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2-Phenyl-3-(quinolizidin-1-yl)-5-substituted indoles as platelet antiaggregating agents

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Abstract

A set of ten 2-phenyl-3-(quinolizidin-1-yl)-5-substituted indoles was prepared through the Fischer cyclization of lupinyl- and *epi*-lupinylphenylketone 4-substituted phenylhydrazones.Compounds were tested for antiaggregating activity on human platelets activated by adenosine diphosphate (ADP), collagen and adrenaline. At 2.5×10^{-4} M concentration most compounds strongly inhibited the aggregation induced by all the agonists considered and many of them still displayed good activity at 0.625×10^{-4} M concentration. The least active (**1c**) and one of the most active (**1d**) compounds were also tested for antiaggregating activity on rabbit platelets activated by ADP, PAF and sodium arachidonate. Both the compounds were active against ADP and PAF, but only **1d** inhibited the arachidonate-induced aggregation (100% at 8×10^{-6} M concentration) and increased the bleeding time in mice. The same compounds were subjected to a general pharmacological screening and found to display several activities; of particular interest was the dose dependent reduction of serum cholesterol and heparin precipitating betalipoproteins in hypercholesterolemic mice exerted by **1c**, which was still significant at the oral dose of 10 mg/kg.

Keywords: Quinolizidine derivatives; Indoles; Platelet antiaggregating activity; Hypocholesterolemic activity

1. Introduction

Thromboembolic diseases are a leading cause of mortality and disability in developed countries. Present approaches for their treatment and/or prevention include the use of anticoagulant, thrombolytic and antiplatelet drugs.

An optimal antiplatelet drug should block platelet-dependent thrombus formation in a reversible manner and regardless of the nature of the activating agent, and should be endowed with a proper balance of antithrombotic efficacy and maintenance of hemostasis for long-term prophylactic treatment [1–3].

Since the above requirements are not fulfiled by any available drug, the search for new antiaggregating agents is warranted.

During our long-standing study on many quinolizidine derivatives, synthesized for different pharmacological aims, significant platelet antiaggregating activity was often observed in in vitro assays [4–12].

Among the tested compounds, a 100% inhibition of 150 μM sodium arachidonate-induced aggregation was observed in four compounds [6,8,10,12] at concentrations in the range of 2.46–7.7 μM, while three additional compounds exhibited the same activity at 25 μM concentration [8–10]. The best compound was *S*-[(2-cinnamoylamino) phenyl] thiolupinine [5] which, on the other hand, showed only modest activity against adenosine diphosphate (ADP)- and PAF-induced aggregation, even at much higher concentrations. An even greater selective antagonist for arachidonate vs. ADP and PAF activation was the 3-benzyl-1-lupinyl-6-methoxyquinoxalin-2(1H)-one [6,13].

Generally higher concentrations (in the range 200–300 μM) were needed to antagonize the ADP (1.5 μM)-induced aggregation, which was observed in seven compounds.

The best activity was seen for 10-homolupinanoyl-2-methoxyphenothiazine [9], which inhibited the ADP-induced aggregation for ≈50% at 73 μM concentration; the

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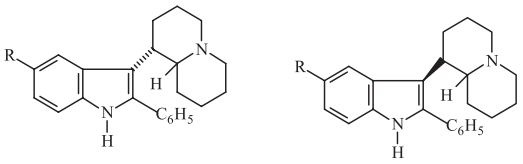
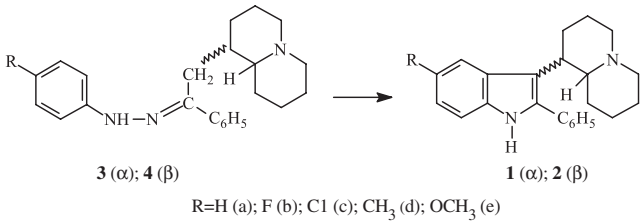


Scheme 1



Scheme 2

same compound was also active against PAF-induced aggregation at ≈25 μM concentration.

Nine compounds (at 20–30 μM concentrations) were active (60–100% inhibition) against PAF (20 ng/ml)-induced aggregation, one of which, 2-ethoxycarboamido-10-homolupinanoylphenothiazine [9], continued to exhibit a 58% inhibition at 2.1 μM concentration.

Activities against ADP- and PAF- induced aggregation were commonly exhibited, though at different concentrations, by the same compounds while lacking activity against arachidonate.

Therefore, the 9-(lupinylthio)xanthene [8] and the 10-homolupinanoylphenothiazine [9] are somewhat outstanding in exhibiting a good activity against the platelet aggregation induced by the three tested agonists.

It is noteworthy that the introduction of different types of substituents on position 2 of the phenothiazine nucleus abolishes the activity against arachidonate-induced aggregation, leaving the activity against ADP and PAF platelet activation unchanged or improved.

In the above situation, we thought it is interesting to undertake a more systematic study of several kinds of quinolizidine derivatives as potential antiaggregating agents.

Therefore, as a first possibility, a set of 2-phenyl-3-quinolizidin-1α/1β-yl-5-substituted indoles of structures **1** and **2** (Scheme 1) is described.

Related 2-unsubstituted or 2-methyl-3-quinolizidin-1α-yl-5-substituted indoles were described by us several years ago [5,7] and some of them were moderately active against ADP-induced platelet aggregation.

Indoles **1** and **2** were tested for antiaggregating activity against ADP-, collagen- and adrenaline- induced platelet aggregation. Moreover, a couple of these (**1c** and **1d**) were subjected to a general pharmacological screening.

2. Chemistry

The required 2-phenyl-3-(quinolizidin-1α/1β-yl)-5-substituted indoles (**1** and **2**) were prepared through a Fischer indole cyclization of lupinyl/*epi*-lupinylphenylketone 4-substituted phenylhydrazones (Scheme 2).

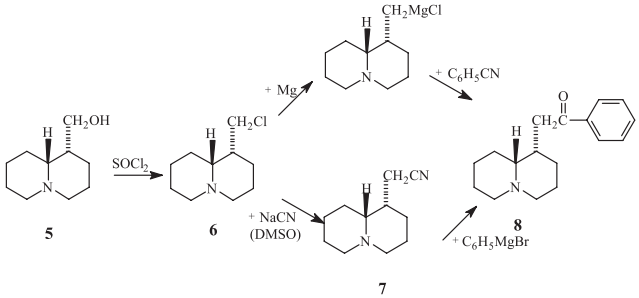
A preliminary attempt of cyclization by refluxing the ethanol solution of phenylhydrazones in the presence of dry hydrogen chloride, as carried out in the past for the analogous derivatives of lupinylmethylketone [5], failed completely. Therefore, the cyclization was effected by heating the mixture of phenylhydrazone and dry zinc chloride (molar ratio 1:15) at 200 °C. In the case of 4-methoxyphenylhydrazone, the last method gave a complex mixture of compounds, from which the indole was not isolated. The desired indole was finally obtained, though in poor yields, by refluxing the absolute ethanol solution of the ketone, 4-methoxyphenylhydrazine hydrochloride and 85% phosphoric acid in a molar ratio of 1:1:4.4.

The required lupinylphenylketone [1-phenyl-2-(quinolizidin-1α-yl)ethanone] (**8**) was prepared in two methods, as outlined in Scheme 3, in all cases starting from l-lupinine, which was extracted from cultivated *Lupinus luteus* L. seeds or from wild Sardinian *L. hispanicus* Boiss et Reut. seeds.

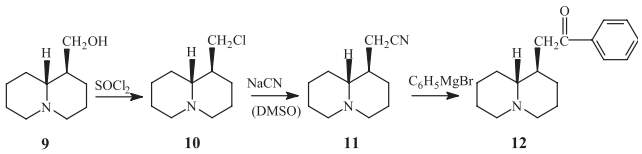
The reaction of cyanolupinane with phenylmagnesium bromide was the most profitable. Therefore, only the latter procedure was applied to obtain the *epi*-lupinylphenylketone [1-phenyl-2-(quinolizidin-1β-yl)ethanone] (**12**), starting from *epi*-lupinine, prepared from lupinine by the action of sodium ethoxide or sodium hydride in refluxing xylene (scheme 4).

Cyanolupinane and *epi*-cyanolupinane were already described by Clemo and Rudinger [14], who obtained them by refluxing the ethanol solution of KCN and the corresponding bromoderivatives, the preparation of which is rather tedious. We found that the reaction of corresponding chloroderivatives with NaCN in DMSO was more profitable.

The cyclization of phenylhydrazones with structures **3** and **4** yielded the expected indoles only in moderate or definitively poor yields. Nevertheless, the formation of byproducts



Scheme 3



Scheme 4

analogous to those obtained in the cyclization of lupinylmethylketone phenylhydrazones [5] was never observed. Indeed, in the latter case a peculiar aminoketone [1(4-aminophenyl)-1-quinolizidin-1α-yl)-propan-2-one] was isolated, as a result of a *para*-benzidine-like rearrangement of the hydrazoene intermediate, which is postulated by the Robinsons mechanism of Fischer reaction [15,16].

Structures of final indoles were supported by elemental analyses and spectral (UV and NMR) data.

^{1}H NMR spectra did not exhibit any peculiar feature, thus only a few are described as examples (see Section 3).

3. Experimental

3.1. Chemistry

Melting points were determined by the capillary method on an electrothermal apparatus and are uncorrected.

Elemental analyses were performed with a CE EA 1110 CHNS-O instrument and the results obtained for the indicated elements were within ±0.3% of the calculated values.

UV and IR spectra were recorded, respectively, on a Varian Techtron model DMS 80 and on a Perkin Elmer 398 spectrophotometer; ^{1}H NMR were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ (or d_6-DMSO) as solvent with TMS as internal standard.

*3.1.1. Cyanolupinane (**7**) and* epi-*cyanolupinane (**11**)*

Sodium cyanide (2 g, 36 mmol, 88% title) was added to a solution of chlorolupinane [17] or *epi*-chlorolupinane [18] (4.75 g, 25 mmol) in 12 ml of freshly distilled DMSO. The solution was heated at 145 °C for 6 h.

The cooled suspension was diluted with water and after having added 3 ml of 6-N NaOH, was thoroughly extracted with ether. The ether solution was washed with a small quantity of water, dried over sodium sulfate and evaporated to dryness. The oily residue was distilled at 80–85 °C and 0.1 torr. In both cases about 3.9 g (87% yield) of nitrile was obtained. Cyanolupinane was oily, while the *epi*-cyanolupinane was solid and, after crystallization from pentane, melted at 60–62 °C [19].

*3.1.2. 1-Phenyl-2-(quinolizidin-1α/1β-yl)ethanones (**8** and **12**) (lupinyl- and* epi-*lupinylphenylketones)*

3.1.2.1. A commercial 3 M ethereal solution of phenylmagnesium bromide (4 ml) was diluted with 20 ml of ether and added dropwise to a solution of cyanolupinane or *epi*-cyanolupinane (2 g, 11.2 mmol) in 20 ml of dry ether. The mixture was heated at reflux for 9 or 18 h, respectively, and then treated with 1 N hydrochloric acid (30 ml). The acid solution was extracted with ether, basified and again extracted with ether.

After removing the solvent, the residue was distilled under reduced pressure (0.01–0.05 torr); b.p. 160–170 °C (air bath temperature). In the case of the *epi*-lupinyl derivative, the distillation was repeated two or three times to completely eliminate the unreacted nitrile.

IR spectra of both ketones, in KBr pellets, exhibited a sharp carbonyl band around 1685 cm^{-1}.

8: oil; 85% yield; analysis (C, H, N) for $C_{17}H_{23}NO$; oxime: m.p. 235–237 °C, analysis for $C_{17}H_{24}N_2O$.

12: m.p. 42–44 °C (dry ether–pentane), 45% yield; analysis (C, H, N) for $C_{17}H_{23}NO$; oxime: m.p. 154–155 °C, analysis (C, H, N) for $C_{17}H_{24}N_2O$.

3.1.2.2. Magnesium turnings (0.97 g, 40 mmol) were covered with 5 ml of dry ether and treated with a crystal of iodine and a few drops of methyl iodide. After a few minutes, a solution of freshly distilled chlorolupinane (7.5 g, 40 mmol) in 25 ml of dry ether was added and the mixture was heated at reflux, while stirring, for 4 h. After cooling, a solution of 4.12 g (40 mmol) of benzonitrile in 25 ml of dry ether was added dropwise and then the mixture was refluxed for 12 h. After cooling, a 10% solution of ammonium chloride was added and the mixture was thoroughly extracted with ether. The ether solution was then shaken with 1 N hydrochloric acid (50 + 30 + 20 ml) and subsequently with water; the acid solution was extracted with ether, basified and again extracted with ether. After removing the solvent, the oily residue was distilled at 0.2 torr to obtain the ketone **8** (yield 75%).

3.1.3. Lupinyl- and epi-*lupinylphenylketone 4-substituted phenylhydrazones (**3a–d, 4a–d**)*

Equimolar amounts (5 mmol) of lupinylphenylketone or *epi*-lupinylphenylketone and 4-substituted phenylhydrazine were heated at 110 °C for 4 h under a slow stream of nitrogen. The crude 4-substituted phenylhydrazones were used as such for the indole cyclization.

*3.1.4. 2-Phenyl-3-(quinolizidin-1α/1β-yl)-5-substituted indoles (**1a–d, 2a–d**)*

The crude phenylhydrazone **3** or **4** (5 mmol) was thoroughly mixed with finely grounded anhydrous zinc chloride in a dry-box. The mixture was gradually heated to 200 °C under a slow stream of nitrogen and maintained at this temperature for 10 min. After cooling, the mixture was taken up with water, basified with a 2-N NaOH solution and extracted with ether. The dried (Na_2SO_4) ether solution was evaporated to dryness and the oily residue was worked up in different manners depending on each compound.

Compounds **2a** and **2b** crystallized after the addition of a few drops of anhydrous ether; crystals were collected and washed with cold dry ether and the ether solution was chromatographed on neutral alumina (activity 1) to obtain a further crop of crystalline compound.

In the case of compounds **1b, 1c, 2c** and **2d**, the oily residue was distilled at 0.1 torr (120–125 °C, air bath temperature) to remove the starting ketones; the residue was then

Table 1
Characteristics of compounds **1a–e** and **2a–e**

Compound	Formula [a]	Solvent [b]	Yield (%)	M.p. (°C)	UV λ_{max} (nm)
1a	$C_{23}H_{26}N_2$ + HCl	a	34	>310	223; 298
1b	$C_{23}H_{25}FN_2$	b	18	166–167	223; 298
1c	$C_{23}H_{25}ClN_2$	c	35	162–163	231; 304
1d	$C_{24}H_{28}N_2$ + HCl + 0.5 H_2O	a	35	>310	223; 299
1e [c]	$C_{24}H_{28}N_2O$	d	14,5	169–171	224; 304
2a	$C_{23}H_{26}N_2$	b	40	249–250	224; 299
2b	$C_{23}H_{25}FN_2$	b	30	260–262	226; 302
2c	$C_{23}H_{25}ClN_2$	b	28,5	239–240	230; 304
2d [d]	$C_{24}H_{28}N_2$	b	18	175–176	228; 302
2e	$C_{24}H_{28}N_2O$ + 0.25 H_2O	b	3	190–192	226; 305

[a] All compounds were analyzed for C, H, N and results were within ±0.3% of calculated values.
[b] a : Absolute ethanol; b: anhydrous ether; c: anhydrous ether/pentane; d: ethanol.
[c] ^{1}H NMR ($CDCl_3$): δ1.16–2.3 (m, 13H, quinolizidine ring); 2.9–3.3 (m, 3H, α-protons on C3, C4 and C6 of quinolizidine ring); 3.65 (s, 3H, OCH_3); 6.8–7.9 (m, 8H, arom.); 8.8 (bs, 1H, exchangeable, NH).
[d] H NMR ($CDCl_3$): δ 0.9–2.55 (m, 13H, quinolizidine ring + s, 3H at δ 2.5, CH_3); 2.8–3 (m, 3H, α-protons on C3, C4 and C6 of quinolizidine ring); 6.82–7.65 (m, 8H, arom.); 7.9 (bs, 1H, exchangeable, NH).

chromatographed on alumina, eluting with dry ether to obtain crystalline compounds.

Finally, in the case of compounds **1a** and **1d,** the oily residue was chromatographed directly on alumina, eluting with ether; indoles were eluted as oils, which were converted to crystalline hydrochlorides.

Characteristics of compounds are reported in Table 1.

*3.1.5. 5-Methoxy-2-phenyl-3-(quinolizidin-1α/1β-yl)indoles (**1e** and **2e**)*

4-Methoxyphenylhydrazine hydrochloride (0.69 g, 4 mmol) and 1.2 ml (17.5 mmol) of 85% phosphoric acid were added to a solution of lupinylphenylketone or *epi*-lupinylphenylketone (1.03 g, 4 mmol) in 12 ml of absolute ethanol. The solution was heated at reflux for 12 h under a stream of nitrogen and then evaporated to dryness under reduced pressure. The residue was dissolved in water, basified with a 2-N NaOH solution and extracted with ether. The solvent was evaporated and the residue was distilled at 120–125 °C (0.1 torr) to remove the unreacted ketone (≈0.7 g). The undistilled residue was chromatographed on neutral alumina (activity 1) eluting with dry ether to obtain the crystalline indoles **1e** and **2e**.

Characteristics of compounds are reported in Table 1.

3.2. Platelet aggregation assays

Human blood samples from normal volunteers were drawn through a 19-gauge needle, carefully avoiding prolonged venous stasis. None of these subjects were treated with any drug known to influence the platelet function.

Blood (36 ml) was collected in plastic tubes containing 3.8% trisodium citrate aqueous solution (4 ml). Platelet-rich plasma (PRP) was obtained by centrifuging the blood at 100 *g* for 20 min. Platelet-poor plasma (PPP) was obtained by centrifuging the remaining blood at 1100 *g* for 15 min.

Platelet count in PRP was maintained at 300,000/mmc.

Platelet aggregation, performed in an Aggrecorder II PA3220 aggregometer (Menarini, Firenze, Italy), was measured according to Born's turbidimetric method [20] and quantified by the maximal light transmission after 5 min.

A first sample of PRP was pre-incubated at 37 °C for 2 min before the addition of the agonist (2 and 5 μM ADP; collagen at final concentrations of 4 and 2 μg/ml, 5 μM adrenaline).

A second sample of PRP was incubated for 2 min with a solution of the tested compound. Platelet aggregation was then performed adding the agonist and comparing the maximal light transmission of the aggregation curves obtained with and without the addition of the tested compound, the percentage of inhibition of platelet aggregation was calculated.

All compounds were used as hydrochlorides.

3.3. General pharmacological screening

A general pharmacological screening was performed by MDS-Panlabs, Bothell, WA, USA, on compounds **1c** and **1d**. This screening consisted in the determination of the maximum tolerated dose in mice (MTD, p.o. and i.p.) with simultaneous behavioral examination (Irwin test) and in 34 primary in vivo tests (using a suitable MTD fraction, depending on the test type) and in 20 in vitro tests, concerning CNS, cardiovascular and gastrointestinal apparatuses, intermediary metabolism, allergy and inflammation.

For in vivo tests, compounds were generally administered orally using a gastric tube in the form of aqueous solutions or finely homogenized suspensions in Tween 80 (2%), when the highest dose (300 mg/kg) was used. In a few cases compounds were introduced i.p. as aqueous solutions (10 ml/kg). Groups of three or five animals (rat or mice) were used.

For in vitro assays, the dissolution of a test compound in a buffer or saline solution was speeded up by means of DMSO;

Table 2
Human platelet antiaggregating activity of compounds **1a–e** and **2a–e**

Compound	Final concentration (μM)	Inhibition of platelet aggregation induced by ADP, collagen and adrenalin in human plasma (%) [a]				
		ADP (5 μM)	ADP (2 μM)	Collagen (4 μg/ml)	Collagen (2 μg/ml)	Adrenalin (5 μM)
1a	250	84	90	93	93	87
	125	21	54	87	88	82
	62.5	24	43	25	32	68
1b	250	39	62	86	87	88
	125	31	51	79	87	86
	62.5	14	17	22	39	84
1c	250	33	52	87	90	85
	125	16	39	20	47	72
	62.5	7	3	11	6	5
1d	250	67	78	92	91	94
	125	43	59	74	87	88
	62.5	9	32	31	46	42
1e	250	67	88	84	89	80
	125	48	65	86	91	86
	62.5	29	46	23	69	65
2a	250	80	67	86	89	100
	125	61	56	74	85	77
	62.5	49	47	11	17	63
2b	250	56	56	83	84	81
	125	35	31	74	83	68
	62.5	11	13	20	55	25
2c	250	56	58	91	89	89
	125	51	51	68	83	31
	62.5	11	36	19	43	5
2d	250	48	75	89	89	86
	125	44	65	45	87	79
	62.5	39	62	11	36	55
2e	250	89	96	92	93	97
	125	58	86	90	89	76
	62.5	50	89	16	78	79
ASA	1000	45	41	41	37	68
	100	9	34	3	3	30
Adenosine	350	55	85			
	240	32	82			
	64	6	15			

[a] Mean value of two or three determinations.

the final concentration of DMSO (not interfering with the tests) was 0.1% for platelet aggregation and 0.5% for all the others.

The procedures for these assays have already been described [7–10,21–23]: a pre-established level of response, which is high enough to suggest significant activity, is indicated for each assay. Doses (mg/kg) or concentrations (mg/ml or μM) indicated in the methods were the highest used routinely, depending on toxicity; when significant activity was detected, lower doses or concentrations were tested in order to define the minimal effective ones and secondary tests were performed to provide some insight for the possible mechanisms of action.

In Tables 3–7 only the results of the more significant assays are collected; the pertinent reference for the method employed is indicated near each assay name.

4. Results and discussion

Antiaggregating activity of compounds **1a–e** and **2a–e** (Scheme 1) was tested on human platelet aggregation stimulated by ADP at 5 and 2 μM concentration, collagen at 4 and 2 μg/ml and adrenaline at 5 μM concentration. Compounds were assayed at 2.5, 1.25 and 0.62×10^{-4} M. Results are collected in Table 2.

Most compounds, when tested at the concentration 2.5×10^{-4} M, strongly inhibited the aggregation induced by all the agonists considered, and many of them maintained good activity at 1.25×10^{-4} M concentration.

The lowest inhibitory effects were observed against ADP, while the highest were against adrenaline. All compounds were definitely more active than acetylsalicylic acid (ASA) at comparable concentrations, and many compounds in the aforementioned concentrations were more active than ASA

Table 3
Inhibition of rabbit platelet aggregation induced by several agents and related in vivo assays

Test	Concentration (μm) Route; dose (mg/kg)	Effect	Inhibition by compound (%)		Reference drug	Concentration (μm) Route; dose (mg/kg)	Inhibition (%)
			1c	**1d**			
Platelet aggregation induced by							
Na arachidonate [8]	27	[a]	0	100			
	8			100	Aspirin	13.8	100
	2.7			0			
ADP [8]	270	[a]	57	100			
	80		9	20	Adenosine	18.7	64
PAF [8]	27	[a]	97	100	RP48740 [d]	20.4	54
	8		48	60			
	2.7		nt	20			
In vivo							
Bleeding time [10]	p.o. 100	[b]	nt	28	Aspirin	p.o. 100	60
PAF antagonism (mice) [8]	p.o. 100	[c]	40	60	L-65273 [e]	p.o. 100	60
	p.o. 30		nt	40			

[a] % inhibition of maximum non-reversible rabbit platelet aggregation induced by: Na arachidonate (50 μg/ml = 153 μM), ADP (0.8 μg/ml = 1.8 μM) or PAF (10–20 ng/ml).
[b] % prolongation of bleeding time relative to a control group of mice.
[c] % inhibition of mortality observed 1 h after the i.v. administration of PAF–acether (200 μg/kg).
[d] 3-(3-Pyridyl)-1H,3H-pyrrolo[1,2-c]thiazole-7-carboxamide.
[e] *trans*-2,5-bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran.

Table 4
Maximal tolerated dose in mice [8] for compounds **1c** and **1d**

Administration route	Dose (mg/kg)	Number of animals dead (and effects) after treatment with compound	
		1c	**1d**
Oral	300	0/3	0/3
		No effect	Slight decrease limb tone and grip
i.p.	100	0/3	0/3
		No effect	Slight tremor and muscolar relax
	30	nt	0/3
			Slight decrease limb tone and grip

Table 5
Analgesic activity in mice

Test	Route; dose (mg/kg^{1})	Effect	Effect produced by compound **1c**	Reference drug	Route; dose (mg/kg)	Effect
Phenylquinone writhing [8]	p.o. 100	[a]	69	Aspirin	p.o. 100	68
	p.o. 30		36	Ibuprofren	p.o. 30	65
Formalin analgesia [21]	p.o. 100	[b]	90	Aspirin	p.o. 300	52
	p.o. 30		59	Ibuprofen	p.o. 30	66
	p.o. 10		9	Morphine	p.o. 10	62
Tail flick [8]	i. p. 30	[c]	10	Tifluadom	i.p. 10	65

[a] Inhibition of number of writhes.
[b] % reduction of induced paw licking time recorded between 20 and 30 min after formalin injection.
[c] % prolongation of the time to elicit pain response.

at 10^{-3} M concentration. Many compounds displayed good activity even at 0.625×10^{-4} M concentration against ADP-, collagen- and adrenaline-induced aggregations; in particular at this concentration compounds **1d, 1e, 2a** and **2c** inhibited ADP-induced aggregation for 40–50%. It is therefore evident that there is a concentration-dependent inhibitory effect, though the curve steepness is very variable for both the tested compounds and the agonist considered.

It is worth noting that, with regard to the structure–activity relationships, the different (α or β) spatial connection of the quinolizidine ring to the indole nucleus does not produce a homogeneous effect and, in any case, the differences observed in the antiaggregating activity are not very high.

A somewhat more marked influence is exerted by the nature of substituents on position 5, with methoxy, methyl and unsubstituted compounds being better than fluoro- and chloroderivatives.

Indeed compounds **1c** and **1d**, which resulted, respectively, the least active and one of the most active as inhibitors of human platelets aggregation are characterized by the pres-

Table 6
In vivo assays for antihyperlipemic [21] and diuretic [8] activities of compounds **1c** and **1d**

Test	Dose mg/kg p.o.	Effect	Response to compound		Reference drug	Dose mg/kg p.o.	Response
			1c	**1d**			
Total cholesterol (in mice)	300	[a]	–30	nt	Bezafibrate	300	–39
	100		–27	0		100	–25
	30		–21	nt			
	10		–16	nt	D-thyroxine	10	–30
	3		–7	nt			
Heparin precipitating HPL (in mice)	300	[b]	–35	nt			
	100		–29	0	Bezafibrate	100	–30
	30		–23	nt			
	10		–21	nt	D-thyroxine	10	–35
	3		–3	nt			
Ratio HPL/total cholesterol	300	[c]	0.93	nt			
	100		0.97	0.96	Bezafibrate	100	0.93
	30		0.97	nt			
	10		0.94	nt	D-thyroxine	10	0.93
Urine volume (in rats)	30	[d]	–20	+50	Amiloride	20	+60
	10		nt	+20			
Saluresis (in rats)	30	[e]	+30	+200	Furosemide	5	+200
	10		nt	+20			
Kaluresis (in rats)	30	[e]	–30	+20			

[a] Average percent reduction in serum cholesterol concentration ≥ 15% relative to hypercholestrolemic control mice indicates significant activity (established control animals have a serum total cholesterol content of 350 ± 28 mg/dl).
[b] Reduction of serum HPL (heparin precipitating betalipoproteins) concentration ≥ 20% indicates significant activity.
[c] Reduction of ratio below 0.92 indicates potential anti-atherogenic activity.
[d] % variation of Na^+ or K^+ excretion expressed as μeq/100 g body weight; a variation ≥ 50% indicates significant activity.
[e] Variation of Na^+ or K^+ excretion expressed as μeq/100 g body weight; a variation ≥ 50% indicates significant variety.

ence of a chlorine and a methyl group, respectively. They were also tested for antiaggregating activity on rabbit platelets against ADP, PAF and sodium arachidonate activation.

The activity against ADP-induced aggregation was confirmed by both the compounds, which, at lower concentrations, also displayed high activity against the PAF-induced aggregation, comparable or superior to that of the standard agent RP-48740 (3-(3-pyridyl)-1H,3H-pyrrolo[1,2-c] thiazole-7-carboxamide). However, a clear-cut difference was observed in the activity of compounds **1c** and **1d** against the arachidonate-induced aggregation; while the first compound was completely inactive at a 27 μM concentration, the second one still gave a 100% inhibition at 8 μM concentration. Correspondingly, compound **1d** produced a moderate increase of bleeding time in mice.

Both compounds, but in particular **1d**, protected mice from mortality induced by i.v. injection of PAF, resulting comparable to compound L-652731 [*trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran] used as a reference drug. This protective activity is rarely observed [24–26] and therefore, it deserves further investigation to define eventual therapeutic potentialities.

Compounds **1c** and **1d**, when subjected to the general pharmacological screening resulted well tolerated up to the maximum tested dose of 300 mg/kg p.o. and 100 mg i.p.

The 5-chloroderivative **1c** exhibited analgesic activity in phenylquinone writhing test and against formalin algesia, resulting, respectively, comparable or superior to aspirin. In the latter test compound **1c**, at 30 mg/kg p.o., was comparable to morphine at 10 mg/kg p.o. However, compound **1c** was inactive against the carragenan-induced rat paw edema, suggesting that the observed antinociceptive activity may be of central origin.

The relevant compound exerted a strong reduction in serum cholesterol and heparin precipitating betalipoproteins in hypercholesterolemic mice; the reduction was still significant after an oral dose of only 10 mg/kg. Thus, compound **1c** is an interesting hit for the preparation of antihypercholesterolemic agents not structurally related to existing drugs.

The simultaneous presence in the same molecule of hypocholesterolemic and platelet antiaggregating activities represents an outstanding property, which could result useful in the prevention and treatment of thrombotic disorders.

This double-faced activity is shared, as far as we know, only by fibrates and has recently been described by some of us [22] for 5-chloro-2-{4-[3-(dimethylammino)propoxy] phenyl}benzotriazole. The latter compound, as well as clofibric acid, while active against ADP-induced platelet aggregation, was inactive against arachidonic acid-induced aggregation, similarly to compound **1c**.

On the other hand, the 5-methylderivative **1d** did not have analgesic and antihypocholesterolemic activities, but it was endowed with saluretic activity (associated with increased urine volume output in the hydrated rat) and with tracheal

Table 7
Antiarrhythmic activity in mice [8] and some in vitro assays related to cardiocirculatory function

Test	Route; dose (mg/kg) Concentration (μg/ml)	Effect	Effects produced by compounds **1c** and **1d** and reference drugs				
			1c	**1d**	Lidocaine	Quinidine	Others
In vivo							
Chloroform arrhythmia	i.p. 100	[a]	2/3	3/3		3/3	
	i.p. 30		0/3	2/3	3/3[h]		
	i.p. 10		nt	0/3			
Aconitine arrhythmia	i. p. 100	[b]	92	55		108[h]	
	i.p. 30		0	0	113[h]		
Aconitine ventricular tachycardia	i.p. 100	[b]	115	87			
	i.p. 30		0	0	95[h]	89[h]	
In vitro							
Left atria electrical driven (guinea pig) Inotropic effect (guinea pig left atria)	100	[c]	80	nt	46	37[i]	
	30		50	51			
	10		20	0			
	10	[d]	–19	–50	–20[i]		
Chronotropic effect (guinea pig right atria)	10	[d]	0	–13		–18 (diltiazem)	
	3		nt	–7			
Ca^{2+} antagonism (guinea pig ileum)	3	[e]	74	94	65[j] (diltiazem)		
	1		19	78			
	0.3		nt	56			
	0.1		nt	18			
Ca^{2+} antagonism (guinea pig left atria)	3	[f]	17	81	60[k] (diltiazem)		
	1		nt	71			
	0.3		nt	56			
	0.1		nt	15			
Methacoline antag. (guinea pig ileum) CCK antagonist (guinea pig ileum)	100	[g]	100	0	75[l] (atropine)		
	30		68	nt			
	10		41	nt			
	3	[g]	0	52	70[l] (lorglumide)		
	1		nt	27			

[a] Number of protected over treated animals.
[b] % Time increase for onset of arrhythmia and ventricular tachycardia relative to control group.
[c] % Reduction in ability to follow electrically-induced contractile frequency.
[d] % Variation in contractile force or rate.
[e] % Inhibition of Ca^{2+}-induced contraction.
[f] % Reduction of a Ca^{2+}-induced increase in contractile force.
[g] % Reduction of contractile response.
[h] Tested at 50 mg/kg.
[i] Tested at 25 μg/ml.
[j] Tested at 0.01 μg/ml.
[k] : Tested at 2.5 μg/ml.
[l] Tested at 0.1 μg/ml.

relaxant activity in vitro. The latter appears unrelated to β_2-adrenergic agonism, since it was not inhibited by propranolol, but may relate to possible inhibition of cyclooxygenase, suggested by the aforementioned inhibition of arachidonic acid-induced platelet aggregation.

Both compounds **1c** and **1d** exhibited good antiarrhythmic activity in mice, which underwent deep chloroform anesthesia and aconitine infusion, as well as in electrically driven left atria. This activity may relate to calcium antagonism, which was seen in vitro in guinea pig ileum, but not in rat portal vein; calcium antagonism was observed also in guinea pig atria for compound **1d**, but not for **1c**. Moreover, in the case of compound **1d**, the antiarrhythmic activity may be also related to the moderate (but long lasting) local anesthetic activity observed in the guinea pig corneal surface stimulation test. This activity was completely absent in compound **1c**.

Finally, compound **1c** antagonized the methacholine induced, but not the CCK-induced, contractile response in guinea pig ileum; the opposite was observed in compound **1d**.

The results of the general pharmacological screening, though limited to only two compounds, clearly indicate that the nature of the substituent introduced on position 5 of 2-phenyl-3-(quinolizidin-1′-yl)indole may not only give rise to quantitative differences in each activity (as already observed for the human platelet antiaggregating activity), but even to largely different biological properties.

Therefore, the 2-phenyl-3-(quinolizidin-1′-yl)indoles deserve further studies. The preparation of new 5-substituted derivatives and corresponding position isomers will be pursued, while the mechanisms of some of the observed activities will be investigated with particular concern to the possible connection of the antihypercholesterolemic activity with the sigma receptor affinity and/or peroxysome proliferator-activated receptor (PPAR) agonism.

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