacuo, leaving white crystals (0.77 g, 93% yield). Mp: 94−97 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−2.40 (m, 14H of Q + s at 2.21, 6H, $2CH_3$); 2.78−2.90 (m, 2H, Hα near N of Q); 3.40 (br s, 2H, NH_2, collapses with D_2O); 3.80−4.00 (m, 2H, CH_2O); 6.36 (s, 2H, aromatic protons). Anal. ($C_{18}H_{28}N_2O$) C, H, N.

(1*R*,9a*R*)-1-[(2,6-Dimethyl-4-mesylaminophenoxy)methyl]-octahydro-2*H*-quinolizine (10). The foregoing amino compound **9** (0.50 g, 1.73 mmol) was dissolved in pyridine (7 mL) and treated with mesyl chloride (0.13 mL, 1.7 mmol). The mixture was stirred for 10 min at rt and then refluxed for 1.5 h. After cooling, the mixture was poured onto ice, and 0.1 N NaOH solution was added until pH ≈ 8 was reached and then thoroughly extracted with CH_2Cl_2. The solvent was evaporated, and the oily residue was distilled under reduced pressure to remove the pyridine. The residue was chromatographed on alumina (1:30) eluting with chloroform. Thus, 230 mg of **10** (37% yield) was obtained. Mp: 126−127 °C (Et_2O). ^{1}H NMR ($CDCl_3$): δ 1.20−2.20 (m, 14H of Q); 2.27 (s, 6H, $2CH_3$); 2.80−2.94 (m, 2H, Hα near N of Q); 2.98 (s, 3H, SO_2CH_3); 3.80−4.00 (m, 2H, CH_2O); 6.40 (br s, 1H, N*H*SO_2CH_3, collapses with D_2O); 6.89 (s, 2H, aromatic protons). Anal. ($C_{19}H_{30}N_2O_3S$) C, H, N, S.

(1*S*,9a*R*)-1-[(4-Amino-2,6-dimethylphenoxy)methyl]octahydro-2*H*-quinolizine (14). The nitro derivative **13** was reduced as described for the epimeric **8**; the catalyst was filtered and the solvent evaporated to leave an oil that crystallized when rinsed with pentane (0.63 g, 94% yield). Mp: 56−58 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−2.20 (m, 14H of Q); 2.20 (s, 6H, $2CH_3$); 2.80−2.92 (m, 2H, Hα near N of Q); 3.40 (br s, 2H, NH_2, collapses with D_2O); 3.65 (d, J = 4.6 Hz, 2H, CH_2O); 6.36 (s, 2H, aromatic protons). Anal. ($C_{18}H_{28}N_2O$) C, H, N.

(1*S*,9a*R*)-1-[(2,6-Dimethyl-4-mesylaminophenoxy)methyl]octahydro-2*H*-quinolizine (15). The foregoing amino compound **14** was treated with mesyl chloride as described for the preparation of the mesylamino derivative **10**. The separation of the mesylamino derivative **15** from the starting amine was quite laborious; thus, only a 13% yield of pure **15** was obtained. Mp: 157−158 °C (Et_2O). ^{1}H NMR ($CDCl_3$): δ 1.20−2.20 (m, 14H of Q); 2.27 (s, 6H, $2CH_3$); 2.80−2.95 (m, 2H, Hα near N of Q); 2.99 (s, 3H, SO_2CH_3); 3.69 (d, J = 4.4 Hz, 2H, CH_2O); 6.89 (s, 2H, aromatic protons); the signal due to $NHSO_2$ was not detected. Anal. ($C_{19}H_{30}N_2O_3S$) C, H, N, S.

4-Nitro-*N*-[(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]-benzamide (17). Compound **17** was repared as previously described.[15] Mp: 121 °C (EtOH/H_2O). ^{1}H NMR ($CDCl_3$): δ 1.20−2.30 (m, 14H of Q); 2.90−3.05 (m, 2H, Hα near N of Q); 3.65−3.75 (m, 2H, C*H*$_2$NH); 7.90−8.05 and 8.15−8.35 (2 m, 2H each, aromatic para substitution); 9.90 (br s, 1H, NH, collapses with D_2O). The following are data for the hydrochloride. Mp: 154−158 °C dec (absolute EtOH/Et_2O). Anal. ($C_{17}H_{23}N_3O_3$·HCl) C, H, N.

4-Amino-*N*-[(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]-benzamide (18). Compound **18** was prepared as previously described,[15] by reduction of **17** in the presence of 10% palladium on activated carbon instead of PtO_2. Mp: 193−194 °C (EtOH). Anal. ($C_{17}H_{25}N_3O$) C, H, N.

4-Acetylamino-*N*-[(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]benzamide (19). Acetic anhydride (1.5 mL) was added to a solution of the foregoing amino compound **18** (0.7 g, 2.4 mmol) in 2 mL of a mixture of acetic acid and water (1:1), and the mixture was heated in a boiling water bath for 5 min. After cooling, the solution was neutralized with 10% Na_2CO_3 solution, and the precipitate was collected and washed with water. After drying, 0.67 g (84% yield) of **19** was obtained. Mp: 230−231 °C. ^{1}H NMR (DMSO-d_6): δ 1.10−2.00 (m, 14H of Q); 2.06 (s, 3H, CH_3CO); 2.66−2.82 (m, 2H, Hα near N of Q); 3.35−3.48 (m, 2H, C*H*$_2$-NH); 7.55−7.80 (m, 4H, aromatic protons); 8.25−8.40 (m, 1H, N*H*CH_2, collapses with D_2O); 10.15 (s, 1H, N*H*$COCH_3$, collapses with D_2O). Anal. ($C_{19}H_{27}N_3O_2$) C, H, N.

4-Mesylamino-*N*-[(1*S*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methyl]benzamide (20). A solution of mesyl chloride (0.2 mL, 2.6 mmol) in $CHCl_3$ (EtOH-free) (7 mL) was added dropwise to a solution of the amino compound **18** (0.7 g, 2.43 mmol) in $CHCl_3$ (EtOH-free, 7 mL). The mixture was stirred at rt for 1 h and then evaporated to dryness. The residue was taken up in water (7 mL), and the solution was neutralized with 10% Na_2CO_3 solution and extracted with ether, which removed some unreacted amino compound. By careful correction of the pH, a precipitate was obtained that was extracted with $CHCl_3$. After drying (Na_2SO_4), the solvent was evaporated, and the residue was chromatographed on alumina (1:30) eluting with $CHCl_3$. Compound **20** was finally crystallized from pentane, 0.29 g (33% yield). Mp: 179−184 °C. ^{1}H NMR (DMSO-d_6): δ 1.10−2.00 (m, 14H of Q); 3.05 (s, 3H, CH_3SO_2); 2.70−2.85 (m, 2H, Hα near N of Q); 3.30−3.50 (m, 2H, C*H*$_2$NH); 7.18−7.90 (m, 4H, aromatic protons); 8.30−8.42 (m, 1H, N*H*CH_2, collapses with D_2O); 10.05 (br s, 1H, N*H*SO_2CH_3, collapses with D_2O). Anal. ($C_{18}H_{27}N_3O_3S$) C, H, N, S.

Pharmacology. 1. Guinea Pig Atrial Preparations. Female guinea pigs (300−400 g) 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_4·xH_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 Corp., Quincy, MA) using Power Lab software (AD-Instruments Pty Ltd., Castle Hill, Australia). 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 minutes, a length−tension curve was determined, and the muscle length was maintained, which elicited 90% of the maximum contractile force observed at the optimal length. A stabilization period of 45−60 min was allowed before the atria were used to test the 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 activities of the compounds (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 μM) dissolved in PSS. 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.

2. Alternating Current Induced Arrhythmia in Guinea Pig Left Atrial Preparations.[28,29] Left atria from guinea pigs were fixed to platinum wire electrodes and placed between two parallel platinum field electrodes. The preparations were mounted vertically in a 15 mL organ bath containing a modified Krebs−Henselait solution of the following composition (mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 NaH_2PO_4·xH_2O, 24.9 $NaHCO_3$, and 10.1 glucose. The solution was buffered at pH 7.4 by saturation with 95% O_2/5% CO_2 gas, and the temperature was maintained at 35 °C. The preparations were connected to a force transducer. Isometric force contraction was recorded using Power Lab software. Arrhythmias were induced by application of sinusoidal alternating current (50 Hz) of increasing strength to the isolated heart preparations, 2 and 4 mA/(cm^2·s) to induce arrhythmia corresponding to an increase in the field intensity of 0.3 V/(cm·s). The current strength i (mA/cm^2) at which extra beats occurred was called the "threshold of ac-arrhythmia".[37] The preparations were allowed to stabilize for 60 min. Then concentration−response curves for the threshold of ac-arrhythmia were recorded by cumulative application of compounds to the bathing solution every 30 min.

3. 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_2-

PO_4, 25 $NaHCO_3$, and 11 glucose. The solution was 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 mm × 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 pharmacological salt solution (PSS) at 35 °C. Aortic strips were subjected to a resting force of 1 g. The strips were secured at one end to a force displacement transducer (FT 0.3, Grass Instruments Corp.) to monitor changes in the isometric contraction and washed every 20 min with fresh PSS for 1 h after the equilibration period; guinea pig aortic strips were 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 (PSS for all compounds).

4. Guinea Pig Isolated Perfused Heart According to Langendorff. Female guinea pigs (300−400 g) were killed by cervical dislocation. The hearts were quickly removed and rapidly perfused through the aorta at constant flow (11−12 mL min^{-1} g^{-1}) with a modified Krebs−Henselait solution with the following composition (mM): 128 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 15 $NaHCO_3$, 1.2 KH_2PO_4, 11.1 glucose, and 2 sodium pyruvate. The solution was bubbled with a gas mixture (95% O_2/5% CO_2) (pH 7.4−7.5) and maintained at 37 °C. A perfusion pressure of 50−60 mmHg was obtained at this flow rate. The addition of pyruvate to the medium has been shown to confer the same metabolic and functional features of the heart in situ to the isolated heart.[38] A stabilization period of 30 min was given to the heart under normal ECG conditions to keep the frequency of spontaneous beating hearts constant. Surface ECG was recorded by means of two electrodes, one placed near the initial portion of the anterior intraventricular artery and the other on the left ventricular free wall. The main ECG intervals (PR = atrioventricular conduction time; QRS = intraventricular conduction time; QT = duration of ventricular action potential) were measured. The drug-induced changes in conduction velocity of the AV node and ventricular myocardium were calculated as changes in the reciprocal of the PR interval and QRS interval, respectively.[39] The compounds were added with increasing concentrations (0.001, 0.01, 0.1, 1, and 10 μM). During the building of concentration−response curves, the next higher concentration of the compounds was added only after the preparation reached a steady state (about 30 min).

The potency of the drugs, defined as the EC_{50} value, was evaluated from log concentration−response curves (n = 6−8) (GraphPad) in the appropriate pharmacological preparations.[27] All data are presented as the mean ± SEM.[25]

Acknowledgment. Financial support from the Italian MIUR and from the Universities of Bologna and Genova are gratefully acknowledged. We thank Mr. O. Gagliardo for performing the elemental analyses.

Supporting Information Available: Elemental analysis data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM060878M