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Synthesis and pharmacological evaluation of some thiolupinine derivatives

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Abstract

A small set of 9-(lupinylthio)xanthene, -thioxanthenes and α-(lupinylthio)diphenylmethanes was prepared and found to inhibit the angiotensin II-induced contractions of guinea pig ileum. Some of these compounds were also moderately active in vitro as tracheal relaxants and one compound was more active than aspirin against arachidonic acid-induced platelet aggregation.

Keywords: Thiolupinine derivatives; Angiotensin antagonism; Platelet aggregation inhibition; Trachea relaxation

1. Introduction

Recently, Novelli and Sparatore described the preparation of thiolupinine and some of its S-substituted derivatives, several of which exhibited interesting in vitro and in vivo activities [1].

Particularly the 9-(lupinylthio)xanthene (**1**) at the concentration of 30 μg/ml (75 μM) relaxed zigzag cut guinea pig trachea by 71% of the maximal relaxation induced by epinephrine, whereas theophylline at the same w/v concentration (166 μM) exerted a relaxant effect corresponding to 60%.

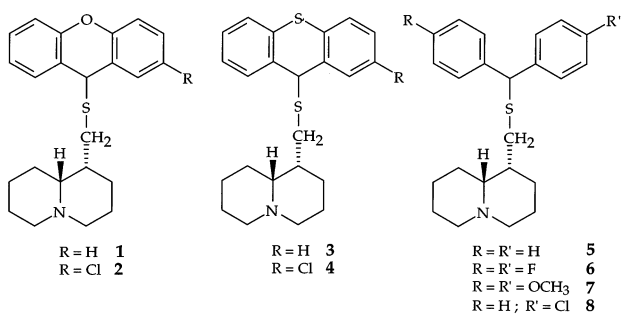
This activity could be related to the cyclooxygenase inhibition inferred from the inhibition, exerted at 10 μg/ml, of arachidonic acid-induced platelet aggregation.

At higher concentrations compound **1** inhibited also the platelet aggregation induced by PAF and ADP, but only a moderate increase in bleeding-time was seen in mice.

Still, compound **1** antagonized guinea pig ileum contractile responses induced by calcium ions, angiotensin I and II, methacoline, cholecystokinin and electrical stimulation. However it did not exhibit any activity on blood pressure in SHR and was inactive against serotonin-induced bradycardia in mice under pentobarbital anesthesia.

Such an unusual behaviour might suggest on one hand a multireceptorial tropism at intestinal level (and therefore a possible therapeutic action against the so-called 'irritable bowel' [2]), but on the other hand, an affinity for AT_2 rather than AT_1 receptors with potential ability to increase cognitive capabilities [3,4].

On this basis a set of thiolupinine derivatives (**2**–**8**) was prepared and investigated to determine what concerns their action on tracheal relaxation, the inhibition of arachidonic acid-induced platelet aggregation and of angiotensin II-induced contractions of guinea pig ileum.



It is of interest that compounds **3** and **6** are thia-homologues of previously described 9-lupinylthioxan-

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thene and of lupinyl-bis[(4-fluoro)phenyl]-methane. The first was endowed with dopamine-releasing activity from striatal and frontal cortex synaptosomes and was able to activate dopamine receptors mediating acetylcholine release inhibition [5–7].

The latter, on the other hand, was endowed with strong activity on the *Mycobacterium tuberculosis* [8].

These activities will be investigated later on.

2. Chemistry

The preparation of compounds **2**–**4** was effected by condensing thiolupinine with the suitable secondary alcohols as for compound **1** synthesis [1]. The reaction between thiols and xanthydrols or thiaxanthydrols was already studied by others [9,10]; we obtained good yields by operating in ethanol–acetic acid (1:1) solution as suggested by Sawicki and Oliverio [9].

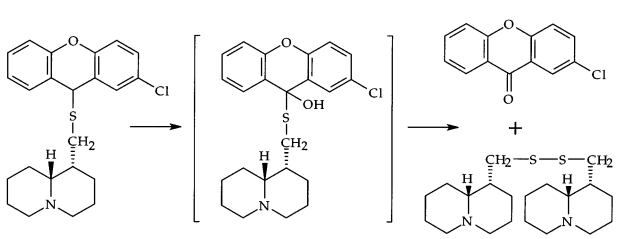
To obtain compounds **5**–**8**, thiolupinine was reacted with the corresponding benzhydryl chlorides, prepared from the corresponding alcohols and thionyl chloride.

The required secondary alcohols were prepared by reducing the ketones with zinc and sodium hydroxide [11]; the quality of zinc is of paramount importance. Freshly-activated zinc following the indications of Fieser and Fieser [12] gave the best results.

Compounds **5**, **6** and **8**, though giving excellent results in the elemental analysis, exhibited two close spots in TLC indicating the presence of two very similar compounds, possibly two isomers (lupinyl and minor amounts of *epi*-lupinyl derivatives). Moreover in the NMR spectra the proton on the carbon bridge gave a split signal with, for instance, δ 5.09 and 5.11 for the simplest compound **5**. However, repeated column chromatography, though in different conditions, did not afford any separation of the purported two epimers, giving only fractions with different ratios between the two signals.

On the other hand, both TLC and NMR spectra seem to indicate that compounds **1**–**4** and **7** were unitary.

It is worth noting that 9-(lupinylthio)xanthenes, and particularly the 2-chloroderivative, are easily oxidizable by air giving rise to xanthone (or 2-chloroxanthone) and dilupinyl disulfide.



3. Experimental

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

The elemental analyses were performed with a CE EA 1110 CHNS-O instrument and the analytical results for the indicated elements were within $\pm 0.3\%$ of the calculated values.

UV spectra were recorded with a Perkin–Elmer model 550 S spectrophotometer; ^{1}H NMR spectra were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ as solvent, with TMS as internal standard.

3.1. Secondary alcohols

The required secondary alcohols, which were not commercially available, were prepared reducing the corresponding ketones as follows.

The ketone (2–3 g), suspended in 20–30 ml of methanol previously saturated at room temperature with NaOH, was treated with zinc dust (2–3 g) activated as described by Fieser and Fieser [12]. A blue colour appeared with increasing intensity that faded away at the end of reduction. If the colour failed to appear rapidly, the reaction mixture was heated at 80°C until it became blue and then was stirred at room temperature for 1–2 h; the progression of the reduction was checked by TLC (alumina; dichloromethane). The solvent was removed under reduced pressure, dichloromethane was added and the suspension was filtered through cellulose powder. The filtrate was washed with water and again with a saturated solution of sodium chloride, dried on sodium sulfate and evaporated to dryness. The alcohol (yield from 83 to 91%) had to be used immediately for the next step, otherwise formation of ether and/or ketone was observed.

An attempt to reduce 2-chloroxanthone with $NaBH_4$ afforded a mixture of 2-chloroxanthydrol and its ethyl ether (m.p. = 43–44°C). *Anal.* (C, H, Cl) for $C_{15}H_{13}ClO_2$. ^{1}H NMR ($CDCl_3$): δ 7.6–7.0 (m, 7H, arom.); 5.73 (s, 1H, H9 xanthene ring); 3.23–3.13 (quart, 2H, CH_2CH_3); 1.12–1.05 (t, 3H, CH_2CH_3).

3.2. Bis(4-methoxyphenyl)-chloromethane

The 4,4′-dimethoxybenzhydrol (2.67 g) (obtained as above described from 4,4′-dimethoxybenzophenone) was partially dissolved in toluene (12 ml) and thionyl chloride (1 ml) was added dropwise. The mixture was refluxed for 3 h and the solvent was removed under reduced pressure. The residue was taken up with ether and the solution was washed twice with 5%

K_2CO_3 solution. After removing the solvent, the oily residue was used directly for the next step.

3.3. 9-(Lupinylthio)xanthenes and 9-(lupinylthio)-thiaxanthenes (**1**–**4**)

The suitable 9-hydroxy-xanthene or -thiaxanthene (5 mmol) dissolved in a mixture of ethanol and acetic acid (1.5 ml each) was treated with 0.93 g (5 mmol) of freshly distilled thiolupinine and the mixture was left for 16 h in a closed tube after flushing with nitrogen. Ethanol was removed under reduced pressure, water was added and acidity was increased with a few drops of 6 N HCl (pH 2). The acid solution was thoroughly extracted with ether to remove the unreacted secondary alcohol, made alkaline with 6 N NaOH solution and filtered (compounds **3** and **4**) or extracted with ether (compounds **1** and **2**). The solid compounds were washed thoroughly with water and dried in vacuo. The ether extract of compounds **1** and **2** was dried (Na_2SO_4) and evaporated to dryness; the oily residue was chromatographed on neutral alumina (1:20) using dry ether as eluent. The free bases were converted to the hydrochlorides by dissolution in 1 N ethanolic HCl; dry ether was added and the precipitate was washed several times with dry ether.

The obtained compounds are described in Table 1.

Table 1
Characteristics of thiolupinine derivatives **2**–**8**

Comp.	Formula [a]	M.p. (°C)	Yield (%)	NMR Ar—CH(S—Q)—Ar'
2	$C_{23}H_{26}ClNOS$	oil	51	[c]
	$C_{23}H_{26}ClNOS \cdot HCl \cdot 0.5H_2O$	[b]		
3	$C_{23}H_{27}NS_2$	163–164	88	5.21
	$C_{23}H_{27}NS_2 \cdot HCl \cdot 0.5H_2O$	[b]		
4	$C_{23}H_{26}ClNS_2$	124–128	37	5.14
	$C_{23}H_{26}ClNS_2 \cdot HCl \cdot 0.5H_2O$	[b]		
5	$C_{23}H_{29}NS$	waxy	66	5.09/5.11
	$C_{23}H_{29}NS \cdot HCl$	200–204		
6	$C_{23}H_{27}F_2NS$	waxy	80	5.03/5.07
	$C_{23}H_{27}F_2NS \cdot HCl \cdot 0.25H_2O$	[b]		
7	$C_{25}H_{33}NO_2S$	waxy	49	5.04
	$C_{25}H_{33}NO_2S \cdot HCl \cdot 0.5H_2O$	[b]		
8	$C_{23}H_{28}ClNS$	waxy	73	5.02/5.08
	$C_{23}H_{28}ClNS \cdot HCl \cdot 0.25H_2O$	[b]		

[a] Analytical results for C, H, N were within $\pm 0.3\%$ of the calculated values.

[b] The hydrochloride was rather hygroscopic and melted in a wide range.

[c] As free base this compound was unstable.

3.4. Oxidation of 2-chloro-9-(lupinylthio)xanthene

A sample of analytically pure 2-chloro-9-(lupinylthio)xanthene was left exposed to air for a few days becoming orange coloured. The elemental analysis of the orange-coloured sample indicated the quantitative absorption of one oxygen atom for each xanthene unit.

Analysis:	found: C, 66.40; H, 6.03; N, 3.36%.
for $C_{23}H_{26}ClNOS + O$	calc.: C, 66.40; H, 6.30; N, 3.37%.
for $(2C_{13}H_7ClO_2 + C_{20}H_{36}N_2S_2)$	calc.: C, 66.57; H, 6.07; N, 3.38%.

3.5. α-(Lupinylthio)diarylmethanes (**5**–**8**)

Freshly distilled α-chlorodiphenylmethane (or the corresponding 4-mono- or 4,4′-bisubstituted derivatives) (2.9 mmol) was mixed with freshly distilled thiolupinine (0.54 g, 2.9 mmol) in a pressure tube that, after being flushed with nitrogen, was closed with the threaded plug and heated at 160°C with stirring for 16 h. After cooling, the solidified material was taken up with ether and acidified water. The ether layer was washed with acidic water that was added to the aqueous layer; the acid solution was extracted with ether, basified and again extracted with ether. After drying, the solvent was removed and the residue chromatographed on neutral alumina (1:30) eluting with dry ether. For compounds **5**, **6** and **8** repeated chromatographies of the main fraction always gave compounds exhibiting two very near spots in the TLC and two close signals for the proton on the carbon bridge.

The compounds obtained (waxy solids) are described in Table 1.

It is worth noting that a lower reaction temperature or a shorter heating time gave poorer results; an attempt at reacting thiolupinine with benzhydryl chloride in ethanol solution at 110°C in a sealed tube failed completely.

4. Pharmacology

The new compounds **2**–**8** were subjected by Panlabs Inc. of Bothell (WA, USA) to in vitro tests for relaxation of zigzag cut guinea pig trachea, inhibition of arachidonic acid-induced rabbit platelet aggregation and angiotensin antagonism on guinea pig ileum. The previously described compound **1** was tested for affinity to AT_2 receptors.

Table 2
In vitro activity of compounds **1**–**8** and of reference drugs

Comp./ref. Drug	Conc. (μM) [a]	Angiotensin II inhibition (%) [b]	Platelet aggregation inhibition (%) [c]	Tracheal relaxation (%) [d]
1	75			71
	25		100	31
	7.5	80	0	
	2.5	67		
	0.75	38		
2	67.4			33
	27.6		0	
	22.5	92		
	6.7	61		
	2.25	33		
3	70			13
	30	100		
	23.4		0	
	10	89		
	3	66		
	1	37		
4	65			0
	30	100		
	10	93		
	3	70		
	1	21		
5	77			25
	30		0	
	25.8	100		
	7.7	94		
	2.6	38		
6	70.7			17
	30	100	0	
	10	96		
	3	86		
	1	16		
7	65.6			35
	30	100		
	21.9		100	
	10	92		
	6.6		100	
	3	29		
	2.2		0	
8	71			0
	30	100	0	
	10	95		
	3	56		
	1	26		
Captopril [e]	0.46	69		
Aspirin [e]	16.6		100	
Theophylline [e]	166			60

[a] See Section 4.1.
[b] % reduction of contractile response of guinea pig ileum segments to angiotensin II (10 ng/ml).
[c] % reduction of maximum non reversible platelet aggregation (rabbit platelet rich plasma) induced by sodium arachidonate (50 μg/ml).
[d] % inhibition of spontaneous contractions of guinea pig trachea segments relative to maximal relaxation induced by 0.3 μg/ml of epinephrine.
[e] Minimal concentration giving a highly significant (>50%) response.

4.1. Materials and methods

All tested compounds were used as hydrochlorides.

For the in vitro assays it was usually necessary to increase the solubility by means of dimethylsulfoxide in a concentration not interfering with tests (0.1% for platelet aggregation and 0.5% for the others).

The procedures for the above-mentioned assays were already described [1]. The concentrations (μg/ml or μM) indicated in the methods were the highest utilized routinely; when significant activity was detected lower concentrations were tested in order to define the minimal effective ones.

Two successive experiments were carried out, each time employing concentrations of test compounds expressed as μg/ml (30, 10, 3, 1 μg/ml) or as μM (30, 10, 3, 1 μM); for the sake of homogeneity in Table 2 concentrations are always indicated as μM.

4.2. Angiotensin AT_2 binding [13]

Membranes prepared from male rabbit adrenal glands were incubated for 45 min at 25°C with 25 pM [^{125}I]CGP-42112A. Non-specific binding is estimated in the presence of 1 μM angiotensin II. Membranes are filtered, washed and counted to determine the bound [^{125}I]CGP-42112A. Compound **1** was screened at 10 μM concentration. Saralasin was used as reference compound, exhibiting $IC_{50} = 1$ nM and $K_i = 0.99$ nM.

5. Results and discussion

The results of the pharmacological assays are collected in Table 2, where the results concerning the previously described compound **1** are also included for comparison.

Among the activities exhibited by the lead compound **1**, only the inhibition of angiotensin II-induced guinea pig contractions was found in all tested compounds, while the remaining ones were seen only occasionally in a few compounds. Thus, compound **7** inhibited the arachidonic acid-induced platelet aggregation at a lower concentration (6.6 μM) than that of **1** (25 μM), but the remaining compounds were inactive, at least in the concentration range explored. Compound **7** was clearly more active than aspirin (MIC = 16.6 μM).

As regards tracheal relaxation, compounds **2**, **5** and **7** exhibited only moderate activity at the highest concentration employed (30 μg/ml = 65–77 μM), while the lead compound **1** was more than twice as active as theophylline on a molar basis.

Compounds **3**, **4**, **6** and **8** inhibited significantly (56–86%) the guinea pig ileum contractile response to angiotensin II at 3 μM concentration, similar to the lead compound **1** (2.5 μM), while the remaining compounds **2**, **5** and **7** were active at higher concentrations (6.7–10 μM).

Thus, xanthene, thioxanthene and diphenylmethane backbones appear equally suitable for this kind of activity; the chloro substitution on the benzene ring of xanthene and thioxanthene does not produce significant change of activity, while among the diphenylmethane derivatives the halogen-substituted compounds **6** and **8** are more active than both the unsubstituted (**5**) and the methoxy-substituted derivatives.

Finally, it is worth noting that in the binding test the lead compound **1** failed to displace the specific ligand [^{125}I]CGP-42112A from angiotensin AT_2 receptors at concentrations up to 10^{-5} M. Therefore any direct interaction of **1** and similar compounds with this receptor should be excluded in the observed inhibition of angiotensin II-induced contraction of guinea pig ileum.

References

[1] F. Novelli, F. Sparatore, Thiolupinine and some derivatives of pharmacological interest, Farmaco 48 (1993) 1021–1049.
[2] W.J. Kinnier, J.F. Kachur, V.H. Audia, C. Kaiser, Irritable bowel syndrome: present and prospective pharmacological intervention, Med. Res. Rev. 9 (1989) 325–342.
[3] C.J. Blankley, J.C. Hodges, S.R. Klutchko, R.J. Himmelsbach, A. Chucholowski, C.J. Connolly, S.J. Neergaard, M.S. Van Nieuwenhze, A. Sebastian, J. Quin, A.D. Essenburg, D.M. Cohen, Synthesis and structure–activity relationships of a novel series of non-peptide angiotensin II receptor binding inhibitors specific for the AT_2 subtype, J. Med. Chem. 34 (1991) 3248–3260.
[4] N.M. Barnes, B. Costall, M.E. Kelly, D.A. Murphy, R.J. Naylor, Cognitive enhancing actions of PD123177 detected in a mouse habituation paradigm, Neurol. Rep. 2 (1991) 351–353.
[5] A. Sparatore, A.M. Caviglia, M. Marchi, M. Raiteri, Pharmacological characterization of the presynaptic muscarinic autoreceptors regulating acetylcholine release from rat cortical synaptosomes, Symp. Neurosci. (Liviana Press, Padua) 3 (1986) 447–452.
[6] A. Sparatore, M. Marchi, G. Maura, G. Bonanno, M. Ulivi, M. Raiteri, Studies on some new indirect dopaminergic receptor agonist, Symp. Neurosci. (Liviana Press, Padua) 5 (1988) 192–196.
[7] A. Sparatore, M. Marchi, G. Maura, M. Raiteri, Effect of some quinolizidine derivatives on the release of serotonin, noradrenaline, dopamine and acetylcholine from rat brain synaptosomes, Farmaco 44 (1989) 1205–1216.
[8] I. Vazzana, F. Novelli, F. Sparatore, A. Sparatore, G. Fadda, G. Manca, Quinolizidine derivatives with antitubercular activity, Farmaco 49 (1994) 105–110.
[9] E. Sawicki, V.T. Oliverio, Reaction of thiaxanthydrol with compounds containing active hydrogen, J. Org. Chem. 21 (1956) 183–189.
[10] J.A. Bristol, E.H. Gold, I. Gross, R.G. Lovey, J.F. Long, Gastric antisecretory agents. 2. Antisecretory activity of 9-[(aminoalkyl)thio]-9*H*-xanthenes and 5-[(aminoalkyl)thio]-5*H*-[1]benzopyrano-[2,3-*b*]-pyridines, J. Med. Chem. 24 (1981) 1010–1013.
[11] M.A. Christie, R.L. Webb, A.M. Tickner, Synthesis of cyclohexylidene–xanthenes via the Wittig–Horner reaction, J. Org. Chem. 47 (1982) 2802–2804.
[12] L.F. Fieser, M. Fieser, Reagents for Organic Synthesis, vol. 1, Wiley, New York, 1967, p. 1276.
[13] S.E. Whitebread, V. Taylor, S.P. Bottari, B. Kamfer, M. de Gasparo, Radioiodinated CGP-42112A: a novel high affinity and highly selective ligand for the characterization of angiotensin AT_2 receptors, Biochem. Biophys. Res. Comm. 181 (1991) 1365–1371.