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Novel Quinolizidinyl Derivatives as Antiarrhythmic Agents: 2. Further Investigation

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Fifteen quinolizidine derivatives have been tested for antiarrhythmic, inotropic, and chronotropic effects on isolated guinea pig (gp) heart tissues and to assess calcium antagonist activity. All compounds exhibited from moderate to high antiarrhythmic activity, and five of them (**3**, **4**, **6**, **13**, and **15**) were more active and potent than the reference drugs (amiodarone, lidocaine, procainamide, and quinidine). These compounds were studied on spontaneously beating Langendorff-perfuse gp heart; even at concentration 17−67 times higher than the corresponding EC_{50} for antiarrhythmic activity, they prolonged the QT intervals only moderately, comparing favorably with amiodarone and quinidine. Compounds **3** and **15** deserve further investigation due to their interesting cardiovascular profiles.

Introduction

Arrhythmia is a complex abnormality of cardiac rhythm, affecting an increasing percent of the population with the increase of age.[1] Atrial fibrillation is the most common cardiac arrhythmia with high risk of thromboembolic stroke in older patients.[2]

Despite the availability of a large number of drugs able to suppress dysrhythmic cardiac activity through different mechanisms (Chart 1), a satisfactory pharmacological therapy has not yet been developed.[3]

Therefore, the search of new antiarrhythmic agents is largely pursued, particularly with the aim to obtain compounds with multiple mechanisms of action,[4] combining, for instance, the electrophysiologic properties of antiarrhythmic drugs of classes Ib and III (Vaughan Williams classification)[5] in order to balance the proarrhythmic risk inherent to the latter type of compounds.

Recently,[6] we have described 18 analogues of lidocaine, mexiletine, and procainamide, whose aminoalkyl chains were replaced with the rigid and cumbersone quinolizidine nucleus (Chart 2). The novel compounds were tested for antiarrhythmic, inotropic, and chronotropic effects on isolated guinea pig (gp[a]) heart tissues and to assess calcium antagonist activity. Most compounds exhibited from moderate to high antiarrhythmic activity, resulting in several cases more active and potent than quinidine and lidocaine, while producing only moderate negative inotropic and, in most cases, negative chronotropic activity. None of them possess vasorelaxant effects. Moreover, while quinidine and amiodarone produced a dose dependent prolongation of all the electrocardiogram (ECG) intervals, our best compounds, even at concentrations 10−20 times higher than EC_{50} for the antiarrhythmic activity, only moderately prolonged the atrioventricular conduction time (PR) and the duration of the ventricular action potential (QT) intervals, leaving unchanged the intraventricular conduction time (QRS) complex.

These interesting characteristics can be related to the presence of the peculiar quinolizidine moiety, and therefore, we deemed worthy to investigate other derivatives of this bicyclic ring (Chart 3). On the whole, 15 compounds have been now considered, 10 of which were synthesized and investigated in the past for other pharmacological aims.[7−11]

With relation to the structure, the presently tested compounds can be grouped in four subsets (**A**−**D**). Compounds of the first and second groups (**1**−**4** and **5**−**7**) represent, respectively, the extensions of homolupinanoyl anilines and of aroylaminolupinanes previously studied (exemplified by **I**, **II**, and **IV** of Chart 2). Compounds of third group (**8**−**11**) still contain an amidic group, but they differentiate from the previous ones for the presence of heterocyclic moieties (benzotriazole and quinoxalin-2(1*H*)one). It is worth noting that the 1-substituted 3-phenylquinoxaline substructure of compound **10** is present in compounds endowed with remarkable positive inotropic activity on isolated frog heart at submicromolar concentration.[12] Finally, the fourth group (**12**−**15**) is formed by sterically hindered *N*-(quinolizidinylalkyl)anilines, with two nitrogen atoms of different basicity, linked each other through an atom sequence of variable length. Moreover, these compounds are further characterized by the lack of any hydrogen-bond accepting carbonyl group.

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[a] Abbreviations: ADP, adenosine diphosphate; CC, column chromatography; CCh, carbachol; DBN, 1,5-diazabicyclo[4.3.0]non-5-ene; ECG, electrocardiogram; gp, guinea pig; GPILSM, guinea pig ileum longitudinal smooth muscle; HR, heart rate; Lup, lupinyl residue (octahydroquinolizin-1-ylmethyl); ms, millisecondi; PAF, platelet activating factor; PR, atrio-ventricular conduction time; Q, octahydroquinolizine ring; QRS, intraventricular conduction time; QT, duration of the ventricular action potential; RR, time duration between two consecutive R waves of the ECG; THF, tetrahydrofuran.

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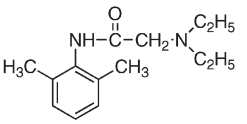
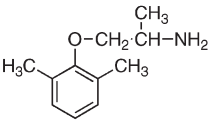
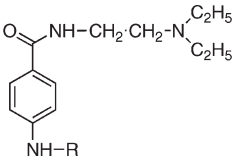
Chart 1. Structures of Representative Antiarrhytmic Drugs



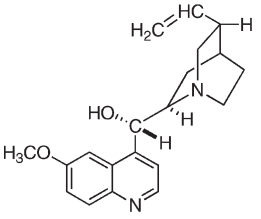
Lidocaine (Class Ib)



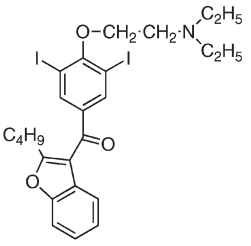
Mexiletine (Class Ib)



R = H, Procainamide (Class Ia)
R = CH_3-CO, Acecainide (Class IV)

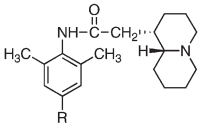


Quinidine (Class Ia)

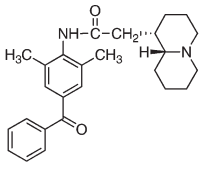


Amiodarone (Class III)

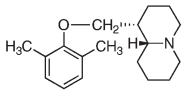
Chart 2. Structures of Some Antiarrhythmic Quinolizidine Derivatives Previously Studied



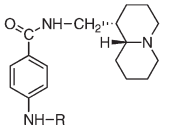
I **a**: R = H (EC_{50} = 5.71 μM)
b: R = NO_2 (inactive)
c: R = NH-SO_2-CH_3 (inactive)



II (EC_{50} = 4.81 μM)



III (EC_{50} = 0.46 μM)
epi-**III** (moderate activity at 10 μM)



IV **a**: R = H (moderate activity at 10 μM)
b: R = $COCH_3$ (EC_{50} = 0.47 μM)

Chemistry

The known compounds **1**−**3** and **5**−**11** were prepared as previously described,[6−11] while the novel compounds **4** and **12**−**15** were obtained as indicated in Scheme 1−3.

Compound **4** was obtained by reacting *N*-(chloroacetyl)-2,6-dimethyl aniline with thiolupinine [(1*R*,9a*R*)octahydro-2*H*-quinolizin-1-ylmethanethiol], which was prepared as indicated by Novelli and Sparatore[13] (Scheme 1).

Amine **12** was prepared by reacting 2,6-dimethylaniline with 1-bromolupinane [(1*R*,9a*R*)-1-bromomethyl-octahydro-2*H*-quinolizine], obtained from lupinine and phosphorus tribromide,[14] while the homolupinyl anilines **13** and **14** were obtained by $LiAlH_4$ reduction of the corresponding anilides **Ia** and **1**, previously obtained from the suitable anilines and homolupinanoyl chloride hydrochloride [(1*S*,9a*R*)(octahydro-2*H*-quinolizin-1-yl)ethanoyl chloride] (Scheme 2).[15]

It is worth noting that the analogous reduction of the anilide **4**, instead of the expected amino compound **15**, gave origin to the cleavage of C−S bond with the formation of the thiolupinine, isolated as lupinyldisulfide (Scheme 3). Therefore, to obtain the amino compound **15**, thiolupinine was reacted with excess of 1,2-dichloroethane in the presence of 1,5-diazabicyclo-[4.3.0]non-5-ene (DBN), and the *S*-(2-chloroethyl)thiolupinine was finally reacted with 2,6-dimethylaniline (Scheme 3).

The structures of all final and intermediate compounds were supported by elemental analyses and ^{1}H NMR spectral data.

Pharmacology

Compounds **1**−**15** were tested for antiarrhythmic activity on isolated gp left atria driven at 1 Hz, on isolated gp driven left and spontaneously beating right atria to evaluate their inotropic and chronotropic effects, respectively, and on K^+-depolarized guinea pig aortic strips to assess calcium antagonist activity (as expression of vasorelaxant activity).

In particular, the antiarrhythmic activity was evaluated inducing arrhythmias by application of sinusoidal alternating current (50 Hz) of increasing strength to the isolated left atria driven at 1 Hz and assessing the "threshold of ac-arrhythmia" (the current strength at which extra beats occur) before and following the compound was added to the tissue bath. Because arrhythmias by alternating current are mainly due to an increase in Na^+ conductance in cardiomyocytes, the method is particularly suitable to study antiarrhythmic agents acting as Na^+ channel inhibitors (class I).[16] Moreover, this model avoids the damage, toxicity, and drug−drug interactions caused by other chemical methods used to induce arrhythmias.[17]

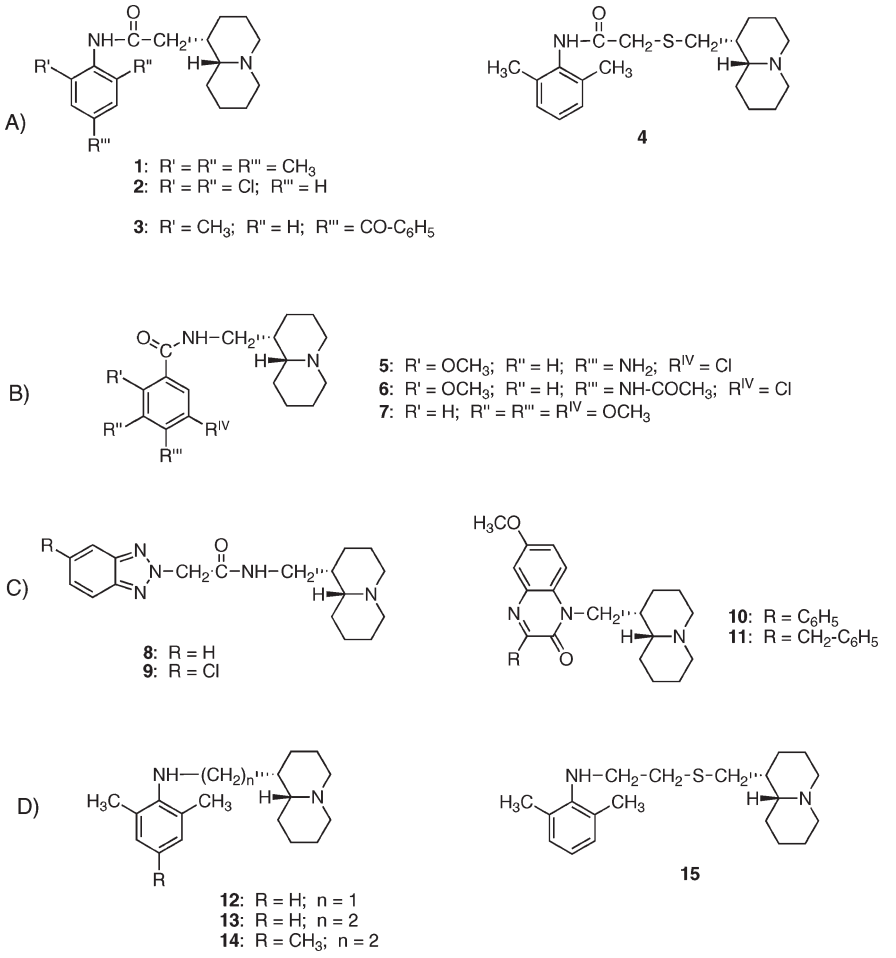
The Langendorff-perfused guinea pig heart was used to assay the whole cardiac activity of compounds **3**, **4**, **6**, **13**, **15**, and of the reference compounds amiodarone and quinidine. Compounds were checked at increasing doses to evaluate changes in heart rate (HR) (chronotropic activity) and in ECG signals: PR, QRS, QT.

For compound **6**, the functional activity was extended at guinea pig ileum longitudinal smooth muscle (GPILSM) and the activity at muscarinic receptor subtypes was determined by the use of the muscarinic M_2 receptor-mediated negative inotropism in guinea pig driven (1 Hz) left atria.

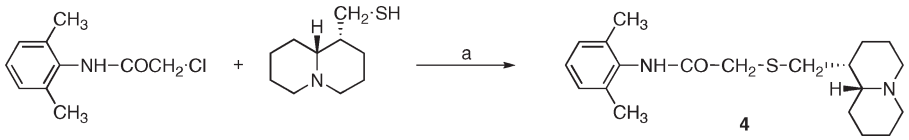
All these methods have been described in detail earlier.[6,16−18] Relevant details are found in the Supporting Information.

Data were analyzed using Student's *t*-test and are presented as mean ± SEM.[19] Because the drugs were added in cumulative manner, only the significance ($P < 0.05$) between

Chart 3. Structures of Quinolizidine Derivatives Tested for Antiarrhythmic Activity

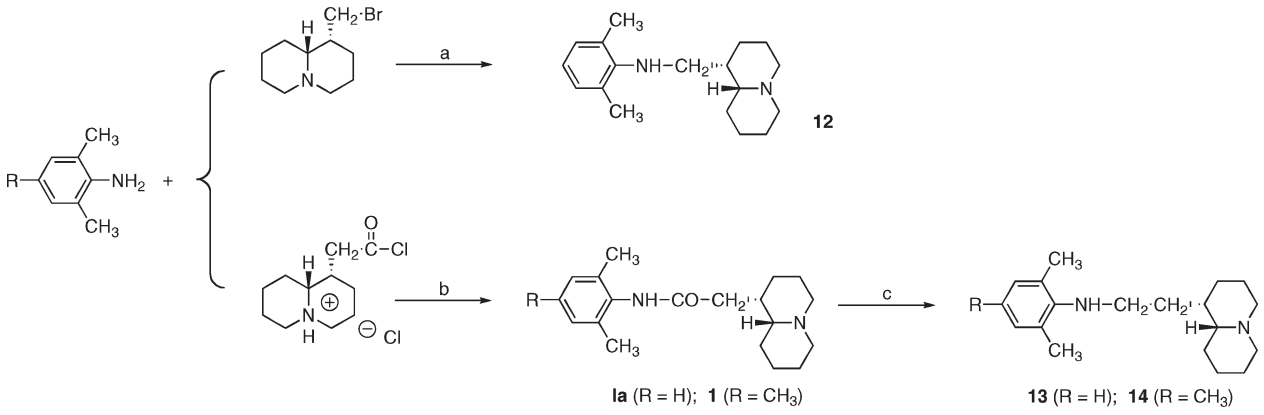


Scheme 1[a]



[a] Reagents and conditions: (a) abs EtOH, N_2, Δ 8 h.

Scheme 2[a]

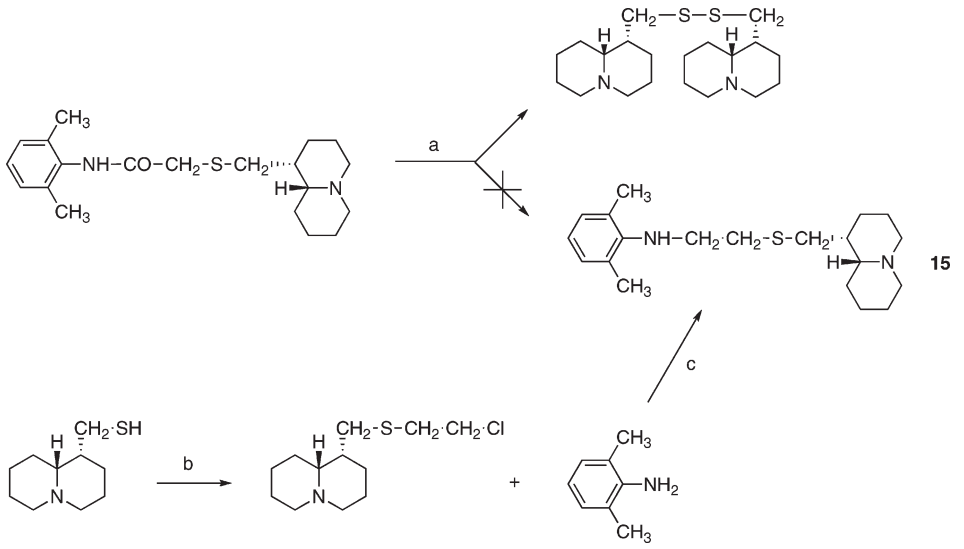


[a] Reagents and conditions: (a) neat, in pressure tube, 110 °C, 36 h; (b) $CHCl_3$, reflux 4 h; (c) $LiAlH_4$, THF, N_2, reflux 24 h.

the control and the experimental values at each concentration was indicated by a letter.

The potency of drugs defined as EC_{50}, EC_{30}, and IC_{50} or pD_2 was evaluated from log concentration−response curves

Scheme 3[a]



[a] Reagents and conditions: (a) $LiAlH_4$, THF, N_2, reflux; (b) Cl-CH_2-CH_2-Cl, DBN, rt 36 h; (c) net, in pressure tube, 110 °C, 72 h.

(Probit analysis using Litchfield and Wilcoxon[20] or GraphPad Prism software[21]) in the appropriate pharmacological preparations.

Results and Discussion

Compounds **1**−**15** reported in Chart 3, together with amiodarone, lidocaine, procainamide, and quinidine as reference drugs, were evaluated in vitro for antiarrhythmic activity on isolated gp 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.

All tested compounds produced from moderate to high increase of the threshold of ac-arrhythmia, and most of them, at a concentration of 50 μM or even lower, were more active than lidocaine and procainamide and comparable or superior to quinidine. Indeed, seven compounds exhibit the maximal activity (26−129%) at concentration of 0.5, 1, 5, and 10 μM, which are respectively, 100, 50, 10, and 5 times lower than the concentration at which lidocaine and quinidine exhibit their maximal activity (34% and 69%). Activity of compound **13**, a *N*-(quinolizidinylalkyl)aniline derivative, was still growing at the maximal tested concentration of 100 μM, at which it produced a 208% increase of threshold of ac-arrhythmia, that is 3-fold that of quinidine.

Moreover, for compounds exhibiting an increase of the threshold higher than 50%, the EC_{50} values were calculated, which indicated that most compounds were not only more efficacious but also more potent than reference drugs. Compounds **3**, **6**, **15**, **13**, and **4**, in decreasing order of potency, exhibited EC_{50} in the range from 0.15 to 0.59 μM and were, therefore, from 68.4 to 17.4 times more potent than quinidine (EC_{50} = 10.26 μM).

It is underlined that antiarrhythmic activity has been observed in all subsets of quinolizidinyl derivatives presently and previously studied, despite the wide structural diversity of the aromatic moieties to which the quinolizidine ring is joined, further supporting the importance of this structural feature for such activity.

In all tested compounds, the substituted methylene is linked axially to the quinolizidine ring, as it is in natural (−)-lupinine from which thay are derived. We consider this disposition as more suitable than the epimeric one (corresponding to (+)-*epi*-lupinine) for the expression of the antiarrhythmic activity.

Indeed, in a preliminary study,[7] the epimer of the most potent compound **3**, resulted devoid of any protective activity against the $CHCl_3$ induced arrhythmia, which was used as a screening test. Moreover, as it was recently shown,[6] the lupinyl ether III

Table 1. Anti-arrhythmic Activity of Compounds **1**−**15**

compd	max % increase of threshold of ac-arrhythmia after pretreatment with compounds[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
1	49 ± 0.4		
2	32 ± 1.3		
3	64 ± 3.2	0.15	0.088−0.21
4	67 ± 1.8	0.59	0.43−0.82
5	113 ± 8.6	6.73	2.48−9.23
6	84 ± 3.9[d]	0.24	0.16−0.34
7	56 ± 3.5[d]	2.62	1.71−3.39
8	26 ± 1.4[e]		
9	28 ± 0.2[f]		
10	30 ± 2.1		
11	14 ± 1.1[g]		
12	28 ± 1.5[d]		
13	208 ± 10.3[c]	0.46	0.33−0.66
14	63 ± 2.4[d]	8.15	7.23−8.63
15	129 ± 2.3[d]	0.31	0.25−0.86
Ia[h]	78 ± 2.5	5.71	4.38−7.46
II[h]	55 ± 3.0	4.81	4.38−5.27
III[h]	176 ± 10.2[d]	0.46	0.30−0.69
IVa[h]	28 ± 1.7[d]		
IVb[h]	92 ± 3.7	0.47	0.29−0.76

[a] Increase of threshold of ac-arrhythmia: increase in the current strength of 50 Hz alternating current required to produce arrhythmia in guinea pig left atria driven at 1 Hz in the presence of each tested compounds at 5×10^{-5} M. For all data $P < 0.05$. [b] Calculated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[20] 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^{-7} M. [f] At 5×10^{-6} M. [g] At 10^{-6} M. [h] Data from ref 6.

Table 2. Influences of Tested Compounds on Cardiovascular Parameters

compd	% 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[f]			5.57	4.93−6.02	3 ± 0.1
lidocaine	88 ± 3.0[g]	29 ± 0.9[f,h,i]	0.017	0.012−0.024			14 ± 0.9[f,j]
procaine	92 ± 1.4[k]	9 ± 0.6[i]	0.014	0.011−0.017			3 ± 0.2
quinidine	71 ± 3.6	86 ± 0.5[f,j]	3.38	2.69−4.25	3.99	3.81−4.06	30 ± 1.6[f,j]
1	54 ± 1.3[l]	6 ± 0.4[j]	0.010	0.0082−0.013			2 ± 0.1
2	93 ± 2.4	15 ± 0.9[f,j]	0.10	0.06−0.18			6 ± 0.3
3	79 ± 3.2[m]	59 ± 1.7[f]	0.044	0.031−0.062	6.14	5.89−6.29	30 ± 2.6[f]
4	48 ± 1.1[l]	30 ± 1.5[f]					18 ± 0.9[f]
5	95 ± 2.1	22 ± 1.3[f,j]	0.35	0.13−0.51			6 ± 0.5
6	93 ± 2.7[h]	6 ± 0.3	0.018	0.012−0.029			4 ± 0.1
7	79 ± 1.5[k]	11 ± 0.6[f,i]	0.017	0.013−0.024			7 ± 0.3
8	98 ± 2.0	34 ± 1.6[f]	0.035	0.025−0.048			4 ± 0.2
9	92 ± 1.6[m]	27 ± 1.3[f]	0.074	0.051−0.15			26 ± 1.3[f]
10	85 ± 2.7[m]	69 ± 3.4[f,j]	0.031	0.028−0.042	15.29	12.05−17.28	11 ± 0.4[f]
11	86 ± 3.5[h]	62 ± 1.7[f,m]	0.11	0.086−0.15	2.75	2.43−3.02	18 ± 0.9[f,j]
12	75 ± 3.1[g]	40 ± 0.3[f,m]	0.023	0.017−0.030			27 ± 0.5[f,j]
13	89 ± 3.9	72 ± 2.7[f,j]	0.34	0.19−0.60	6.89	6.02−7.03	28 ± 2.4[f,j]
14	89 ± 2.2[m]	44 ± 2.1[f,j]	0.034	0.025−0.046			37 ± 2.6[f]
15	37 ± 1.4[m]	62 ± 2.2[f,m]			8.42	7.68−9.23	34 ± 1.6[f]
Ia[n]	95 ± 1.2	8 ± 0.1[g,i]	0.62	0.42−0.94			5 ± 0.1
II[n]	88 ± 2.6	70 ± 1.7[f,k]	0.76	0.51−1.12	3.98	3.15−4.42	31 ± 0.3[f]
III[n]	25 ± 1.9[o]	19 ± 0.8[m]					26 ± 1.4[f]
Iva[n]	95 ± 3.8[e]	13 ± 0.8[i]	0.46	0.32−0.71			3 ± 0.2
IVb[n]	86 ± 0.8[m]	14 ± 1.4[k]	0.059	0.041−0.087			3 ± 0.2

[a] Activity: decrease in developed tension in isolated guinea-pig left atrium at 5×10^{-5} M, expressed as percentage change from the control ($n = 4-6$). For all data $P < 0.05$. The left atria were driven at 1 Hz. [b] Activity: decrease in 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. [c] Calculated from log concentration−response curve (Probit analysis according to Litchfield and Wilcoxon[20] 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 strip at 10^{-4} M. The 10^{-4} M concentration gave the maximum effect for most compounds. [e] At 10^{-4} M. [f] $P < 0.05$. [g] At 10^{-6} M. [h] At 5×10^{-6} M. [i] Positive chronotropic effect. [j] At 5×10^{-5} M. [k] At 5×10^{-7} M. [l] At 10^{-7} M. [m] At 10^{-5} M. [n] Data from ref 6. [o] At 5×10^{-8} M.

(Chart 2) was more active and potent than the corresponding *epi*-lupinyl ether in increasing the threshold of ac-arrhythmia.

To define completely the role of stereochemistry of quinolizidine derivatives in the antiarrhythmic effects, the activities of the other enantiomers corresponding to (+)-lupinine and (−)-*epi*-lupinine should be also evaluated; however these starting materials are not easily available.

Compounds **1**−**4** are closely related to the homolupinanoylanilids of the previous study,[6] exemplified by compounds **Ia**−**c** and **II** of Chart 2, and in comparing their activities, some interesting structure−activity relationships can be drawn. The introduction of a substituents in position para to the amino group of **Ia** has different effects depending on its nature: the introduction of a bulky benzoyl moiety (**II**) has only minor effects on both potency and activity, while the latter is reduced by the introduction of a simple methyl group (**1**) and is abolished by that of a nitro or mesylamino group (**Ib**, **Ic**).[6]

Even more important is the effect of replacing the *N*-homolupinanoyl residue of compound **Ia** (Chart 2) with a lupinylthioacetyl moiety (compound **4**) that, while leaving almost unchanged the activity, strongly enhanced (27 times) the potency. Probably this effect is related to the increased distance of the basic nitrogen from the aromatic ring, as it will be discussed later.

A pronounced enhancement of potency (32-fold) and activity is observed by exchanging an hydrogen atom for one of the two methyl group of compound **II** to give compound **3**. Although the number of atoms interposed between the basic nitrogen and the aromatic nucleus has remained unchanged, the lower steric hindrance in compound **3** might allow a more appropriate spatial arrangement of the basic side chain in respect to the aromatic moiety. Compound **3** appears of potential therapeutic interest in the prophylaxis of thromboembolic stroke and in the treatment of postinfarctual patients, embodying simultaneously a very potent antiarrhythmic activity and the ability to strongly inhibit the arachidonic acid-induced platelet aggregation,[7] even if the ADP-induced aggregation was only moderately affected. It is worth noting that classic antiarrhythmic drugs, as quinidine and lidocaine, are devoid of any antiaggregating activity.[22]

Finally, it is observed that the exchange of methyl groups of compound **Ia** with two chlorine atoms (**2**) markedly reduced the activity.

Compounds **5**−**7** are related to the previously studied *N*-lupinylbenzamides, exemplified by compound **IVa**−**b** (Chart 2). The introduction of chlorine and methoxyl group in the benzene ring of **IVa** and **IVb** clearly increased the potency of each of them, yet maintaining the higher potency of the 4-acetylamino- (**6**) compared to the corresponding nonacetylated derivative (**5**).

Compound **5** was already studied in the past[8] as a lupinyl analogue of metoclopramide (2-methoxy-5-chloroprocainamide), a drug largely used as antiemetic and gastroprokinetic and whose activities are related to the overlapping of D_2 and 5-HT_3 antagonistic and 5-HT_4 agonistic effects. Compounds **5** enhanced intestinal transit rate (charcoal bolus progression test) two times more than metoclopramide, but its activity should rely on a more complex mechanism because it

exhibited a quite poor affinity to D_2 and 5-HT_3 receptors and only a moderate relaxing activity on previously contracted rat esophageal tunica muscolaris mucosae (a 5-HT_4 activation effect).[23]

Despite a wide distribution in CNS, gastroenteric apparatus, and other peripheral structures, 5-HT_4 receptors have a sole cardiac localization on atria and the increase of the Ca^{2+} and pacemaker currents in the atrium are mediated through activation of these receptors.[24−27]

Cisapride, a gastro-prokinetic agent and 5-HT_4 agonist more potent than metoclopramide, has been withdrawn from the market because of its potential to induce serious and occasionally fatal cardiac arrhythmias (QT interval prolongation),[28] while metoclopramide, maybe for the weaker 5-HT_4 partial agonism, even in large endovenous doses, did not show significant effect on intracardiac conduction and certain other hemodynamic parameters.[29] According to Marmo et al.,[30] metoclopramide even displayed antiarrhythmic activity in experimental animals, which was evaluated as quali-/quantitatively similar to that of procainamide. In our experimental conditions, compounds **5** and **6** resulted manyfold more active and potent than procainamide and than its lupinyl analogue **IVa**.

Such a large increase in antiarrhythmic potency with a negligeable variation of QT interval (see further) might be related to an even weaker partial agonism of our compounds, or to a shift to a frank antagonism for 5-HT_4 receptor, as a consequence of the introduction of the rigid quinolizidinyl framework in place of the flexible basic chain of metoclopramide. Indeed, a shift from agonism to antagonism among 5-HT_4 receptor ligands has been observed even after modest structural variation,[31,32] and on the other hand, 5-HT_4 antagonists have been suggested as a valuable therapeutic alternative for the treatment of atrial fibrillation (without depressive effects on the ventricle) and for thromboembolic stroke.[32] Therefore, further investigation on compound **6**, concerning its binding affinity to 5-HT_4 receptor and its agonistic or antagonistic profile, are warranted.

It is worth noting that both metoclopramide and its lupinyl analogue **5** inhibit platelet aggregation induced by ADP and PAF, with **5** being more potent than the former; however, only metoclopramide was able to inhibit arachidonic acid-induced aggregation.[8] Considering the importance of a simultaneous antiaggregating activity, it will be interesting to assess how the acetylation of the amino group of **5** (to give **6**) modifies the antiaggregating properties of the latter, besides strongly increasing (28-fold) its antiarrhythmic potency.

The third compound of this subset (**7**) was prepared long time ago[7] and found to exert long lasting antihypertensive activity in the rabbit,[33] probably due to peripheral adrenergic antagonism. At present, compound **7** displayed high activity against ac-arrhythmia with $EC_{50} = 2.62\,\mu M$, therefore clearly superior to quinidine.

The quinolizidinyl derivatives of heteroaromatic ring systems **8**−**11** were previously prepared, together with many other analogues, which were found endowed either with local anesthetic (benzotriazoles[10]) or analgesic activity (quinoxalinones[34]), both of high intensity and long lasting.

The four compounds displayed an antiarrhythmic activity that is higher than that of procainamide and, for three of them (**8**−**10**), proximal to that of lidocaine. Such activity might be related to the previously shown local anesthetic activity and hence to Na^+ channel block for the benzotriazole derivative **8** and **9** and to the strong negative chronotropic action in the case of quinoxalinone **10** and **11** (see further).

The 3-benzylquinoxalinone **11**, despite its lowest activity against the ac-arrhythmia among the whole set of compounds studied, is considered interesting for the simultaneous high inhibitory action against the arachidonic acid-induced platelet aggregation, which is comparable to that observed for compound **3**. The 3-phenylquinoxalinone **10**, as well as the benzotriazoles **8** and **9**, were devoid of any antiaggregating activity (unpublished observations).

The compounds of the last subset **12**−**15**, characterized by the presence of two basic nitrogen atoms (even if of quite different basicity), exhibit increasing activity and potency against ac-arrhythmia with the increase of the length of the linker between quinolizidine and benzene rings.

The positive effect of chain elongantion was already pointed out when the activities of the homolupinanoyl anilide **Ia** (Chart 2) and of lupinylthioacetylanilide **4** were compared.

Amino compounds **13**, **14**, and **15** correspond, respectively, to the anilides **Ia**, **1**, and **4**, and it is observed that the exchange of a methylene for the carbonyl group increases, even if in different measures, the antiarrhythmic potency (up to 12 times for **13** in comparison to the anilide **Ia**).

The introduction of a third methyl group on the benzene ring of compound **13**, giving rise to compound **14**, produces a 17.7-fold decrease of potency, confirming the activity lowering effect observed for compound **1** in respect to **Ia**.

Finally, 2,6-dimethyl-*N*-lupinyl aniline (**12**) exhibits a quite lower activity and potency in respect to 2,6-dimethyl-*O*-lupinylphenol (**III**; Chart 2) previously studied, thus indicating that both the length and the nature (NH in place of O) of the linker are determinants of the antiarrhythmic activity. Therefore, it is expected that the increase of the linker length in the already very potent lupinyl ether **III**, previously studied,[6] will produce an interesting further enhancement of the antiarrhythmic action.

To better define the pharmacological profile of the investigated antiarrhythmic agents **1**−**15**, we compared their influence on additional cardiovascular parameters with that elicited by the four reference compounds (Table 2).

With the exception of compound **4** and **15**, all compounds strongly decreased the developed tension on driven left atria with EC_{50} in the range 0.010−0.34 μM; the most potent, in decreasing order, were compounds **1**, **7**, **6**, and **12**, whose EC_{50} values (0.010−0.023 μM) were yet comparable with that of lidocaine (0.017 μM).

Only a weak negative inotropic activity was observed for compound **15** that decreased the developed tension for 37% at 10 μM concentration, thus resulting less depressive that quinidine ($EC_{50} = 3.38\,\mu M$).

It is important to observe that even small structural modifications can affect the negative inotropic activity and that these effects may be either in the same direction or to the opposite of those exerted on the antiarrhythmic activity, discussed above.

Thus the suppression of one methyl group of **II** (Chart 2) to give **3** strongly increased both the antiarrhythmic (32-fold) and the negative inotropic potency (17-fold). Similarly, the acetylation of the primary amino group of **IVa** and **5**, to give **IVb** and **6** respectively, besides increasing the antiarrhythmic activity enhanced 7.8 and 19.4 times, respectively, the negative inotropic action.

On the other hand, the introduction of a methyl in position para to the amino group of **Ia** and **13** to have **1** and **14**, while reducing the antiarrhythmic activity, enhanced 62 and 10 times, respectively, the negative inotropic effect.

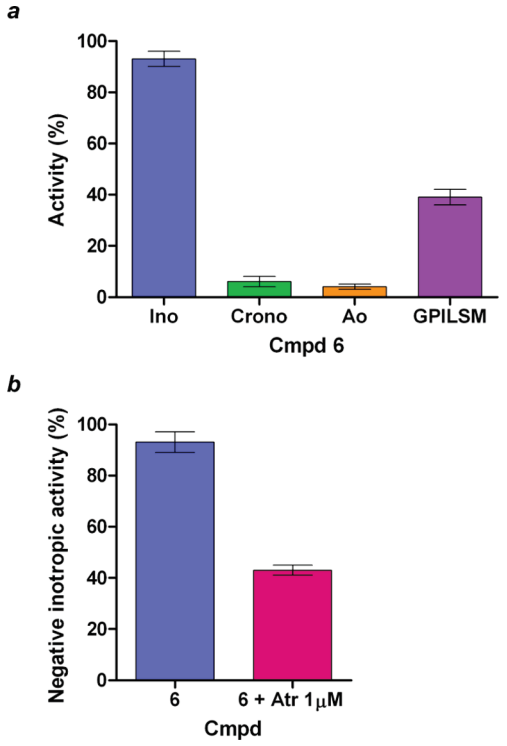


Figure 1. (a) Intrinsic activity of **6** on driven left atria (Ino) and spontaneously beating right atria (Chrono), calcium antagonist activity on K^+ depolarized aortic strips (Ao) and ileum longitudinal smooth muscle (GPLSM). (b) Muscarinic agonist activity (α = 0.93) of **6** alone and in presence of 1 μM atropine (Atr.).

The potentiating effect of linker elongation on the inhibition of ac-arrhythmia, observed in compounds **4** and **15**, was associated with an interesting reduction of negative inotropism.

The negative chronotropic activity (Table 2) detected on spontaneously beating right atria was generally minor compared to that of quinidine and amiodarone; only compound **11** exhibited a EC_{30} value (2.75 μM) lower than quinidine (EC_{30} = 3.99 μM). *N*-Lupinyl-3,4,5-trimethoxybenzamide **7** showed a weak positive chronotropic activity as observed for lidocaine and procainamide.

Also the vasorelaxant activity (Table 2), as expressed by the inhibition of calcium induced contraction on K^+-depolarized (80 mM) guinea pig aorta strips, was rather modest. Most compounds showed inhibition values in the range 11−37% at 50−100 μM, similarly to lidocaine and quinidine, while compounds **1**, **2**, and **5**−**8** were practically inactive, as were amiodarone and procainamide.

The observed negative inotropic activity of these compounds may be related to interaction with cholinergic system. Indeed, several quinolizidine alkaloids were shown to bind to muscarinic receptor,[35] as did, even more potently, a number of synthetic quinolizidine derivatives, prepared by some of us.[7,8,36]

Compound **6** has intrinsic negative inotropic activity [93 ± 2 at 5 × 10^{-6} M concentration (α = 0.93)]. This intrinsic activity does not involve calcium channels; in fact, **6** did not affect contraction either on guinea pig aorta strips and on guinea pig longitudinal smooth muscle from ileum (GPILSM) depolarized with potassium 80 mM (Figure 1a). This selective negative inotropic activity was inhibited by atropine (Figure 1b) as well as for carbachol (CCh), a known muscarinic agonist.

Furthermore the onset of **6** was different in comparison to CCh because the maximum effect elicited by each concentration of compound was reached after 30 min of incubation. The delay in the onset could be explained whith a modulation of the G protein coupled to the muscarinic receptor.[18] Of course, additional in-depth investigations are needed to clarify the observed muscarinic agonistic behavior but such studies are beyond the scope of the present work.

For the most interesting compounds, **3**, **4**, **6**, **13**, and **15**, the cardiovascular profile was extended to the Langendorff retrogradely perfused guinea pig spontaneously beating heart to detect the influence on ECG parameters.[6]

The data are reported in Table 3, compared to those of the reference drugs amiodarone and quinidine. None of the compounds, even tested at concentrations that are from 17 to 67 times higher than the corresponding EC_{50} values for antiarrhythmic activity, show effects on the parameters considered worthy of note, in line with what has been shown in the set of compounds previously described.[6]

Conclusions

Fifteen quinolizidine derivatives, largely differing for the aromatic moieties to which the basic bicycle is linked, have been tested for antiarrhythmic, inotropic, and chronotropic effects on isolated guinea pig heart tissues and to assess calcium antagonist activity, in comparison with amiodarone, lidocaine, procainamide, and quinidine.

Even if with different degrees of potency, antiarrhythmic activity has been observed in all the studied subsets of quinolizidine derivatives, as previously found with other subsets of derivatives, supporting the existence of a rather peculiar interaction between the rigid and bulky quinolizidine ring and the biological structures involved in the regulation of heart activity.

Compounds **3**, **4**, **6**, **13**, and **15** resulted in being endowed with very high antiarrhythmic activity, exhibiting EC_{50} values in the range from 0.15 to 0.59 μM, thus comparing favorably with the four reference drugs, the most potent of which, quinidine, exhibited EC_{50} = 10.3 μM.

The five most active compounds were studied on spontaneously beating Langendorff-perfused gp heart; even at concentrations 17−67 times higher than the corresponding EC_{50} for antiarrhythmic activity, they prolonged the QT intervals only moderately (from 9.6 to 11.4%), comparing favorably with amiodarone (13%) and quinidine (45).

Compound **3**, embodying simultaneously a very potent antiarrhythmic activity and the ability to strongly inhibit the arachidonic acid-induced platelet aggregation might be of potential therapeutic interest in the prophilaxis of thromboembolic stroke and in the treatment of postinfarctual patients.

Interestingly, compound **3** resulted as being well tolerated by rats and mice when administered at the dose of 100 mg/kg, either po or ip, with only a slight decrease in limb tone.[7] At the same dosage ip in mice, lidocaine resulted in being quite toxic (LD_{50} = 105 mg/kg).[37]

Compound **15** exhibits an interesting cardiovascular profile, being endowed with high antiarrhythmic activity and potency with only modest negative inotropic and chronotropic effects and vasorelaxant activity.

Both compounds **3** and **15** deserve further investigation to define more completely their pharmacological profile and mechanisms of action, which will allow the design and

Table 3. Influence of **3**, **4**, **6**, **13**, and **15** on ECG Parameters Evaluated in Spontaneously Beating Langendorff-Perfused Guinea Pig Heart in Comparison with The Reference Compounds Quinidine and Amiodarone

		beat/min (HR) or interval time (ms): mean ± SEM (n = 4−5)					
compd	parameters	basal	10^{-9}M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
amiodarone[a]	HR[b]	187 ± 11	187 ± 15	180 ± 13	171 ± 12	164 ± 9[c]	132 ± 8[c]
	PR[d]	55 ± 4	54 ± 3	55 ± 5	55 ± 2	56 ± 6	58 ± 7
	QRS[e]	20 ± 1.5	20 ± 1.7	20 ± 1.9	21 ± 2	22 ± 2.1	24 ± 1.8
	QT[f]	161 ± 8	161 ± 7	161 ± 11	169 ± 9	174 ± 10	182 ± 7[c]
quinidine[a]	HR[b]	165 ± 13	160 ± 10	153 ± 11	140 ± 10[c]	123 ± 12[c]	107 ± 9[c]
	PR[d]	52 ± 2	53 ± 4	54 ± 2	57 ± 1[c]	60 ± 3[c]	70 ± 4[c]
	QRS[e]	24 ± 1	25 ± 2	27 ± 1	31 ± 2[c]	35 ± 1[c]	39 ± 4[c]
	QT[f]	158 ± 5	158 ± 5	170 ± 8	182 ± 10[c]	200 ± 11[c]	229 ± 15[c]
3	HR[b]	175 ± 5	177 ± 3	171 ± 3	158 ± 5[c]	145 ± 2[c]	137 ± 2[c]
	PR[d]	54 ± 1	54 ± 3	54 ± 2	53 ± 1	56 ± 3	57 ± 1
	QRS[e]	22 ± 2	23 ± 1	20 ± 1	23 ± 2	22 ± 1	25 ± 2
	QT[f]	155 ± 2	158 ± 3	154 ± 3	155 ± 1	162 ± 3[c]	170 ± 1[c]
4	HR[b]	180 ± 5	181 ± 3	175 ± 2	171 ± 3[c]	166 ± 4[c]	156 ± 3[c]
	PR[d]	50 ± 2	52 ± 1	51 ± 1	53 ± 2	55 ± 3	60 ± 2[c]
	QRS[e]	22 ± 2	22 ± 1	22 ± 1	24 ± 2	24 ± 2	23 ± 1
	QT[f]	152 ± 2	150 ± 3	155 ± 1	157 ± 1	164 ± 4[c]	168 ± 4[c]
6	HR[b]	177 ± 6	172 ± 4	170 ± 3	168 ± 4	167 ± 2	165 ± 5
	PR[d]	52 ± 4	52 ± 3	54 ± 2	53 ± 4	56 ± 3	56 ± 3
	QRS[e]	20 ± 1	20 ± 2	20 ± 2	21 ± 2	22 ± 1	22 ± 1
	QT[f]	140 ± 8	139 ± 4	141 ± 4	150 ± 5	152 ± 4[c]	155 ± 6[c]
13	HR[b]	186 ± 6	182 ± 3	171 ± 4	163 ± 3[c]	142 ± 5[c]	136 ± 2[c]
	PR[d]	54 ± 3	53 ± 2	56 ± 1	57 ± 4	58 ± 2	59 ± 5
	QRS[e]	19 ± 1	19 ± 1	19 ± 2	20 ± 2	20 ± 2	21 ± 3
	QT[f]	155 ± 6	154 ± 5	157 ± 6	160 ± 5	170 ± 4[c]	175 ± 2[c]
15	HR[b]	190 ± 5	186 ± 4	185 ± 5	179 ± 3[c]	158 ± 2[c]	142 ± 3[c]
	PR[d]	55 ± 3	55 ± 1	55 ± 2	55 ± 2	57 ± 4	60 ± 5
	QRS[e]	23 ± 2	23 ± 3	23 ± 2	24 ± 1	25 ± 1	26 ± 2
	QT[f]	157 ± 3	160 ± 5	162 ± 4	168 ± 2[c]	170 ± 6[c]	175 ± 5[c]

[a] Data from ref 6. [b] HR: heart rate calculated from RR interval on ECG signal. [c] $P < 0.05$. [d] PR: atrioventricular conduction time (ms). [e] QRS: intraventricular conduction time (ms). [f] QT: the duration of the ventricular action potential (ms).

development of ever more active and potent antiarrhythmic agents.

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 $CDCl_3$ on Varian Gemini 200 spectrometer; chemical shifts (δ) are reported in ppm from internal Me_4Si; values of the coupling constants (J) are reported in Hz; Lup = lupinyl (octahydroquinolizin-1-yl-methyl) residue, Q = octahydroquinolizine ring. Column chromatography (CC) was effected by using silica gel 60 (Merck) or basic alumina (Across). Elemental analyses were performed on Carlo Erba EA 1110 CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmaceutical Sciences of Genoa University. The analytical results are within ±0.3% of calculated values. The results of NMR spectra and elemental analyses indicated that the purity of all compounds was higher than 95%.

***N*-(2,6-Dimethylphenyl)-2-[(1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1-yl)methylthio]acetamide (4).** To a solution of *N*-(chloroacetyl)-2,6-dimethylaniline[38] (0.65 g, 3.3 mmol) in abs EtOH (15 mL), a solution of thiolupinine[13] (0.61 g, 3.3 mmol) in abs EtOH (5 mL) was added. The mixture was heated to reflux for 8 h under nitrogen. After evaporation of solvent, the residue was taken up in diluted hydrochloric acid and washed with ether to remove the unreacted amide.

The acidic phase was alkalinized and extracted with ether. After drying over Na_2SO_4, the solvent was evaporated and the residue was crystallized with dry ether (0.87 g; yield = 76%); mp 106−107 °C. ^{1}H NMR ($CDCl_3$): δ 1.03−2.06 (m, 14H of Lup), 2.18 (s, 6H, $2CH_3$), 2.64−2.98 (m, 2H, Hα near N of Q + 2H, S-CH_2-Q), 3.21−3.45 (m, 2H, C(O)-CH_2-S), 6.94−7.14 (m, 3H, aromatic protons), 8.23 (s, 1H, CONH, collapses with D_2O). Anal. Calcd for $C_{20}H_{30}N_2OS$: C, 69.22; H, 8.73; N, 8.08; S, 9.25. Found: C, 68.99; H, 8.74; N, 8.10; S, 9.25.

***N*-[(1*S*,9a*R*)(Octahydro-2*H*-quinolizin-1-yl)methyl]-2,6-dimethylaniline (12).** A mixture of bromolupinane[14] (0.58 g, 2.5 mmol) and 2,6-dimethylaniline (0.31 mL, 2.5 mmol) was introduced into an Aldrich pressure tube flushed with nitrogen. The sealed tube was heated at 110 °C for 36 h. The residue was treated with 0.1 N HCl and the acidic phase (pH = 3) was extracted with ether to remove the unreacted 2,6-dimethylaniline. The aqueous phase was alkalinized and entracted with CH_2Cl_2. After drying (Na_2SO_4), the solvent was evaporated and the residue was chromatographed on alumina (1:25) eluting with dry ether. Thus 0.38 g (yield = 56%) of 12 were obtained. Oil. ^{1}H NMR ($CDCl_3$): δ 1.03−2.18 (m, 15H of Lup), 2.24 (s, 6H, $2CH_3$), 2.69−2.87 (m, 2H, Hα near N of Q), 3.03−3.22 (m, 2H, CH_2−NH), 6.68−7.02 (m, 3H, aromatic protons). Anal. Calcd for $C_{18}H_{28}N_2$: C, 79.36; H, 10.36; N, 10.28; S. Found: C, 79.06; H, 10.50; N, 10.58.

***N*-[2-(1*S*,9a*R*)(Octahydro-2*H*-quinolizin-1-yl)ethyl]-2,6-dimethylaniline (13).** A solution of anilide **Ia**[6] (0.41 g, 1.36 mmol) in dry THF (20 mL) was added dropwise to a suspension of powdered $LiAlH_4$ (0.31 g, 8.17 mmol) in dry THF (10 mL) cooled to 0 °C by ice−water bath. The reaction mixture was allowed to

warm to ambient temperature, refluxed under nitrogen for 24 h, and then quenched with sequential addition of H_2O (1 mL), NaOH 6N (1.5 mL), and H_2O (1 mL). The precipitate was filtered and thoroughly washed with THF.

After evaporation to dryness, the residue was taken up in dry ether and filtered to remove the insoluble part. The organic solution was evaporated and the oily residue was chromatographed on alumina (1:30) eluting with dry ether. Thus 180 mg of **13** (yield = 46%) were obtained.

Data for free base: oil. ^{1}H NMR ($CDCl_3$): δ 1.16−2.14 (m, 16H of Lup), 2.31 (s, 6H, $2CH_3$), 2.79−3.14 (m, 2H, Hα near N of Q + 2H, CH_2-NH + 1H, NH, collapses with D_2O), 6.75−7.05 (m, 3H, aromatic protons).

Data for hydrochloride: mp 184−186 °C. Anal. Calcd for $C_{19}H_{30}N_2 \cdot HCl$: C, 70.67; H, 9.68; N, 8.67; S. Found: C, 70.77; H, 9.42; N, 8.65.

***N*-[2-(1*S*,9a*R*)(Octahydro-2*H*-quinolizin-1-yl)ethyl]-2,4,6-trimethylaniline (14).** A solution of anilide **1**[6] (0.4 g, 1.27 mmol) in dry THF (20 mL) was added dropwise to a suspension of powdered $LiAlH_4$ (0.33 g, 8.7 mmol) in dry THF (15 mL) cooled to 0 °C by ice−water bath. The reaction mixture was allowed to warm to ambient temperature, refluxed under nitrogen for 24 h, and then quenched with sequential addition of H_2O (1 mL), NaOH 6N (1.5 mL), and H_2O (1 mL). The mixture was filtered, and the inorganic residue was thoroughly washed with THF.

The solution was concentrated to remove some THF, and the aqueous phase was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated. The residue was treated with dry ether and pentane to obtain 0.12 g (yield = 32%) of the title compound. Oil. ^{1}H NMR ($CDCl_3$): δ 1.04−2.58 (m with superimposed two s at 2.15 and 2.18, 18H of Lup + 6H, 2 *o*-CH_3 + 3H, *p*-CH_3), 2.64−2.98 (m, 2H, Hα near N of Q + 1H, NH, collapses with D_2O), 6.62−6.88 (m, 2H, aromatic protons), 8.23 (s, 1H, CONH, collapses with D_2O). Anal. Calcd for $C_{20}H_{32}N_2$: C, 79.94; H, 10.73; N, 9.32. Found: C, 79.95; H, 10.69; N, 9.31.

***N*-{2-[(1*R*,9a*R*)-(Octahydro-2*H*-quinolizin-1-yl)methylthio]-ethyl}-2,6-dimethylaniline (15).** A mixture of *S*-(2-chloroethyl)-thiolupinine (0.18 g, 0.72 mmol) and 2,6-dimethylaniline (0.09 mL, 0.72 mmol) was introduced into an Aldrich pressure tube flushed with nitrogen. The sealed tube was heated at 110 °C for 72 h. The mixture was treated with 1N HCl, and the acidic phase was extracted with ether to remove the unreacted 2,6-dimethylaniline. The aqueous phase was alkalinized and extracted with CH_2Cl_2. After drying (Na_2SO_4), the solvent was evaporated and the residue was chromatographed on alumina (1:30) eluting with dry ether. Thus 0.05 g (yield = 21%) of **15** was obtained. Oil. ^{1}H NMR ($CDCl_3$): δ 1.18−2.32 (m, 14H of Lup), 2.47 (s, 6H, $2CH_3$), 2.72−3.11 (m, 2H, Hα near N of Q + 2H, CH_2-S, + 2H, S-CH_2-Q + 1H, NH collapses with D_2O), 3.22−3.43 (m, 2H, N−CH_2), 6.92−7.24 (m, 3H, aromatic protons). Anal. Calcd for $C_{20}H_{32}NS$: C, 72.23; H, 9.70; N, 8.42; S, 9.64. Found: C, 72.48; H, 9.56; N, 8.25; S, 9.92.

2-[(1*R*,9a*R*)-(Octahydro-2*H*-quinolizinyl-1-yl)methylthio]-1-chloroethane. A mixture of 1,2-dichloroethane (4.7 mL, 60 mmol), thiolupinine[13] (0.56 g, 3 mmol), and 1,5-diazabicyclo-[4.3.0]nonene (0.4 mL; 3 mmol) was introduced into an Aldrich pressure tube flushed with nitrogen. The mixture was maintained at room temperature for 36 h with magnetic stirring. The solution was evaporated to dryness, and the residue was taken up in 0.5N hydrochloric acid (15 mL). The acidic phase was washed with ether, alkalinized, and extracted with ether. The solvent was dried over Na_2SO_4 and evaporated to obtain an oily residue that was chromatographed on alumina (1:20) eluting with dry ether. Part of the crude *S*-(2-chloroethyl)thiolupinine (0.18 g) was reacted with 2,6-dimethylaniline without further purification. The compound was purified by chromatography (Al_2O_3, eluting with dry ether). Oil. ^{1}H NMR ($CDCl_3$): δ 1.13−2.16 (m, 14H of Lup), 2.08−3.02 (m, 2H, Hα near N of Q + 2H, CH_2-S, + 2H, S-CH_2-Q), 3.57−3.76 (m, 2H, −CH_2-Cl). Anal. Calcd for $C_{12}H_{22}ClNS$: C, 58.16; H, 8.95; N, 5.65; S, 12.94. Found: C, 58.46; H, 9.10; N, 5.78; S, 12.92.

Functional Studies. All methods used for the functional studies have been already described[6,10−18] and are provided, with some additional details, as Supporting Information.

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Supporting Information Available: Further details for functional assays and additional references. This material is available free of charge via the Internet at http://pubs.acs.org.

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