Eur J Med Chem (1992) 27, 527–535

Short communication

Synthesis and pharmacological activities of some pyrido[2,1-*b*]oxazines

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(Received 29 July 1991; accepted 11 February 1992)

Summary — A novel kind of fused heterocyclic compounds, with the pyrido[2,1-*b*]oxazine ring have been prepared and tested for their pharmacologic properties. Some of them have shown long-term antihypertensive-bradycardic effects as well as anti-inflammatory, spasmolytic and other effects.

pyrido-oxazine / antihypertensive activity / bradycardic activity / anti-inflammatory activity / spasmolytic activity

Introduction

In our search for new antihypertensive agents we have recently prepared several oxazolo[3,2-*a*]pyridines that show discrete but prolonged antihypertensive effects accompanied by bradycardia [1]. As a continuation of such research we now report the preparation and evaluation of some substances containing the hitherto undescribed heterocyclic system of pyrido[2,1-*b*]-oxazine (I) that have been assayed *in vitro* on guinea-pig *Tenia coli* and *in vivo* on genetically hypertensive rats.

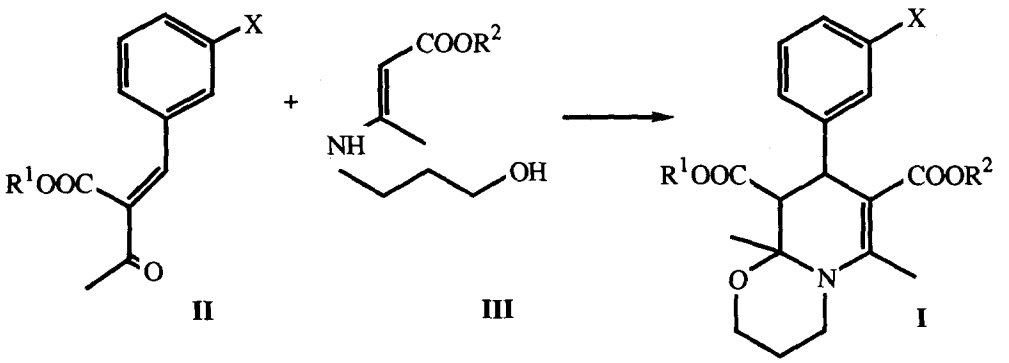
Results and discussion

Chemistry

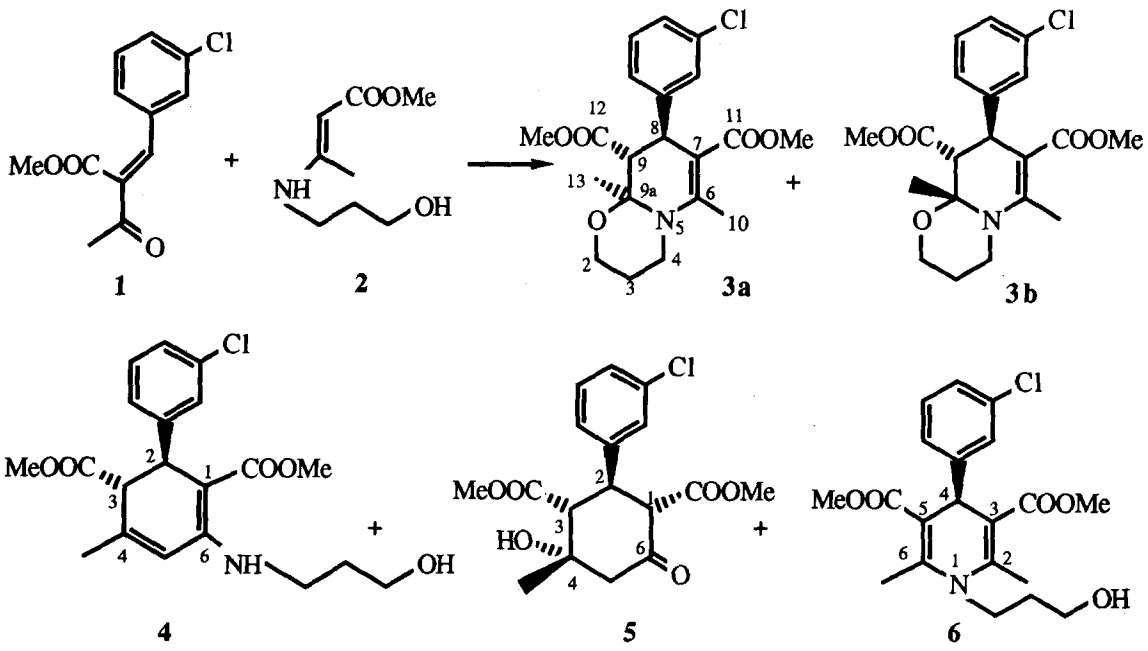
The preparation of pyrido[2,1-*b*]oxazines (I) has been achieved through condensation between conjugated enones of Knoevenagel adducts type II and enamines from 3-aminopropanol and β-ketoesters (III) (scheme 1).

The reaction was carried out by refluxing in methanol over 20–24 h, followed by column chromatography of the crude product and crystallization of the isolated compounds. In all cases the reaction product was a mixture, but the complete structural study of secondary products was performed only for the reaction between **1** and **2** (scheme 2). In this case, the main product was the pyrido-oxazine **3a**, which resulted in a 31% yield, whereas the by products **3b**, **4**, **5** and **6** were obtained respectively, in 0.6, 13, 9 and 6% yields.

The structure of compound **3a** was readily ascertained by comparison of its spectral data with those of some oxazolo[3,2-*a*]pyridines prepared previously [1] and on the basis of the difference between the



Scheme 1.



Scheme 2.

starting enamines in both cases. Its stereochemistry was established in the light of the coupling between H_8 and H_9 (J = 12.3 Hz), which revealed the *anti*-pseudodiaxial disposition of both protons, and of results of some NOE experiments (table I) which demonstrate the spatial proximity of Me_{13} and H_8. Thus, the relative configuration and conformation for **3a** is proposed as depicted in figure 1.

Compound **3b** showed analytical and spectral data very close to those of **3a** and its structure was established through the analysis of the differences in the NMR spectra of both substances, which mainly lay in the shift of the Me_{13} resonance, shielded 0.35 ppm in **3b**, and in the value of the H_8-H_9 coupling (J = 5.2 Hz), which denotes a non-diaxial arrangement for both protons. Contrarily to the case of **3a**, irradiaton of Me_{13} induced NOE upon H_9 instead of H_8 (table I). Thus the structure depicted in figure 1 was proposed for **3b**. Its formation as a very minor product in relation to **3a** agrees with the lesser stability expected for the corresponding cisoid conformer of the Knoevenagel adduct, as well as with the possible occurrence of 1,3-pseudodiaxial interactions between the phenyl and methyl groups in **3b**.

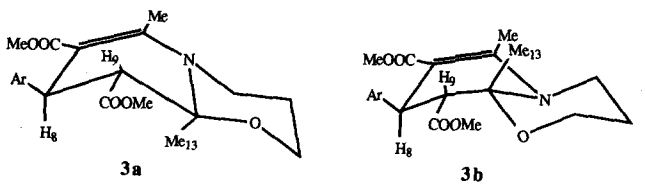


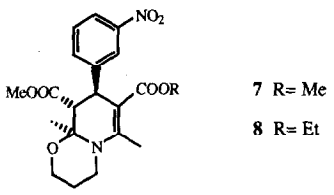
Fig 1.

The structures of compounds **4** and **6** were easily deduced through comparison with two by-products formed in the reaction of the preparation of oxazolo[3,2-*a*]pyridines. The only difference lay in the homologous character of the starting enamines. Compound **5** was identical to the previously reported hydroxyketodiester formed in that reaction [1].

With the aim of testing their pharmacological properties compounds **7** and **8** were prepared, varying the reagents. The reactions coursed in a similar fashion, giving rise to similar by-products and the yield achieved was 21% in both cases (fig 2).

Table I. NOE differences observed for **3a** and **3b**.

Irrad Hi in 3a	*Detected NOEs*
Ar	8, 9
Me_{13}	8
Irrad Hi in 3b	
Ar	8, 9
Me_{13}	9
8	Ar
9	Ar, Me_{13}



7 R= Me

8 R= Et

Fig 2.

Pharmacology

Substances **3a**, **7** and **8** were subjected to several activity assays. Owing to their structural resemblance to the 1,4-dihydropyridines, the antihypertensive and tachycardic effects were tested, as well as other activities considered secondary in nifedipine such as the anti-inflammatory, spasmolytic, anti-ulcerous, antiserotoninic and antihistaminic effects.

Calcium antagonist activity

Compounds **3a**, **7** and **8** were evaluated in guinea pig *Tenia coli* at 10^{-6} M concentration. The substances showed weak activity with pA_2 values of 6.19 (± 0.00), 6.06 (± 0.23) and 6.19 (± 0.01) respectively. In a comparative assay the pA_2 value for nifedipine was 9.49 (± 0.09).

These results are in agreement with the fact that N-substituted 1,4-dihydropyridines are much less active as calcium antagonists [2] than their unsubstituted analogues.

Antihypertensive and bradycardic activity

Compounds **3a**, **7** and **8** were evaluated in genetically hypertensive rats at three or four different oral doses.

As can be observed (table II, fig 3), they showed low antihypertensive effects, much lower than those produced by nifedipine at comparable concentrations. Nevertheless, the maximum effect for nifedipine occurred at, or before, 0.5 h from administration, whereas with pyrido-oxazines the maxima were observed after three or more hours. This fact could be of interest showing that one of the clinical problems with nifedipine is the shortness of its action.

In relation to the overall dose/response curves represented in figure 4, it can be deduced that for compound **3a** the maximum effective dose was not attained, whereas for **7** the maximum effect corresponded to doses between 50 and 100 mg/kg and for the case of **8** doses greater than 50 mg/kg seemed to reduce its antihypertensive effectivity.

Owing to the small calcium antagonistic activity showed by these pyrido-oxazines, their long-term antihypertensive effect could be explained either through their biotransformation into the corresponding 1,4-dihydropyridines or through a different mechanism. The metabolic proposal would be based on the oxidative dealkylation at their C_4-N_5 bond, followed by elimination of a molecule of glycolic acid to generate the second double bond of the 1,4-dihydropyridine.

Regarding the effects on heart rate (table III, figs 5 and 6), compound **3a**, at doses of 25 and 50 mg/kg, produced decrease of rhythm reaching a maximum

Table II. Antihypertensive activity of some pyrido-oxazines, at different oral doses in the genetically hypertensive rat. Statistical evaluation: Student's *t*-test for paired data.

Compound	*Dose (mg/kg)*	*Decrease in systolic arterial pressure in mmHg (X ± SEM) (h since administration)*			
		0.5	1	3	5
3a	100	36 ± 2.5*	57 ± 5.1*	47 ± 6.8*	42 ± 3.6*
	50	15 ± 6.7	20 ± 6.1*	45 ± 3.9*	39 ± 4.8*
	25	16 ± 4.8*	18 ± 7.2	36 ± 7.5*	32 ± 6.0*
7	100	36 ± 7.5*	41 ± 9.0*	69 ± 8.5*	59 ± 7.0*
	50	39 ± 4.0*	53 ± 8.1*	62 ± 7.2*	67 ± 5.1*
	25	22 ± 4.3*	35 ± 2.7*	29 ± 3.7*	30 ± 4.2*
	12.5	27 ± 6.6*	31 ± 4.7*	31 ± 7.4*	27 ± 3.2*
8	100	22 ± 3.3*	22 ± 2.9*	58 ± 6.7*	63 ± 4.1*
	50	46 ± 1.2*	57 ± 3.2*	95 ± 6.7*	90 ± 5.4*
	25	54 ± 15.6*	54 ± 16.1*	56 ± 11.2*	50 ± 9.3*
	12.5	12 ± 3.2*	17 ± 8.6	30 ± 9.8	14 ± 9.0
Nifedipine	10	76 ± 5.4*	74 ± 3.7*	58 ± 5.2*	38 ± 5.4*
	5	61 ± 4.3*	60 ± 3.9*	33 ± 5.0*	15 ± 4.5*
	1	39 ± 4.8*	36 ± 3.9*	16 ± 4.1*	15 ± 3.4*
Control	–	1 ± 1.1	–1 ± 1.7	–5 ± 1.8*	–6 ± 1.9*

*Statistically significant with respect to basal value for $P \leq 0.05$. Number of animals per experimental group: 4–9, 9–10 for nifedine and 24 for the control.

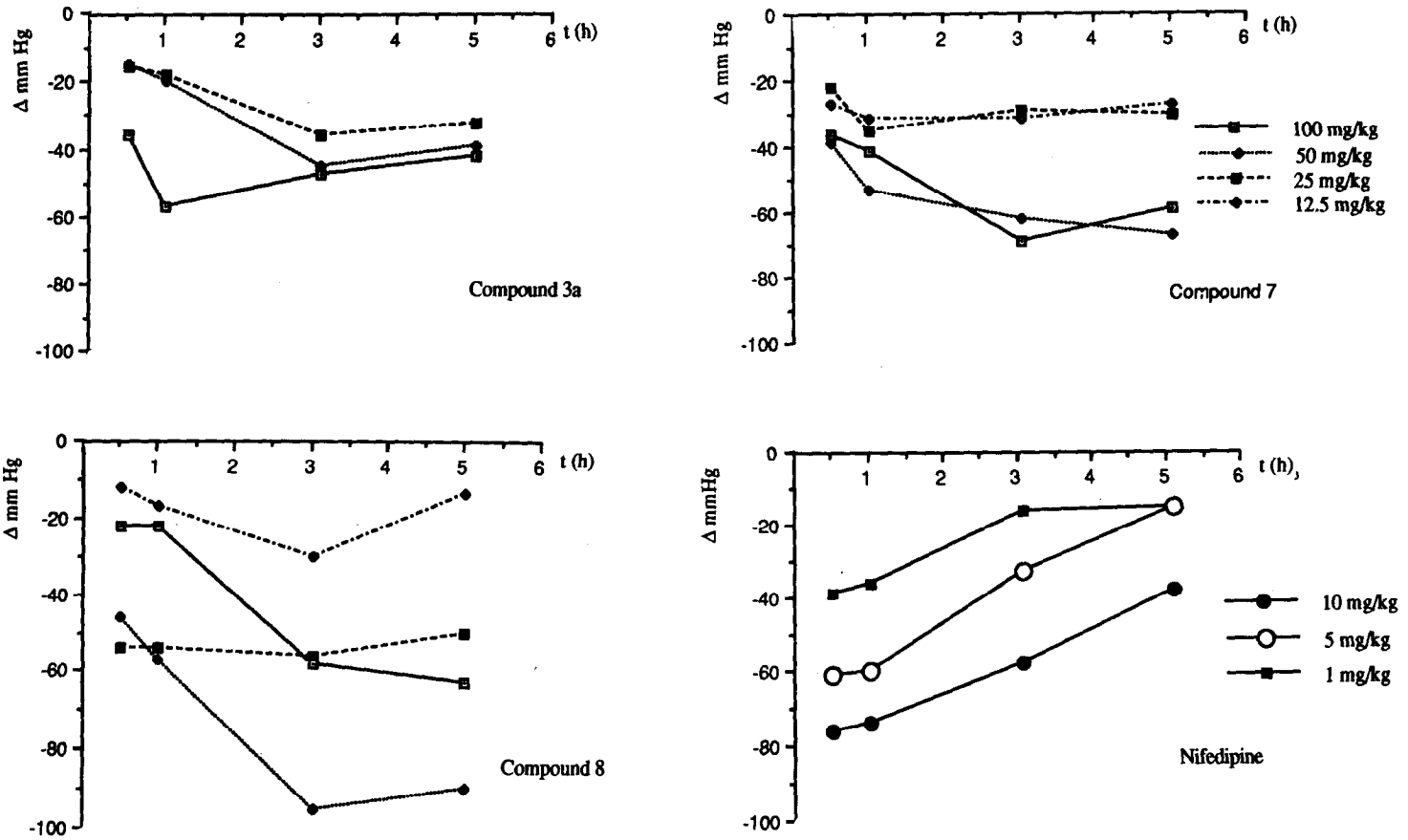


Fig 3. Pressure decrease *vs* time at different doses for pyrido-oxazines **3a**, **7** and **8** and Nifedipine.

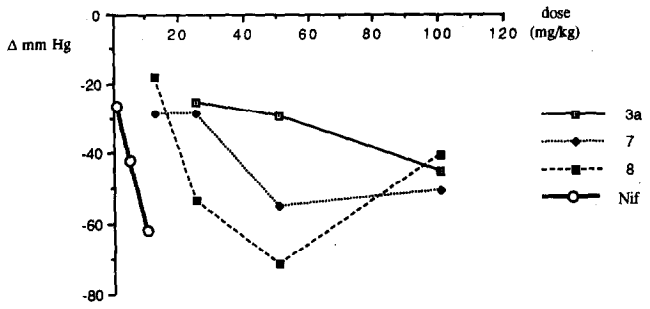


Fig 4. Global dose/response curves (media of all data for each compound) for pyrido-oxazines and nifedipine.

effect for the dose of 25 mg/kg at 3 h from administration. Compound **7** provoked bradycardia at doses of 12.5 and 25 mg/kg but increased tachycardia at doses of 50 and 100 mg/kg. Compound **8** produced irregular effects on the rhythm. For the three substances the maxima of these bradycardic or tachycardic effects occurred at 3 to 5 h. Although not absolute, these facts seem to be in agreement with the metabolic transformation of pyrido-oxazines into nifedipine or its analogues.

Anti-inflammatory activity

Compounds **3a** and **7** were tested for anti-inflammatory activity in the carragenan model and showed important inhibitory effects, particularly compound **7**, which produced an inhibitory effect as high as 74.5% 4 h after treatment (table IV).

Spasmolytic activity

Compounds **3a** and **7** were evaluated for spasmolytic activity in the intestine transient model in mice. They showed activities in of 1/3 and 1/2, respectively to butyl-scopolammonium bromide (table V).

Table III. Activity of pyrido-oxazines on heart rate in genetically hypertensive SHR rats at different oral doses. Statistical evaluation: Student's *t*-test for paired data.

Compound	*Dose (mg/kg)*	*Increase in heart rate in beats/min (X ± SEM) (h since administration)*			
		0.5	1	3	5
3a	100	12 ± 21.5	−12 ± 10.1	20 ± 14.1	10 ± 17.9
	50	−24 ± 27.7	−32 ± 12.4	−28 ± 21.3	−16 ± 16.9
	25	−24 ± 16.0	−16 ± 13.3	−34 ± 25.8	12 ± 15.6
7	100	37 ± 11.0*	67 ± 23.0	95 ± 5.0*	82 ± 5.0*
	50	16 ± 18.1	16 ± 12.9	54 ± 6.0*	38 ± 8.6*
	25	−10 ± 14.1	−18 ± 16.9	− 2 ± 12.8	−30 ± 16.7
	12.5	−22 ± 18.0	−32 ± 16.0	−67 ± 19.3*	−55 ± 13.2*
8	100	55 ± 27.4	52 ± 21.2	66 ± 22.4*	58 ± 21.5
	50	49 ± 10.4*	55 ± 21.8	77 ± 6.3*	32 ± 4.8*
	25	− 2 ± 18.8	− 2 ± 31.2	−25 ± 30.7	−32 ± 27.2
	12.5	12 ± 11.0	17 ± 17.0	12 ± 28.4	35 ± 20.0
Nifedipine	10	42 ± 17.9*	53 ± 8.6*	− 2 ± 9.7	5 ± 9.7
	5	63 ± 8.5*	53 ± 9.3*	5 ± 8.7	14 ± 12.5
	1	12 ± 11.5	3 ± 11.4	− 3 ± 17.6	−19 ± 16.7
Control	–	−10 ± 3.8	− 3 ± 4.7	− 3 ± 5.9	0 ± 4.9

*Statistically significant with respect to basal value for $P \leq 0.05$. Number of animals per experimental group: 4–9, 9–10 for nifedine and 24 for the control.

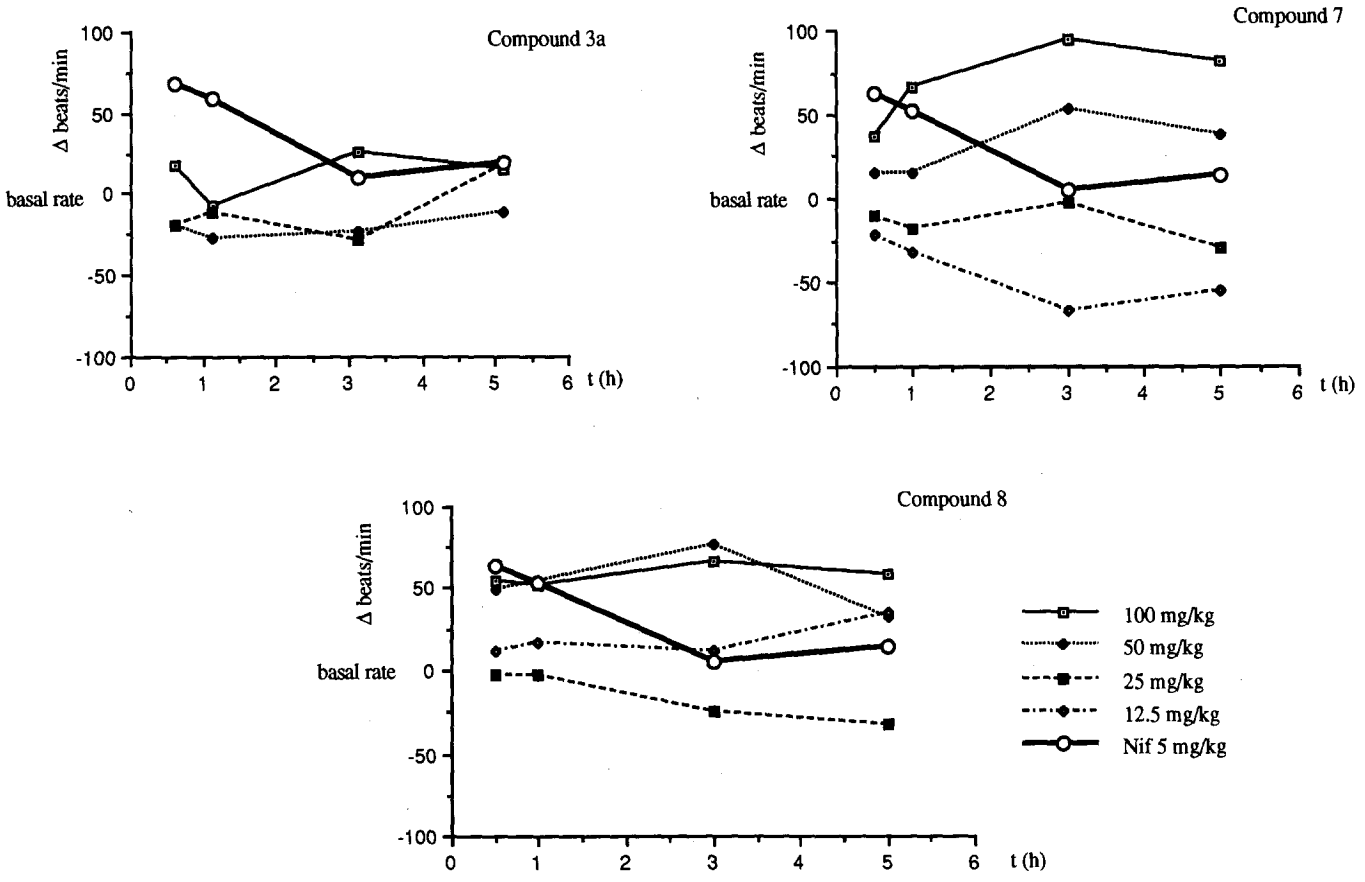


Fig 5. Variation of the influence of pyrido-oxazines and nifedipine on heart rate at different doses.

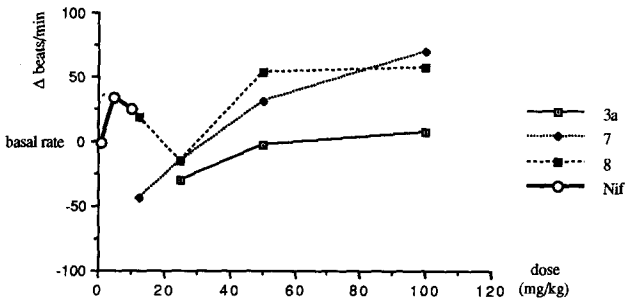


Fig 6. Global dose/heart rate curves (media of all data for each compound) for pyrido-oxazines and nifedipine.

Other activities

Compound **7** also showed some anti-ulcerous and antiserotoninic activity (table VI), whereas compound **3a** showed 45% of the antihistaminic potency of diphenhydramine in the experimental conditions assayed.

Experimental protocols

Chemistry

General experimental information

Mps were determined in capillaries on a Buchi 510 instrument and are uncorrected. UV spectra were recorded in EtOH on a Hitachi 100-60 spectrometer. IR spectra were obtained in KBr disks on a Beckman Acculab VIII spectrophotometer. ^1H NMR (200.13 MHz) and ^{13}C-NMR (50.3 MHz) spectra were measured in $CDCl_3$ or DMSO-d_6 with TMS as internal standard on a Bruker WP 200 SY. δ values are expressed in ppm. EIMS (70 eV) run on a VG-TS-250 mass spectrometer.

Preparation of enamines 2

The enamines were prepared by addition of propanolamine (10 mmol) to the alkyl acetoacetate (10 mmol) with stirring. The reaction mixture was allowed to stand at room temperature for 24 h and was then extracted with CH_2Cl_2, washed with water, dried and evaporated to give the enamines in 95–98% yield.

General procedure of preparation of 3,4,9,9a-tetrahydro-2H, 8H-pyrido[2,1-b]oxazines (3)

A solution of the Knoevenagel adduct **1** (30 g, 125 mmol) and enamine **2** (21.6 g, 125 mmol) in MeOH (100 ml) was refluxed for 24 h. The solvent was removed and the pyrido[2,1-*b*]-oxazines were isolated from the reaction product after chromatographic separation over silica gel and crystallization.

Table IV. Anti-inflammatory activity in the test of carragenine-induced oedema. Statistical evaluation: Duncan's test of analysis of variance.

Compound	*Weight g (X ± SEM)*	*Volume of right hind leg in ml*		*Inflammation*		
		t = 0 h	*t = 4 h*	*ml*	*%*	*% inh*
Control	160 ± 4	1.52 ± 0.03	2.23 ± 0.04	0.74 ± 0.5	47.8 ± 2.4	–
Piroxicam	154 ± 3	1.52 ± 0.02	1.85 ± 0.03	0.33 ± 0.03	22.4 ± 2.0	53.1*
Nifedipine	164 ± 5	1.54 ± 0.05	1.76 ± 0.09	0.21 ± 0.06	13.5 ± 3.4	71.6*
3a	147 ± 8	1.67 ± 0.05	2.05 ± 0.09	0.38 ± 0.06	22.7 ± 3.3	52.5*
7	142 ± 5	1.64 ± 0.05	1.83 ± 0.07	0.19 ± 0.06	12.2 ± 3.9	74.5*

*Statistically significant with respect to the control for $P < 0.05$. The number of animals used was 10 in each case with the exception of the control that included 35.

Table V. Spasmolytic activity of the active carbon pap intestinal transit test. Number of animals per experimental group 10 except the control group and the butylscopolammonium group that included 15. Statistical evaluation: Duncan's test of analysis of variance.

Compound	*Weight g (X ± SEM)*	*Total intest length cm (X ± SEM)*	*Path length (X ± SEM)*		*% inh*
			in cm	*in %*	
Control	29 ± 0.6	55 ± 1.3	32 ± 3.2	58 ± 4	–
Bu-scopol	28 ± 0.7	56.9 ± 0.8	13.3 ± 1.8	23 ± 3	60.0 *
Nifedipine	29 ± 1.1	57.1 ± 0.8	12.1 ± 0.9	21 ± 2	63.45*
3a	29 ± 1.0	58.2 ± 1.4	26.0 ± 2.5	45 ± 4	23.3 *
7	28 ± 1.0	54.5 ± 1.5	21.7 ± 1.5	40 ± 3	31.0 *

*Statistically significant with respect to the control for $P \leq 0.05$. All compounds were administered at a dose of 50 mg/kg.

Table VI. Anti-ulcerous, antiserotoninic and antihistaminic activities. Statistical evaluation carried out with Duncan's test of analysis of variance.

Activity	*Compound*	*Weight g (X ± SEM)*	*No animals*	*Surface area weal/ulcer in mm*	*% inh*
Anti-ulcerous					
	Control	180 ± 4	40	22.32 ± 2.71	–
	Cimetidine	179 ± 4	40	3.45 ± 0.81*	84.54
	Nifedipine	178 ± 7	10	3.60 ± 1.33*	83.87
	3a	184 ± 4	10	19.30 ± 1.33	13.53
	7	227 ± 13	9	14.00 ± 5.34	27.37
Antiserotoninic					
	Control	212 ± 9	17	115 ± 5	–
	Cyproheptadine	296 ± 7	18	14 ± 2	87.8*
	Nifedipine	201 ± 12	6	91 ± 3	20.9*
	3a	182 ± 15	6	112 ± 9	13.5
	7	222 ± 17	6	74 ± 4	37.4*
Antihistaminic					
	Control	164 ± 5	18	89 ± 7	–
	Diphenhydramine	177 ± 5	18	17 ± 3	81.0*
	Nifedipine	167 ± 6	12	81 ± 4	9.3
	3a	211 ± 9	4	17 ± 7	25.6
	7	205 ± 13	5	79 ± 17	11.5

*Statistically significant with respect to the control for $P \leq 0.05$. All compounds were administered at a dose of 10 mg/kg except cimetidine and ciproheptadine, which were administered at 50 mg/kg.

*Dimethyl 8-(3-chlorophenyl)-6,9 a-dimethyl-3,4,9,9a-tetrahydro-2H,8H-pyrido[2,1-b]oxazine-7,9-dicarboxylate (**3a**)*
Yield: 31%, mp = 110–112°C (MeOH). IR: 1730, 1690, 1580 cm^{-1}. ^{1}H-NMR: 1.52 (s, 3H, Me_{13}), 1.60–2.00 (m, 2H, H_3), 2.36 (d, 3H, J = 1.1 Hz, Me_{10}), 2.96 (d, 1H, J = 12.3 Hz, H_9), 3.10–3.20 (m, 2H, H_4), 3,23 (s, 3H, C_7-COO*Me*), 3.47 (s, 3H, C_9-COO*Me*), 3.60–3.80 (m, 2H, H_2), 4.10 (d, 1H, J = 12.3 Hz, H_8), 7.00–7.20 (m, $4H_{arom}$). UV λ max: 295 y 213 nm (ε = 15354 y 10275). Anal calculated for $C_{20}H_{24}ClNO_5$ (C, H, N). ^{13}C-NMR: 12.8 (Me_{10}), 15.9 (Me_{13}), 25.1 (C_4), 39.4 (C_3), 43.9 (C_8), 49.9 (C_7-COO*Me*), 51.4 (C_9-COO*Me*), 58.6 (C_9), 58.9 (C_2), 86.0 (C_{9a}), 100.4 (C_7), 125.2 ($C_{5'}$), 126.2 (C_6), 126.9 ($C_{4'}$), 129.2 ($C_{2'}$), 133.7 (C_6), 146.6 ($C_{3'}$), 151.9 ($C_{1'}$), 168.4 (C_{11}), 171.8 (C_{12}).

*Compound (**3b**)*
Yield: 0.6%, mp 146–148°C (MeOH). IR (CH_2Cl_2): 1730, 1690, 1550, 1470 cm^{-1}. ^{1}H NMR: 1.17 (s, 3H, Me_{13}), 1.20–2.00 (m, 2H, H_3), 2.38 (s, 3H, Me_{10}), 2.99 (d, 1H, J = 5.2 Hz, H_9), 3.20–3.40 (m, 2H, H_4), 3.26 (s, 3H, C_7-COO*Me*), 3.56 (s, 3H, C_9-COO*Me*), 3.60–4.0 (m, 2H, H_2), 4.18 (d, 1H, J = 5.2 Hz, H_8), 7.00–7.20 (m, $4H_{arom}$). Anal calculated for $C_{20}H_{24}ClNO_5$ (C, H, N). ^{13}C NMR: 16.7 (Me_{10}), 20.3 (Me_{13}), 25.1 (C_4), 40.1 (C_3), 40.1 (C_8), 50.5 (C_7-COO*Me*), 51.6 (C_9-COO*Me*), 57.1 (C_9), 60.0 (C_2), 83.4 (C_{9a}), 98.8 (C_7), 125.9 ($C_{5'}$), 126.3 ($C_{6'}$), 127.9 ($C_{4'}$), 129.4 ($C_{2'}$), 134.1 (C_6), 147.1 ($C_{3'}$), 151.7 ($C_{1'}$), 169.6 (C_{11}), 171.2 (C_{12}).

*Dimethyl 2-(3-chlorophenyl)-6-(3-hydroxypropylamino)-4-methylcyclohexa-4,6-diene-1,3-dicarboxylate (**4**)*
Yield: 16%, oil. IR: 3360, 1730, 1600, 1590 cm^{-1}. ^{1}H-NMR: 1.81–1.88 (m, 2H, NH-CH_2-*C*H_2-CH_2OH), 1.83 (s, 3H, Me_7), 3.10 (s, 1H, H_1), 3.52 (s, 3H, C_1-COO*Me*), 3.53 (m, 2H, NH-CH_2-CH_2-*C*H_2OH), 3.54 (s, 3H, C_2-COO*Me*), 3.55 (m, 2H, NH-*C*H_2-CH_2-CH_2OH), 4.54 (s, 1H, H_2), 6.32 (s, 1H, H_5), 7.13–7.16 (m, $4H_{arom}$), 8.90 (t, 1H, J = 6.1 Hz, NH). UV λ max: 230 nm (ε = 9400). Anal calculated for $C_{19}H_{24}ClNO_6$ (C, H, N). ^{13}C-NMR: 24.3 (Me_{17}), 33.3 (NH-*C*H_2-CH_2-CH_2OH), 39.5 (NH-*C* H_2-CH_2-CH_2OH), 40.2 (C_2), 44.0 (C_3-COO*Me*), 50.4 (C_1-COO*Me*), 58.8 (C_1), 59.5 (NH-CH_2-CH_2-*C*H_2OH), 86.1 (C_3), 117.9 (C_5), 125.4 ($C_{6'}$), 126.4 ($C_{4'}$), 127.3 ($C_{2'}$), 129.0 ($C_{5'}$), 133.9 ($C_{3'}$), 142.6 (C_6), 146.5 ($C_{1'}$), 155.2 (C_4), 170.3 (C_{11}), 171.8 (C_{12}).

*Dimethyl 2-(3-chlorophenyl)-4-hydroxy-4-methyl-6-oxocyclohexan-1,3-dicarboxylate (**5**)*
Yield: 9%, mp 164–166°C (MeOH). IR: 3480, 1725, 1600, 1480 cm^{-1}. ^{1}H-NMR: 1.24 (s, 3H, Me_7), 2.37 (d, 1H, J = 13.5 Hz, H_5), 2.94 (d, 1H, J = 13.5 Hz, H_5), 3.34 (s, 3H, C_3-COO*Me*), 3.36 (d, 1H, J = 12.2 Hz, H_3), 3.43 (s, 3H, C_1-COO*Me*), 3.85 (d, 1H, J = 12.6 Hz, H_1), 4.01 (dd, 1H, J_1 = 12.6, J_2 = 12.2 Hz, H_2), 7.20–7.50 (m, $4H_{arom}$). Anal calculated for $C_{17}H_{19}O_6$ (C, H). ^{13}C-NMR: 28.2 (Me_7), 43.7 (C_2), 50.9 (C_3-COO*Me*), 51.4 (C_1-COO*Me*), 53.4 (C_5), 56.1 (C_3), 61.4 (C_1), 72.6 (C_4), 127.1 ($C_{4'}$), 127.1 ($C_{6'}$), 127.7 ($C_{2'}$), 130.0 ($C_{5'}$), 132.8 ($C_{3'}$), 142.8 ($C_{1'}$), 168.6 (C_9), 170.8 (C_8), 202.8 (CO).

*Dimethyl 4-(3-chlorophenyl)-1-(3-hydroxypropyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarboxylate (**6**)*
Yield: 2%, oil. IR (CH_2Cl_2): 3500, 1710, 1680, 1570, 1390 cm^{-1}. ^{1}H-NMR: 1.50–1.54 (m, 2H, NH-CH_2-*C*H_2-CH_2-OH), 2.49 (s, 3H, H_7), 2.49 (s, 3H, H_{10}), 3.20–3.30 (m, 2H, NH-CH_2-CH_2-*C*H_2OH), 3.72 (s, 3H, C_3-COO*Me*), 3.72 (s, 3H,

C_5-COO*Me*), 3.70–3.80 (m, 2H, NH-C*H*$_2$-CH_2-CH_2OH), 5.14 (s, 1H, H_4), 7.04–7.26 (m, 4H_{arom}). ^{13}C-NMR: 16.40 (Me_7), 16.40 (Me_{10}), 33.3 (NH-*C*H_2-CH_2-CH_2OH), 37.7 (C_4), 39.5 (NH-CH_2-*C*H_2-CH_2OH), 51.41 (C_3-COO*Me*), 51.41 (C_5-COO*Me*), 59.5 (NH-CH_2-CH_2-*C*H_2OH), 106.3 (C_3), 106.3 (C_5), 126.2 ($C_{4'}$), 126.8 ($C_{6'}$), 129.5 ($C_{5'}$), 133.7 ($C_{3'}$), 148.0 (C_6), 148.1 (C_2), 149.4 ($C_{1'}$), 166.3 (C_8), 166.3 (C_9).

Dimethyl 8-(3-nitrophenyl)-6,9a-dimethyl-3,4,9,9a-tetrahydro-2H,8H-pyrido[2,1-b]oxazine-7,9-dicarboxylate (7)
Yield: 21.1%, mp 147–148°C (MeOH). IR: 1725, 1690, 1580 cm^{-1}. ^{1}H-NMR: 1.56 (s, 3H, Me_{13}), 1.60–2.40 (m, 2H, H_3), 2.42 (d, 3H, J = 1.5 Hz, Me_{10}), 3.01 (d, 1H, J = 12.3 Hz, H_9), 3.20–3.40 (m, 2H, H_4), 3.22 (s, 3H, C_7-COO*Me*), 3.45 (s, 3H, C_9-COO*Me*), 3.80–3.90 (m, 2H, H_2), 4.27 (d, 1H, J = 12.3 Hz, H_8), 7.40–8.00 (m, 4H_{arom}). MS *m/z* (%): 404 (28), 389 (8), 373 (21), 345 (100), 286 (87), 210 (28), 196 (6), 69 (60). Anal calculated for $C_{20}H_{24}N_2O_7$ (C, H, N). ^{13}C-NMR: 15.9 (Me_{13}), 12.9 (Me_{10}), 24.9 (C_4), 39.3 (C_3), 43.7 (C_7-COO*Me*), 51.4 (C_9-COO*Me*), 58.4 (C_9), 58.9 (C_2), 85.8 (C_{9a}), 99.2 (C_7), 121.1 ($C_{4'}$), 121.6 ($C_{5'}$), 128.3 ($C_{6'}$), 133.3 ($C_{2'}$), 147.1 (C_6), 148.1 ($C_{1'}$), 152.9 ($C_{3'}$), 167.8 (C_{11}), 171.3 (C_{12}).

Ethyl 8-(3-nitrophenyl)-6,9a-dimethyl-9-carbomethoxy-3,4,9,9a-tetrahydro-2H,8H-pyrido[2,1-b]oxazine-7-carboxylate (8)
Yield: 21%, mp = 135–136°C. IR: 1725, 1680, 1580 cm^{-1}. ^{1}H-NMR: 0.74 (t, 3H, J = 7.1 Hz, CH_2-*C*H_3), 1.55 (s, 3H, Me_{13}), 1.91 (m, 2H, H_3), 2.42 (s, 3H, H_{10}), 2.99 (d, 1H, J = 12.4 Hz, H_9), 3.20–3.30 (m, 2H, H_2), 3.80–4.00 (m, 2H, H_1), 3.46 (s, 3H, C_7-COO*Me*), 3.71 (c, 2H, J = 7.1 Hz, *C*H_2-CH_3), 4.27 (d, 1H, J = 12.4 Hz, H_8), 7.30–8.00 (m, 4H_{arom}). MS *m/z* (%): 418 (36), 387 (9), 373 (19), 359 (89), 345 (28), 314 (4), 282 (100), 224 (41), 128 (17), 59 (41). Anal calculated for $C_{21}H_{27}N_2O_7$ (C, H, N). ^{13}C-NMR: 13.5 (CH_2-*C*H_3), 13.7 (Me_{10}), 16.2 (Me_{13}), 25.2 (C_4), 39.5 (C_3), 44.1 (C_7), 51.6 (C_7-COO*Me*), 59.1 (C_2), 58.7 (C_9), 53.9 (*C*H_2-CH_3), 86.1 (C_{9a}), 121.3 ($C_{5'}$), 122.1 ($C_{4'}$), 128.9 ($C_{6'}$), 133.6 ($C_{2'}$), 147.4 (C_6), 148.3 ($C_{1'}$), 153.1 ($C_{3'}$), 167.8 (C_7-*C*OOMe), 171.7 (C_9-*C*OOMe).

Pharmacology

Calcium antagonist activity
Male albino Dunkin-Hartley guinea-pigs weighing about 300 g, supplied by Biocentre were used. Essentially, the method described by Hashimoto *et al* [3] was used for *in vitro* studies on *Tenia coli*. All compounds were assayed at 10^{-6} M concentration in Krebs-Henseleit media with 0.04% EtOH.

Antihypertensive and bradycardic activities
Genetically hypertensive female rats of the SHR strain older than 10 weeks, supplied by Mollegaard (Copenhagen) through Interfauna Ibérica SA, were used. The method described by Ishii H *et al* [4] was followed. Statistical evaluation was carried out using Student's *t*-test for paired values. The compounds assayed were administered orally through a stomach tube and were vehicled in an aqueous solution of carboxymethylcellulose and Tween 80, both at 0.1%. Substances **3a**, **7** and **8** were administered at doses of 100, 50, 25 and/or 12.5 mg/kg, Nifedipine was administered at doses of 10, 5 and 1 mg/kg.

Anti-inflammatory activity
Male Sprague-Dawley rats weighing 140–160 g were used. Essentially, the method of Winter was followed [5]. The anti-inflammatory activity as compared with nifedipine and piroxicam was evaluated in the subplantar oedema by carragenine in the rat [6]. Statistical evaluation was made on the relative arithmetic means. For each group the Duncan test [7] of analysis of variance for a P value of ≤ 0.05 was applied.

The compounds assayed were administered orally in an aqueous solution of carboxymethylcellulose and Tween 80, both at 0.1%, at doses of 100 mg/kg except piroxicam, which was administered at 10 mg/kg. The carragenine was administered at 1% in physiological solution by subplantar injection in the right hind foot of the animals at a dose of 0.1 ml per animal.

Piroxicam (Chemo Ibérica SA Batch 8812). Lambda-type Carragenine (Sigma C-3889).

Spasmolytic activity
Male Swiss mice of about 30 g were used. The method of Janssen *et al* was followed [8]. The total length of the small intestine was measured from the pylorus to the caecum and the length of the trajectory followed by an active carbon pap. The values were used to calculate the trajectory followed by the pap considering the total path as 100 and then the percentage of inhibition with respect to the control. The Duncan test of analysis of variance was applied to the value of the percentage run. Compounds **3a** and **7**, scopolammonium bromide and nifedipine were administered peritoneally at a dose of 50 mg/kg in a solution of carboxymethylcellulose and Tween 80, both at 0.1% in physiological solution. The activated charcoal was administered in the form of a suspension at 10% in a solution of gum arabic (5%) through a stomach tube. Scopolamine (butyl N-bromide) (Sigma, Batch S-7882). Activated charcoal (Abelló).

Antiulcerous activity
Male Sprague-Dawley rats weighing 180–220 g were used. The assay was performed following the protocol reported by Bhargava *et al* [9] modified by Takeda M *et al* [10]. Evaluation of the ulcers was carried out according their length in mm. For each group, the mean, the standard deviation and the mean standard error were calculated. The percentage of inhibition with respect to the control group was calculated. The Duncan test was applied. The compounds were administered orally through a stomach tube in an aqueous solution of carboxymethylcellulose and Tween 80, both at 0.1% at a dose of 100 mg/kg, except cimetidine, which was at 50 mg/kg. Indomethacin was injected intraperitoneally at a dose of 20 mg/kg.

Cimetidine (Kerna Española, Batch M-50/85), indomethacin (Sigma, Batch I-7378).

Antiserotoninic activity
Female Sprague-Dawley rats weighing 150–200 g. The assay was essentially the same as that performed for histamine [11, 12]. The antiserotoninic activity was evaluated in comparison with nifedipine and cyproheptadine, in the experimental model that employs skin weals induced by serotonin in the Sprague-Dawley rat. The surface area of each weal was measured in mm^2 together with the mean of this for each rat. The statistical analysis used the Duncan test of analysis of variance. The substances were administered at a dose of 100 mg/kg orally through a stomach tube in an aqueous suspension of carboxymethylcellulose and Tween 80, both at 0.1%. Cyproheptadine was administered at a dose of 15 mg/kg and serotonin was administered intradermally in a 0.9% saline solution. Serotonin creatine sulfate (Fluka AG, 281217 988).

Antihistaminic activity

Female Sprague-Dawley rats weighing 150–180 g were used. The classic method in animal pharmacology for the evaluation of antihistaminic substances was employed [11, 13]. The activity of the substances was evaluated in comparison with nifedipine and diphenhydramine by the experimental histamine-induced skin weal model for the Sprague-Dawley rat. The surface area of each weal was calculated in mm^2 as was the mean of this value per rat. Statistical evaluation was performed with Duncan's test of analysis of variance. All substances were administered at a dose of 100 mg/kg orally through a stomach tube in an aqueous solution of carboxymethylcellulose and Tween 80, both at 0.1%. The histamine was injected intradermally.

Diphenhydramine hydrochloride (Aldaba Juliá). Histamine hydrochloride (Merck, Batch 707K4054770).

In all cases the animals were kept under constant stabling conditions at a temperature of 22 ± 2°C at a relative humidity of 60% and a controlled light-dark cycle. They were fed a standard diet for rodents (UAR Panlab) and had access to tap water *ad libitum*.

Nifedipine (IESEBE, SA, no of analysis at Dr Andreu Lab 88124).

Acknowledgments

The authors would like to express their gratitude to Dr B Macías (Departamento de Química Inorgánica, Facultad de Farmacia, Salamanca) for the micro-analytical determinations and to Dr JM Hernandez for MS measurements.

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