Eur J Med Chem (1993) 28, 683–691

Synthesis and bradycardic activity of a series of substituted 3-aminoalkyl-2,3-dihydro-4*H*-1,3-benzoxazin-4-ones as potent antiischemics

JP Rieu[1], A Duflos[2], JC Tristani[2], JF Patoiseau[1], J Tisne-Versailles[4], AM Bessac[3], R Bonnafous[3], A Marty[3], Y Verscheure[3], DCH Bigg[2]

[1]*Division Chimie Médicinale II, Centre de Recherches Pierre-Fabre, 17, avenue Jean-Moulin, Castres;*
[2]*Division Chimie Médicinale I, Centre de Recherches Pierre-Fabre, 17, avenue-Jean-Moulin, Castres;*
[3]*Division Pharmacologie Cardiovasculaire II, Centre de Recherches Pierre-Fabre, 17, avenue Jean-Moulin, 81106 Castres Cedex;*
[4]*Projets cardiovasculaires, Pierre Fabre Médicament-Développement, rue Jean Rostand, BP 687, 31319 Labege Innopole Cedex, France*

(Received 4 December 1992; accepted 6 April 1993)

Summary — A series of 2,3-dihydro-4*H*-1,3-benzoxazin-4-ones substituted at the 3-position with an arylalkyaminoalkyl group have been synthesized and their specific bradycardic and antiarrythmic activities evaluated. Bradycardia is optimal when the aromatic rings are substituted with methoxy groups and the side-chain amine is *N*-methylated. *N*-Demethylated and 2-alkylated derivatives show the highest antiarrhythmic activity but have little bradycardic action. Most of the compounds described are more potent than Falipamil. Compound **1m** (F 3226) has been selected for further pharmacological tests and the bradycardic activity has been confirmed when administered orally to anesthetized rats without notable side effects.

3-aminoalkyl-2,3-dihydro-4*H*-1,3-benzoxazine-4-ones / bradycardic, antiarrhythmic and antiischemic agents

Introduction

One approach to treating myocardial ischemia is to lower heart rate in order to reduce myocardial oxygen consumption [1, 2]. The most frequently used bradycardic agents to treat this disease are the β-adrenoceptor antagonists [3] and certain types of calcium antagonists [4]. However, for most of these derivatives bradycardia is associated with negative inotropic effects and hypotension. Recently, 2 new classes of bradycardic agents devoid of negative inotropic or hypotensive activity, and possessing full specific negative chronotropic activity have been found. These are aminoimidazoline derivatives such as alinidine, and benzolactams such as falipamil, zatebradine (UL-FS49) and cilobradine (DK-AH-3) (fig 1). These new compounds have been the subject of recent pharmacological attention [5, 6] and are presently undergoing clinical studies. Protective effects have been shown in angina resulting from effort, with Alinidine, Falipamil or Zatebradine [7–9]. Alinidine reduces emotional tachychardia [10] and the frequency of anginal episodes. Furthermore, it potentiates Atenolol bradycardia [10] and decreases reflex tachycardia induced by Nifedipine and Trinitrine [11]. Such compounds therefore have therapeutic potential in the treatment of chronic stable angina and heart failure. We describe in this paper the synthesis and pharmacological properties of a series of 4*H*-1,3-benzoxazine-4-ones of general formula **1** [12, 13] which have potent bradycardic and antiarrhythmic properties.

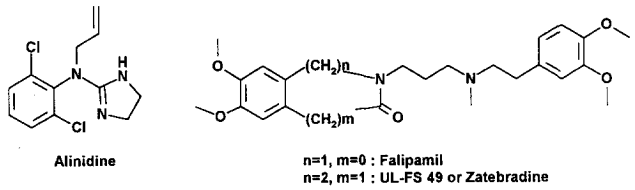
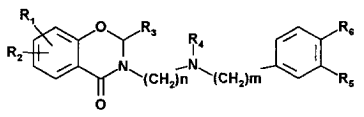


Fig 1. Structures of falipamil, zatebradine and cilobradine.



Formula 1.

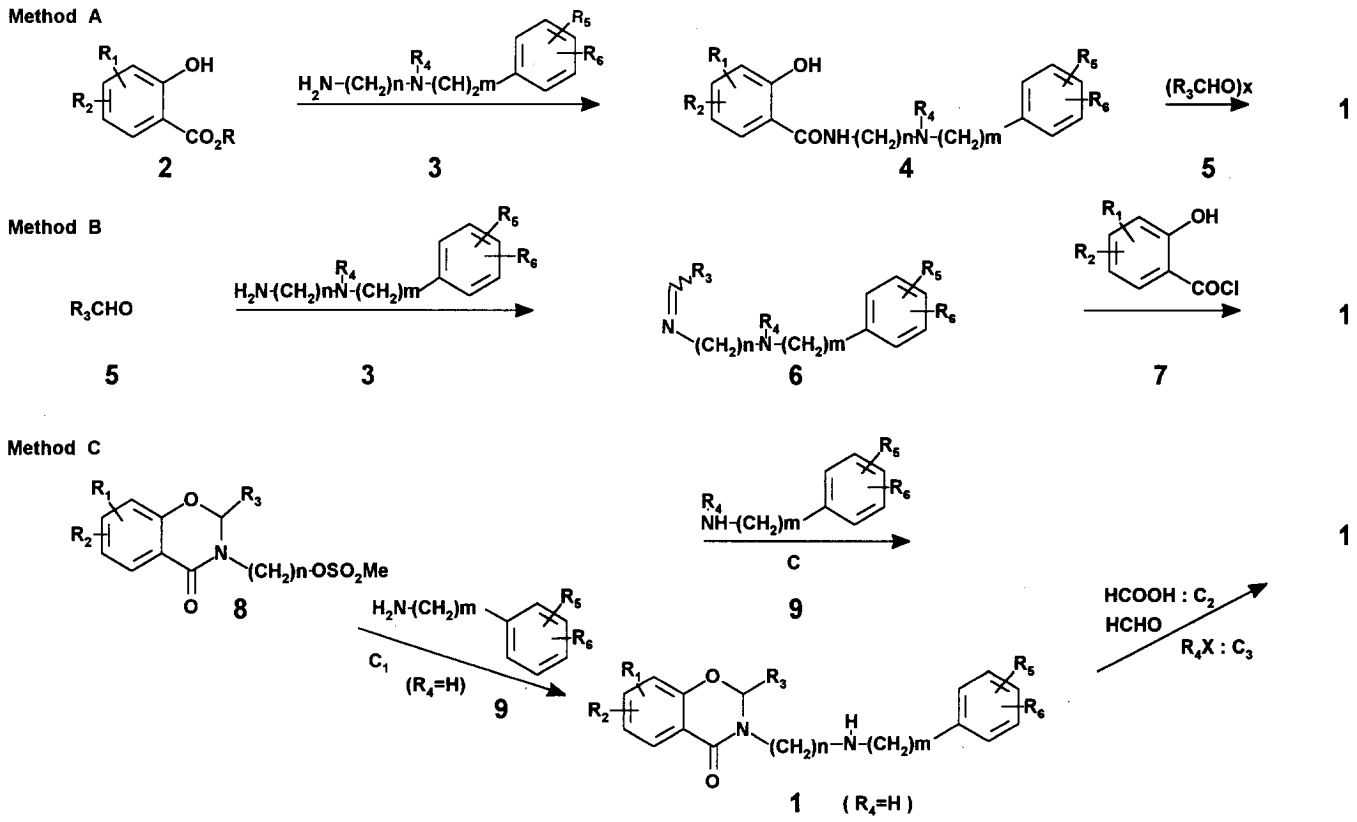
Chemistry

2,3-Dihydro-4*H*-1,3-benzoxazin-4-ones **1** were prepared according to the methods shown in scheme 1. In *Method A*, reaction of alkyl salicylates **2** with diamines **3** [14–16] afforded the salicylamides **4** which were converted to compounds **1** by condensation with an aldehyde (or a precursor) [14, 17]. No cyclisation was, however, observed in the case of aromatic aldehydes. The 2-aryl benzoxazin-4-ones (**1** R_3 = aryl) were prepared according to *Method B*. Thus Schiff bases obtained by classical procedures [18], afforded **1** (R_3 = aryl) on reaction with acid chlorides **7** [19]. Compounds **1** (R_4 = 4) could also be prepared *via* the mesylates **8**, accessible from the appropriate alcohols [17], by reaction with phenethylamines **9**, and *N*-alkyl derivatives were prepared by alkylation or by Eschweiler–Clarke methylation [20, 21] as the direct alkylation of *N*-alkyl phenethylamine gave lower yields (*Method C*). The chemical structures of the compounds described in this paper, their method of preparation and the melting point of their salts are shown in table I.

Pharmacology

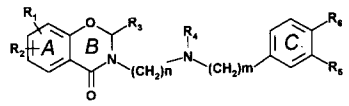
Pentobarbitone-anaesthetized rats were used to study the effect of the compounds on heart rate. Variations in heart rate were recorded 1 min after injection and expressed as percentage changes.

The ligature of coronary artery according to Clark *et al*'s model [22] induced dysrythmia in anaesthetized rats with artificial ventilation. The protective activity of compounds indicated their anti-ischemic and/or antidysrhythmic properties. The results are expressed as percentage protection. The compound **1m** (F3226) was selected for further evaluation. Thus, the bradycardic effect was studied using awake normotensive rats after oral gavage. The effect of bradycardia on haemodynamic activity was also explored using the anaesthetized dog.



Scheme 1.

Table I. Physicochemical and pharmacological properties of compounds **1**.



Comp n°	R_1	R_2	R_3	R_4	R_5	R_6	n	m	Meth.	Yield %	Formula of salt	m.p. °C	F.C.[a]	A.A.[b]
1a	H	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	68	$C_{22}H_{28}N_2O_4$, HCl	168	-17	-9
1b	6-CH_3	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	59	$C_{23}H_{30}N_2O_4$, HCl	147	-26	-5
1c	7-CH_3	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	35	$C_{23}H_{30}N_2O_4$, HCl	148	-18	-1
1d	8-CH_3	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	50	$C_{23}H_{30}N_2O_4$, HCl	149	-16	-43
1e	6-CH_3O	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	56	$C_{23}H_{30}N_2O_5$, HCl	142	-23	-21
1f	7-CH_3O	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	C	30	$C_{23}H_{30}N_2O_5$, HCl	148	-28	-71
1g	8-CH_3O	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	25	$C_{23}H_{30}N_2O_5$, HCl	143	-15	-22
1h	6-Cl	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	C_2	82	$C_{22}H_{27}ClO_4N_2$, HCl	150	-19	+2
1i	7-Cl	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	35	$C_{22}H_{27}ClN_2O_4$, HCl	158	-16	-37
1j	6-Br	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	45	$C_{22}H_{27}BrN_2O_4$, HCl	159	-16	-1
1k	6-NO_2	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	C_2	88	$C_{22}H_{27}N_3O_6$,$C_2H_2O_4$	120	-10	+77
1l	6-AcNH	H	H	CH_3	3-CH_3O	4-CH_3O	3	2	*		$C_{24}H_{31}N_3O_5$, HCl	192	-5	-47
1m	6-CH_3O	7-CH_3O	H	CH_3	3-CH_3O	4-CH_3O	3	2	A	40	$C_{24}H_{32}N_2O_6$, HCl	174	-32	-38
									C_2	90				
									C	50				
1n	6-Cl	8-Cl	H	CH_3	3-CH_3O	4-CH_3O	3	2	C_2	85	$C_{22}H_{26}N_2Cl_2O_4$, HCl	139	-12	-9
1o	6-CH_3O	7-CH_3O	CH_3	CH_3	3-CH_3O	4-CH_3O	3	2	A	70	$C_{25}H_{34}N_2O_6$, HCl	149	-11	-50
1p	6-CH_3O	7-CH_3O	$CH_3(CH_2)_6$	CH_3	3-CH_3O	4-CH_3O	3	2	A	58	$C_{31}H_{46}N_2O_6$, HCl	117	-5	-72
1q	6-CH_3O	7-CH_3O	C_6H_5	CH_3	3-CH_3O	4-CH_3O	3	2	B	12	$C_{30}H_{36}N_2O_6$, HCl	161	-10	-63
1r	6-CH_3O	7-CH_3O	H	H	3-CH_3O	4-CH_3O	3	2	C_1	70	$C_{23}H_{30}N_2O_6$, HCl	155	-10	-66
1s	6-CH_3O	7-CH_3O	H	C_7H_7	3-CH_3O	4-CH_3O	3	2	C_3	68	$C_{30}H_{36}N_2O_6$, HCl,H_2O	75	-5	-16
1t	6-CH_3O	7-CH_3O	H	CH_3	H	H	3	2	A	85	$C_{22}H_{28}N_2O_4$, HCl	174	-11	-47
1u	6-CH_3O	7-CH_3O	H	CH_3	H	4-Cl	3	2	C_2	71	$C_{22}H_{27}ClN_2O_4$, HCl	190	-13	-14
1v	6-CH_3O	7-CH_3O	H	CH_3	H	4-CH_3O	3	2	C_2	85	$C_{23}H_{30}N_2O_5$, HCl	188	-22	-28
1w	6-CH_3O	7-CH_3O	H	CH_3	H	H	3	3	C_2	78	$C_{23}H_{30}N_2O_4$, HCl	150	-21	+5
1x	6-CH_3O	7-CH_3O	H	CH_3	H	H	3	4	C_2	96	$C_{24}H_{32}N_2O_4$, HCl	171	-13	-75
1y	6-CH_3O	7-CH_3O	H	CH_3	3-CH_3O	4-CH_3O	2	2	C	45	$C_{23}H_{30}N_2O_6$, HCl	140	-5	-29
1z	6-CH_3O	7-CH_3O	H	CH_3	3-CH_3O	4-CH_3O	4	2	C_2	90	$C_{25}H_{34}N_2O_6$, HCl	160	-25	-72
falipamil													-17	-35[c]
zatebradine													-17	-42

[a]% variation in heart rate in the anaesthetized rat 1 min after intravenous injection (1 mg·kg^{-1}); [b]% variation in dysrythmia after coronary ligation in the anaesthetized rat after intravenous injection (2.5 mg·kg^{-1}); [c]at 5 mg·kg^{-1} iv; *see *Experimental*

Results and discussion

The chronotropic and antiarrhythmic activities of 1,3-benzoxazin-4-ones are shown in table I. The comparison of biological data for derivatives **1a–1n** illustrates the influence of aromatic ring A substituents. In the case of monosubstitution, the presence of an electron donating group such as methyl (**1b–1d**) or methoxy (**1e–1g**) increased bradycardia, whereas antiarrhythmic activity was more influenced by a methoxy group. The presence of halogens (**1h–1i**) had little effect on frequency, while improvement in cardiac electrical disturbances was observed only in the case of a substitution in the 7 position. A nitro group (**1k**) or an acetamido group (**1l**) negatively influenced the 2 cardiac parameters studied. The low aqueous solubility of compound **1k** required dissolution in DMSO which, by itself, can affect the antiarrhythmic data. Disubstitution by methoxy groups (**1m**) increased the antiarrhythmic effect and provided optimal bradycardic action as in the benzazepinone series [23]. Two chlorine atoms (**1n**) were unfavorable for activity. The presence of an alkyl or aryl group in position 2 of ring B (**1o–1q**) increased the antiarrhythmic activity but with loss of bradycardia. The replacement of an *N*-methyl group (**1m**) by an *N*-benzyl group (**1s**) or an NH (**1r**) reduced the bradycardic activity, in contrast to the benzazepinone series [23]. Substitution of ring C (compounds **1m**, **1t–1w**) induced similar biological effects to those observed for ring A, with potent bradycardic activity observed for the dimethoxy compound **1m** (homoveratrylamine derivative). The influence of the chain length between the nitrogen of the amine function and the aromatic ring (derivatives **1t**, **1w**, **1x**) was contradictory: the bradycardic effect was optimal for **1w** ($n = 3$), but was associated with a decrease in antiarrhythmic activity. Finally, a hydrocarbon chain with 2 carbon atoms between the 2 nitrogen atoms (**1y**) decreased activity while dysrhythmia was minimal with $n = 3$ (compound **1m**).

Most of the compounds studied showed bradycardic activity equal or superior to that of Falipamil and Zatebradine. Compound **1m** (F3226) which had

Table II. Effects of compound **1m** (F3226) and Falipamil on heart rate and arterial pressure in the conscious normotensive rat.

Compounds and doses : Days		Heart rate (bts/min)[a]					Arterial pressure (mm Hg)[a]				
		T0	+ 1 h	+ 2 h	+ 4 h	+ 6 h	T0	+ 1 h	+ 2 h	+ 4 h	+ 6 h
	1	370	304	282	292	304	117	112	111	110	114
		(10)	(8*)	(7*)	(7*)	(7*)	(3)	(3)	(3)	(3)	(3)
1m (F 3226)	3	372	310	314	308	312	115	118	116	111	105
50 mg/kg/j PO		(8)	(10*)	(10*)	(8*)	(7*)	(2)	(2)	(3)	(4)	(3*)
	5	350	310	310	320	308	116	113	115	111	116
		(10)	(7*)	(8*)	(9)	(7*)	(4)	(4)	(3)	(2)	(3)
Falipamil	1	398	374	376	376	372	140	136	142	137	131
50 mg/kg PO		(5)	(8*)	(6)	(8)	(6*)	(3)	(3)	(4*)	(2)	(6)
Zatebradine	1	360	246	240	244	262	121	107	108	104	105
50 mg/kg		(11)	(7*)	(8*)	(8*)	(9*)	(3)	(2*)	(3*)	(4*)	(3*)

[a]Means ± SEM; *Student's *t*-test.

the best profile was selected for further testing. The pharmacological profile of this compound is shown in tables II and III.

The bradycardia was confirmed after oral administration in the normotensive rat (table II). F3226 induced a decrease in heart rate with a rapid onset of action. This effect was maintained after chronic treatment (5 d). After the first administration, F3226 decreased the heart rate more strongly than Falipamil (–24% *versus* –7%). Zatebradine induced the most potent bradycardia (–33%). But contrary to the 2 other compounds, Zatebradine showed a statistically

Table III. Effects of compound **1m** (F3226) (1 mg/kg iv) on cardiovascular haemodynamic in the anaesthetized dog[a].

Parameters	Time in minutes								
	0	1	5	15	30	60	120	180	240
Mean arterial pressure (mm Hg)	143 (11)	136 (10)	133 (10)	133 (10)	138 (10)	140 (11)	141 (10)	143 (9)	142 (11)
Heart rate (beats/min)	135 (4)	112 (4*)	87 (2*)	89 (3*)	91 (2*)	100 (4*)	113 (6*)	120 (4*)	126 (4)
(+) dp/dt max. (mm Hg/sec)	3108 (289)	2941 (238)	2977 (320)	2857 (278)	2867 (252)	2868 (322)	3186 (208)	3221 (160)	3259 (91)
Left ventricular end diastolic pressure (mm Hg)	11.5 (1.6)	14.6 (1.2)	16.2 (2.7)	16.1 (3.0)	16.5 (3.9)	16.2 (2.5)	16.6 (3.8)	14.1 (1.5)	15.5 (2.5)
Cardiac output (l/min)	4.4 (0.3)	4.6 (0.1)	4.3 (0.2)	4.4 (0.2)	4.3 (0.3)	4.6 (0.1)	4.7 (0.3)	4.6 (0.2)	4.8 (0.1)
Stroke volume (ml)	32.6 (2.5)	41.3 (2.0*)	49.2 (3.2*)	49.5 (2.8*)	46.9 (2.9*)	46.4 (4.3*)	42.2 (1.8)	38.1 (1.2)	38.4 (1.3)
Total peripheral resistance (dynes/sec/cm-5)	2714 (358)	2381 (141)	2490 (123)	2467 (242)	2635 (245)	2439 (172)	2403 (158)	2524 (103)	2365 (152)
Buckberg index	1.02 (0.11)	1.39 (0.16)	1.88 (0.14*)	2.17 (0.54)	1.64 (0.17*)	1.36 (0.08*)	1.26 (0.08*)	1.17 (0.11)	1.09 (0.07)
Katz index	19358 (2025)	15202 (1214*)	11595 (828*)	11947 (1188*)	12648 (1074*)	14178 (1496*)	16026 (1729*)	17236 (1620)	17945 (1462)

[a]Means ± SEM; *Student's *t*-test.

significant hypotensive action, which lasted more than 6 h. At the doses studied, none of the compounds induced adverse effects.

Table III shows the haemodynamic activity of F3226 in the anaesthetized dog. The compound decreased the heart rate and the effect was statistically significant for 3 h. This bradycardic activity was not associated with a myocardial depressive action, since the index of contractility (+ dp/dt max) and the left ventricular end-diastolic pressure was not significantly increased. The cardiac output was not decreased and an improvement in the ejection fraction was shown. The Buckberg index increased strongly and showed an improvement in transmural coronary blood flow. The oxygen consumption was decreased as shown by the Katz index. F3226 was able to decrease the heart rate without reducing the myocardial contractility and improved the myocardial oxygen delivery by an increase in diastolic time. Experiments in the anaesthetized dog showed that F3226 did not modify the intracardiac conduction times. Its sole action was to lengthen the electric diastole, that part of the cycle during which the coronary blood flow is maximal. Cellular electrophysiological studies showed a specific action on the sinoatrial node [13].

In conclusion, 2,3-dihydro-4*H*-1,3-benzoxazin-4-ones substituted with basic groups in the 3 position constitute a new family of specific bradycardic agents. Among these derivatives, F3226 has shown particularly attractive antiischemic properties in animal models; complementary biological tests are in progress.

Experimental protocols

Chemistry

Elemental analysis of the compounds was carried out by the Analytical Chemistry Department at the Pierre Fabre Research Centre; results obtained were within ± 0.4% of the theoretical values. ^{1}H-NMR spectra were recorded on a Bruker AC spectrometer at 200 MHz and chemical shifts were expressed in ppm relative to tetramethylsilane. IR spectra were recorded on a Perkin–Elmer 1600 spectrograph, series FT-IR. Melting points were determined using a Köfler block and were not corrected. TLC was carried out on Merck silica gel 60 (F254) precoated aluminium plates using a mixture of chloroform/methanol/33% ammonia (90:09:01) as eluent. R_f were calculated after exposure to 254 nm UV radiation and by dyeing with ninhydrin spray and heating to 150°C. The following examples represent a typical procedure for the different methods of preparation.

Method A

2,3-Dihydro-6,7-dimethoxy-3-[3-(methyl-phenethylamino)-propyl]-4H-1,3 benzoxazin-4-one hydrochloride ***1t***

4,5-Dimethoxy-N-[3-(methyl-phenethylamino)propyl] salicylamide ***4***. A mixture of 3-(methyl-phenethylamino) propylamine (6.3 g, 25 mmol) and methyl dimethoxy-4,5 salicylate (3.96 g, 25 mmol) was heated at 120°C for 2 h. After cooling to room temperature, the mixture was stirred with a solution of hydrochloric acid (1 N, 30 ml) in water (250 ml). After extraction with ether (3 x 70 ml) the aqueous phase was basified with a saturated solution of sodium bicarbonate (200 ml). The substituted salicylamide was extracted with methylene chloride, washed with water (2 x 100 ml), brine (50 ml) and dried (Na_2SO_4). Concentration of the organic extract gave a light yellow oil which was used for the next step without further purification, m: 5.32 g (68%).

1,3,5-Trioxane (4.3 g, 47.5 mmol) was added portionwise over 1 min to a mixture of 4,5-dimethoxy-*N*-[-3-(methyl-phenethylamino)propyl]salicylamide (4.4 g, 13 mmol), glacial acetic acid (50 ml), and 2 N hydrogen chloride solution in ethyl acetate (50 ml). The solution was stirred, slowly heated to 45–50°C and kept at this temperature for 2 h. The reaction mixture was allowed to cool and the solvent evaporated under reduced pressure. The residue was poured into iced water and basified to pH 9 by addition of caustic soda, and extracted with ethyl acetate. The extracts were washed with water, brine and dried (Na_2SO_4). Filtration and evaporation of the solvent yielded a viscous oil which was dissolved in a small amount of ethyl acetate and the hydrochloride was precipitated by addition of a 2 N solution of hydrogen chloride in the same solvent to give 4.3 g (85%) of **1t**; mp: 174°C; IR (KBr) 1670 (C=0) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.15–2.45 (m, 2H), 2.83 (d, 3H), 2.9–3.4 (m, 6H), 3.65 (tr, 2H), 3.88 (d, 6H), 5.24 (m, 2H), 6.46 (s, 1H), 7.1–7.4 (m, 6H), 12.47 (s, broad, 1H); R_f: 0.65; Anal: $C_{22}H_{28}N_2O_4$•HCl (C, H, N, Cl). For the synthesis of the compounds **1o** and **1p**, 1,3,5-trioxane was replaced by paraldehyde or octanal respectively.

Method B

2,3-Dihydro-6,7-dimethoxy-3-[3-(methyl-3,4-dimethoxyphenethylamino)propyl]-2-phenyl-4H-1,3-benzoxazin-4-one hydrochloride ***1q***

A mixture of *N*-methyl *N*-(3,4-dimethoxyphenethyl) propanediamine (5 g; 20 mmol) and benzaldehyde 2.16 g (20 mmol) in benzene (30 ml) was heated at reflux in a Dean–Stark apparatus to remove water, after which the solvent was evaporated *in vacuo* to give *N'*-benzylidene *N*-methyl *N*-(3,4-dimethoxyphenethyl) propanediamine (6.8 g). A solution of 4,5-dimethoxy salicylyl chloride (2 g, 9.2 mmol) in benzene (60 ml) was cooled to 0°C and cautiously treated with the preceding Schiff base (3.13, 9.2 mmol). The mixture was allowed to stand at room temperature for 36 h. The precipitate which formed was collected by filtration, dried and purified by chromatography over silica gel (eluent: chloroform/methanol/aqueous 33% ammonia, 97:3:0.2). After evaporation under reduced pressure, the residual product was converted to the hydrochloride in ethyl acetate as described above. m: 0.6 g (12%); mp: 161°C; IR (KBr) 1660 (C=O) cm^{-1}; NMR ($CDCl_3$) δ: 2–2.4 (m, 2H), 2.7–2.8 (m, 3H), 2.85–3.5 (m, 7H), 3.8–4.15 (m, 13H), 6.3–6.45 (m, 2H), 6.65–6.9 (m, 3H); 7.2–7.5 (m, 7H); R_f: 0.72; Anal: $C_{30}H_{36}N_2O_6$•HCl (C,H,N,Cl).

Method C

2,3-Dihydro-7-methoxy-3-[3-(methyl-3,4-dimethoxyphenethylamino)propyl]-4H-1,3-benzoxazin-4-one hydrochloride ***1f***

A conglomerated mixture of 2,3-dihydro-7-methoxy-3-[3-(methanesulfonyloxy)propyl]-4*H*-1,3-benzoxazin-4 one (1.35 g, 4.2 mmol) and *N*-methylhomoveratrylamine hydrochloride

(1.46 g, 6.3 mmol) was heated with stirring at 50°C for 10 h in dry DMF (30 ml) containing triethylamine (10 ml). The solution was allowed to cool to room temperature, diluted with water and extracted with ethyl acetate (5 x 60 ml). The organic layer was then washed with water and brine, dried (Na_2SO_4) and evaporated under reduced pressure. The washed residue (1.2 g) was purified by chromatography over silica gel using chloroform/ methanol/aqueous 33% ammonia (95:4.5:0.5) as eluent. The oil obtained was taken up in ethyl acetate and converted to the hydrochloride in the usual manner. m: 550 mg (30%); mp: 148°C; IR (KBr) 1665 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2–2.2 (m, 2H), 2.82 (d, 3H), 3–3.33 (m, 6H), 3.65 (t, 2H), 3.8–3.9 (m, 9H), 5.2–5.32 (m, 2H), 6.44 (d, 1H), 6.5 (dd, 1H), 6.7–6.81 (m, 3H), 7.8 (d, 1H), 12.4 (s, broad, 1H); R_f: 0.61; Anal: $C_{23}H_{30}N_2O_5$·HCl (C, H, N, Cl).

Method C_1

2,3-Dihydro-6,7-dimethoxy-3-[3-(3,4-dimethoxyphenethylamino)propyl]-4H-1,3-benzoxazin-4-one hydrochloride ***1r***

A mixture of 6,7-dimethoxy-2,3-dihydro-3-[3-(methanesulfonyloxy)propyl]-4*H*-1,3-benzoxazin-4-one (64.6 g, 0.187 mol) and homoveratrylamine (203 g, 1.1 mol) was heated for 45 min at 120°C under a nitrogen atmosphere. The excess of homoveratrylamine was recovered by distillation (0.02 m bar). The reaction mixture was cooled to 50°C and the residue partitioned between toluene and water buffered to pH 7. The organic layer was separated and the aqueous layer was extracted with toluene. The aqueous layer was basified with caustic soda to pH 9.7 and again extracted with toluene (4 x 100 ml). The combined organic extracts were washed with water, brine, then dried (Na_2SO_4), and concentrated to give a crude basic oil (80 g). The oil was taken up in 2-propanol (500 ml) and the hydrochloride formed by addition of a 2 N solution of hydrochloric acid in ethyl acetate. The white crystals of **1r** were collected, washed and dried. m: 61 g (70%); mp: 155°C; IR (KBr) 1645 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.05–2.4 (m, 2H), 2.9–3.3 (m, 6H), 3.6–3.8 (t, 2H), 3.85–4.0 (m, 12H), 5.2 (s, 2H), 6.42 (s, 1H), 6.7–7 (m, 3H), 7.23 (s, 1H), 9.8 (s, broad, 1H); R_f: 0.43; Anal: $C_{23}H_{30}N_2O_6$·HCl (C, H, N, Cl).

Method C_2

2,3-Dihydro-6,7-dimethoxy-3-[3-(methyl-3,4-dimethoxyphenethylamino)propyl]-4H-1,3-benzoxazin-4-one hydrochloride ***1m***

A mixture of 2,3-dihydro-6,7-dimethoxy-3-[3-(3,4-dimethoxyphenethylamino)propyl]-4*H*-1,3-benzoxazin-4-one (8 g, 18.5 mmol) in formic acid (2.1 ml, 56 mmol) was stirred at room temperature for 10 min and 40% aqueous formaldehyde (1.6 ml, 23 mmol) added. The reaction flask was placed in an oil bath preheated to 80°C and vigorously agitated for 10 min. After cooling to room temperature the residue was dissolved in methylene chloride, treated with iced caustic soda and the layers were separated. The aqueous layer was extracted with the same solvent (3 x 80 ml) and the combined extracts were washed with brine, dried (Na_2SO_4) and evaporated. The residue was dissolved in 2-propanol and the hydrochloride was precipitated by addition of 2 N hydrochloric acid in ethyl acetate. m: 8 g (90%); mp: 174°C ; IR (KBr) 1650 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.3 (m, 2H), 2.62 (d, 3H), 2.9–3.3 (m, 6H), 3.65 (t, 2H), 5.24 (m, 2H), 6.45 (s, 1H), 6.6–6.9 (m, 3H), 7.28 (s, 1H), 12.45 (m, 1H); R_f: 0.61; Anal: $C_{24}H_{32}N_2O_6$·HCl (C, H, N, Cl).

Method C_3

2,3-Dihydro-6,7-dimethoxy-3-[3-(benzyl-3,4-dimethoxyphenethylamino)propyl]-4H-1,3-benzoxazin-4-one hydrochloride, monohydrate ***1s***

To a solution of 2,3-dihydro-6,7-dimethoxy-3-[3-(3,4-dimethoxyphenethylamino)propyl]-4*H*-1,3-benzoxazin-4-one hydrochloride (2.2 g, 4.7 mmol) in anhydrous DMF (25 ml) cooled in an ice bath, was added triethylamine (3 ml, 21.5 mmol), and benzyl bromide (0.88 g, 5.2 mmol) and the reaction mixture stirred for 1 h at 0°C and 5 h at room temperature. The solvent was evaporated under reduced pressure and the residue dissolved in water, basified with 30% caustic soda, and extracted with ethyl acetate (4 x 40 ml). The combined organic extracts were washed with water, brine, dried (Na_2SO_4) and evaporated to give an oil which was subjected to silica gel column chromatography using chloroform/methanol 95:5 as eluent. The oily residue was dissolved in anhydrous diethyl ether and the hydrochloride was precipitated by addition of 2 N hydrochloric acid in ethyl acetate, yielding white crystals. m: 1.75 g (68%); mp: 78–82°C; IR (KBr) 1660 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.2–2.45 (m, 2H), 3–3.35 (m, 6H), 3.45–3.75 (m, 2H), 3.8–4 (m, 12H), 4.24 (d, 2H), 5.1–5.2 (m, 2H), 6.45 (s, 1H), 6.55–6.8 (m, 3H), 7.0 (s, broad, 1H), 7.29 (s, 1H), 7.32–7.45 (m, 3H), 7.5–7.7 (m, 2H); R_f: 0.65; Anal: $C_{30}H_{36}N_2O_6$·HCl, H_2O (C, H, N, Cl).

Synthesis of 6-acetamido-2,3-dihydro-3-[3-(methyl-3,4-dimethoxyphenethylamino)propyl]-4H-1,3-benzoxazin-4-one hydrochloride ***11***

A solution of 2,3-dihydro-3-[3-(methyl-3,4-dimethoxyphenethylamino)propyl]-6-nitro-4*H*-1,3 benzoxazin-4-one, hydrogenooxalate (7.4 g, 17.3 mmol) **1k** in anhydrous methanol was hydrogenated in the presence of 10% palladium on charcoal at room temperature and at atmospheric pressure. The catalyst was removed by filtration and the filtrate concentrated. The residual oil (7 g) was purified by silica gel column chromatography using a mixture of chloroform/methanol/33% aqueous ammonia (95:4.5:0.5) as eluent. The residue (4.6 g) was used without further purification in the next step. An aliquot of the amine intermediate was purified for analysis (C, H, N) as the dipicrate salt with a melting point at 202°C.

A solution of the above organic base (2.87 g, 7 mmol) in anhydrous THF (20 ml) was cooled to –5°C and a solution of acetyl chloride (0.62 g, 8 mmol) in the same solvent (10 ml) was added dropwise over 15 min with stirring. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirring continued for a further 16 h. The insoluble white product was collected by filtration, washed (THF), and dried. m: 2.27 g (66%); mp: 192°C; IR (KBr) 1660 (C=O), 1685 (CONH) cm^{-1}; ^{1}H-MMR ($CDCl_3$) δ: 2.2–2 (m, 5H), 2.78 (d, 3H), 2.8–3.4 (m, 8H), 3.4–3.7 (m, 2H), 3.73 (s, 6H), 5.1 (s, 2H), 6.5–6.8 (m, 4H), 7.7–7.9 (m, 2H), 9.4 (s, 1H), 11.8 (s, broad, 1H); R_f: 0.35; Anal: $C_{24}H_{31}N_3O_5$·HCl (C, H, N, Cl).

Synthesis of ***8****: 2,3-dihydro-6,7-dimethoxy-3-[3-(methanesulfonyloxy)propyl]-4H-1,3-benzoxazin-4-one* ***8m***

4,5-Dimethoxy-N-(3-hydroxypropyl) salicylamide. A mixture of methyl-4,5-dimethoxy-2-hydroxybenzoate (100 g, 0.471 mol) (26) and 3-aminopropanol (106 g, 1.41 mol) was heated at 120°C for 1 h. The reaction mixture was added to 300 ml of water and acidified to pH 7.1. The insoluble white product was collected by filtration, washed (water) dried and recrystallized from ethyl acetate. m: 107.2 g (89%); mp: 130°C; IR (KBr) 1635 (C=O), 3380 (OH), 3510 (NH) cm^{-1}; ^{1}H-MMR ($CDCl_3$)

δ: 1.6–2.1 (m, 2H), 3.2–4.0 (m, 11H) 6.25 (s, 1H), 6.95 (s, 1H), 7.9 (m, 1H), 12.0 (s, broad, 1H). Anal $C_{12}H_{17}NO_5$ (C, H, N).

2,3-Dihydro-6,7-dimethoxy-3-(3-hydroxypropyl)-4H-1,3-benzoxazin-4-one. To a solution of 4,5-dimethoxy-*N*-(3-hydroxypropyl) salicylamide (107 g, 0.419 mol) in acetic acid (300 ml) and a 2 N solution of hydrochloric acid in ethyl acetate (300 ml) was added 1,3,5-trioxane (75.5 g, 0.84 mol) and the mixture stirred for 16 h at room temperature. The mixture was poured into iced-water (500 g) and basified to pH 8.5. The insoluble matter was extracted with ethyl acetate (3 x 200 ml), washed with water and dried (Na_2SO_4). After filtration, the solution was concentrated to give 130 g (quantitative) of 2,3-dihydro-6,7-dimethoxy-3-(3-acetoxypropyl)-4*H*-1,3-benzoxazin-4-one. IR (neat) 1740 (O-C=O), 1660 (N-C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.75–2.1 (m, 5H), 3.57 (t, 2H), 3.86 (s, 6H), 4.12 (t, 2H), 5.12 (s, 2H), 6.43 (s, 1H), 7.33 (s, 1H). To the crude 2,3-dihydro-6,7-dimethoxy-3-[3-acetoxypropyl]-4*H*-1,3 benzoxazin-4-one (130 g, 0.42 mol) in methanol (700 ml) was added 30% caustic soda (85 ml, 0.84 mol) and the mixture stirred for 45 min at room temperature. The dark-coloured solution was poured into crushed ice (600 g) and acidified with a 6 N solution of hydrochloric acid to pH 8.5. The aqueous phase was extracted with chloroform (2 x 250 ml), washed with water, and dried (Na_2SO_4). The viscous oil obtained after evaporation was stirred in diisopropyl ether (500 ml) to give 105 g (94%) of 2,3-dihydro-6,7-dimethoxy-3-[3-hydroxypropyl]-4*H*-1,3-benzoxazin-4-one; mp: 88°C; IR (KBr) 1645 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.6–2.0 (m, 2H), 2.3–2.65 (m, 5H), 2.72 (s, 6H), 4.97 (s, 1H), 6.25 (s, 1H), 7.65 (s, 1H), 11.5 (s, 1H); Anal: $C_{13}H_{17}NO_5$ (C, H, N).

2,3-Dihydro-6,7-dimethoxy-3-[3-(methane sulfonamido)propyl]-4H-1,3 benzoxazin-4-one ***8m***. A suspension of 2,3-dihydro-6,7-dimethoxy-3-[3-hydroxypropyl]-4*H*-1,3 benzoxazine-4-one (50 g, 0.187 mol) in dry THF (500 ml) cooled to –5°C was treated at once with triethylamine (52 ml, 0.374 mol) and methanesulfonyl chloride (42.85 g, 0.374 mol). After stirring for 5 min at this temperature, the mixture was hydrolyzed with ice (200 g) and the mesylate was extracted with methylene chloride (2 x 300 ml), washed with water, and dried (Na_2SO_4). The residue was stirred with 2-propanol at room temperature and the mesylate recovered by filtration; m: 64 g (98%); mp: 123°C; IR (KBr) δ: 2.0–2.3 (m, 2H), 3.0 (s, 3H), 3.62 (t, 2H), 3.88 (d, 6H), 4.30 (t, 2H), 5.16 (s, 2H), 6.46 (s, 1H), 7.32 (s, 1H); Anal: $C_{14}H_{19}NO_7S$ (C, H, N, S).

Pharmacology

Determination of the bradycardic action in the anaesthetized rat

Sprague–Dawley male rats (320–490 g) were anaesthetized using sodium pentobarbitone (50 mg·kg^{-1} ip). Electrocardiograms were recorded in DII standard derivation on a Siemens mingograph. Parameters were followed for 60 min. Each animal received only 1 dose and 1 product. Each group comprised at least 5 rats. The compounds were dissolved in physiological saline and injected at 0.1 ml·100 g^{-1} through a catheter placed in the vein of the penis.

Determination of the antidysrhythmic activity

Sprague–Dawley male rats (320–490 g) were used. After anaesthesia using sodium pentobarbitone (60 mg·kg^{-1} ip), a tracheotomy allowing artificial ventilation was carried out. The electrocardiogram was recorded in DI, DII, DIII derivations with a Siemens mingograph and the heart rate calculated. A coronary ligature was performed according to the method of Clark *et al* [22]. After left thoracotomy, a silk thread was placed under the left coronary artery. The heart was then replaced in its cavity and the ligature tightened in order to block coronary flow. Dysrhythmia was followed for 30 min. The results observed after drug treatment were compared with those obtained in the placebo group (physiological saline). Each group comprised at least 5 rats. The results were expressed as percentage variations. The compounds were dissolved in physiological saline and injected in 0.1 ml·100 g^{-1} through a catheter placed in the pudendal vein. Compound **1m**, which displayed excellent pharmacological properties, was further studied.

Effect on the heart rate and blood pressure of conscious normotensive rats

Groups of 10 Sprague–Dawley male rats (290–370 g) were used for these studies. The systolic arterial pressure was plethysmographically recorded at the tail according to Gerold and Tschirky [24] and the heart rate calculated. The compounds were studied after acute or sub-chronic administration (5 d) at 50 mg·kg^{-1} *po*. The parameters were evaluated before, then 1, 2, 4, 6 and 24 h after oral gavage. Compounds were administered at 1 ml·100 g^{-1} after dissolution in distilled water.

Haemodynamic effect in the anaesthetized dog with closed chest

Dogs weighing 20–30 kg were anaesthetized by sodium pentobarbitone (30 mg·kg^{-1} iv). An intravenous perfusion of this anaesthetic dissolved in physiological saline and mixing with flaxedyl was given throughout the duration of the experiment. The dogs were maintained under artificial ventilation. The following parameters were evaluated :

– mean arterial pressure recorded in the carotid artery;
– heart rate calculated *via* ECG;
– left ventricular pressure (LVP) recorded with a catheter inserted *via* a femoral artery. The LVP amplification permitted measurement of the end diastolic ventricular pressure. The myocardial contractility was estimated on positive maxima dp/dt. These parameters were computerized on an IBM microprocessor and recorded on Gould ES 1000 polygraph. The computer also determined total peripheral resistance, stroke volume, Katz index, coronary pressure perfusion and Buckberg index [25]. This index represents the ratio between the oxygen supply and the oxygen demand;
– cardiac output: a Swan–Ganz catheter, placed inside the pulmonary artery *via* the right jugular vein, was used to quantify cardiac output by the thermodilution method.

Acknowledgment

The assistance of H Calmel is gratefully acknowledged.

References

1 Sonnenblick EH, Ross J Jr, Braunwald E (1968) *Am J Cardiol* 32, 328–336
2 Braunwald E (1976) *Am J Cardiol* 27, 416–432
3 Connolly ME, Kersting F, Dollery CT (1976) *Prog Cardiovasc Dis* 19, 203–234
4 Mac Allister RG (1982) *Prog Cardiovasc Dis* 25, 83–102
5 Kobinger W, Lillie C (1987) *Eur Heart J* (suppl 4), 8, 7–15

6 Kobinger W (1989) *Antiarrhythmic Drugs* (Vaughan Williams EM, Campbell TJ, eds) Springer-Verlag, Berlin, 424–452
7 Harron DW, Shanks RG (1985) *Eur Heart J* 6, 728–729
8 Hilaire J, Broustet JP, Colle JP, Theron M (1983) *Br J Clin Pharmacol* 16, 627–631
9 Kobinger W, Lillie C (1988) *Cardiovasc Drug Rev* 6, 35–53
10 Auböck J, Konzett H, Olbrich E (1982) *Eur J Clin Pharmacol* 21, 467–471
11 Harron DG, Deering AH, Scott M, Mc Mahon M, Shanks RG (1989) *Br J Clin J* 27, 23–29
12 Rieu JP (1990) Pat Fr 2636065
13 Tisné-Versailles J, Masse C, Bonnafous R, Bessac AM, Marty A, Verscheure Y, Sassine A (1990) *Eur J Pharmacol* 183, 799–800
14 Finkelstein J, Chiang E (1968) *J Med Chem* 11, 1038–1040
15 Lauressergues H, Stenger A, Culetto M, Mouzin G, Cousse H (1975) *Eur J Med Chem* 10, 629–632
16 Knoll AG (1961) UK pat 874206
17 Horrom BW, Zaugg HE (1950) *J Am Chem Soc* 72, 721–724
18 Krapcho J, Turk CF, Piala JP (1968) *J Med Chem* 11, 361–364
19 Zeigler E, Hanus HD (1965) *Monatsch Chem* 96, 411–417
20 Tichy M, Vasickova S (1974) *Collect Czech Chem Commun* 39, 555–565
21 Icke RN, Wisegarver BB (1955) *Org Synth.* John Wiley and Sons Inc, NY, III, 723
22 Clark C, Foreman MI, Kane KA, Mac Donard FM, Parratt JR (1980) *J Pharmacol Methods* 3, 357–368
23 Reiffen M, Eberlein W, Muller P, Psiorz M, Noll K, Heider J, Lillie C, Kobinger W, Luger P (1990) *J Med Chem* 33, 1496–1504
24 Gerold M, Tschirky H (1968) *Arzneim Forsch* 18, 1285–1287
25 Buckberg G, Fixler O, Archie J, Hoffman J (1972) *Circ Res* 30, 67–81
26 Chatterjea JN, Achari K, Jain NC (1970) *J Indian Chem Soc* 47, 6, 590-596