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Original article

Synthesis of oxypropanolamine derivatives of 3,4-dihydro-2*H*-1,4-benzoxazine, β-adrenergic affinity, inotropic, chronotropic and coronary vasodilating activities

Kriton Iakovou[a]*, Michalis Kazanis[a], Andreas Vavayannis[a], Giancarlo Bruni[b], Maria Raffaella Romeo[b], Paola Massarelli[b], Shuji Teramoto[c], Hiroyuki Fujiki[c], Toyoki Mori[c]

[a]*Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis Zografou, GR-157 71 Athens, Greece*
[b]*Istituto di Farmacologia, Universita di Siena, 53100 Siena, Italy*
[c]*2nd Tokushima Institute of New Drug Research, Otsuka Pharmaceutical Co. Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-01, Japan*

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Abstract – A series of oxypropanolamine derivatives of 3,4-dihydro-2*H*-1,4-benzoxazine were synthesized and evaluated for inotropic, chronotropic and coronary vasodilating activities in the canine heart, affinity to β_1-adrenergic receptor in turkey erythrocytes and affinity to the β_2-adrenergic receptor in the rat lung. Among these compounds, 4-acetyl-6-(3-*tert*-butylamino-2-hydroxy)propoxy-3,4-dihydro-2*H*-1,4-benzoxazine showed 2.1-fold more potent affinity to the β_1 receptor than propranolol and 7-(3-*tert*-butylamino-2-hydroxy)propoxy-*N*-butyryl-3,4-dihydro-2*H*-1,4-benzoxazine showed 2.5-fold more potent affinity to the β_2 receptor and furthermore 4 386-fold more potent selectivity to the β_2 receptor than propranolol. In addition, 4-acetyl-6-[3-(3,4-dimethoxybenzyl)amino-2-hydroxy]propoxy-3,4-dihydro-2*H*-1,4-benzoxazine showed 1.1-fold more potent affinity to the β_1 receptor than propranolol and also 1 147-fold more potent selectivity to the β_1 receptor. With a few exceptions, negative inotropic and chronotropic actions of these compounds were dependent on the size of the 4-substituent obeying the order: unsubstituted < acetyl < propanoyl < butanoyl, while the benzoyl substituent conferred even stronger negative actions in the 6-oxypropanolamine derivatives. Neither negative inotropic and chronotropic actions related with affinity to β_1-adrenoceptor nor coronary vasodilator action related with affinity to β_2-adrenoceptor were observed. 4-acetyl-7-[3-(3,4-dimethoxybenzyl)amino-2-hydroxy]propoxy-3,4-dihydro-2*H*-1,4-benzoxazine exerted potent positive inotropic, chronotropic and coronary vasodilating actions. The inotropic and chronotropic actions of the latter compound may be attributed to the release of intrinsic catecholamines, as concluded by the absence of β_1-adrenoceptor affinity and disappearance of activity in the presence of a β-blocker. © 1999 Éditions scientifiques et médicales Elsevier SAS

1,4-benzoxazine / oxypropanolamines / β-adrenoceptor affinity / cardiovascular effects

1. Introduction

A number of 1,4-benzoxazine derivatives [1–4] have been synthesized so far and various pharmacological activities have been reported with this class of molecules. Ethanolamine and oxypropanolamine derivatives of 1,4-benzothiazine active on the adrenergic system are already known [5, 6], as well as of the isoster nuclei 1,4-benzoxazine [7–10] and 3,4-dihydro-2(1*H*)-quinolinone [11, 12]. Among the carbostyril derivatives, 5-(3-*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-2(1*H*)-quinolinone (carteolol) [13, 14] is a β-blocker more potent than propranolol and is apparently devoid of the side effects which are usually associated with β-blocking therapy as it retains an intrinsic sympathomimetic activity. Oxypropanolamine derivatives of 1,4-benzodioxin [15, 16], which are analogues of 1,4-benzoxazine, have been recently synthesized. The 4-acyl derivatives of 7-oxypropanolamine-3,4-dihydro-2*H*-1,4-benzoxazines which are included in the present work may be consid-

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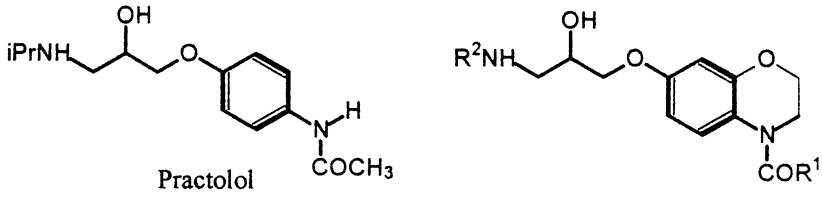


Figure 1. Structure of the synthesized 4-acyl derivatives of 7-oxypropanolamine-3,4-dihydro-2*H*-1,4-benzoxazines compared to practolol.

ered as dicyclic analogues of practolol, a prototype cardioselective blocking agent, as shown in *figure 1* [9, 17, 18].

It is well known that the catecholamine β-stimulants (arylethanolamines and aryloxypropanolamines) have been used as cardiotonic agents in the management of heart failure. Unfortunately these compounds have some disadvantages, such as powerful vasodilating effects, which cause a reflex rise in heart rate, lack of oral absorption and short half-life [19]. On the other hand, some non-oxypropanolamine [20] or oxypropanolamine derivatives of 2(1*H*)-quinolinone [21, 22], have demonstrated remarkable positive inotropic activities, the latter in some cases without having β-agonistic action. Furthermore, a series of non-oxypropanolamine derivatives of 3,4-dihydro-1,4-benzoxazine [23] have exhibited potent, long-acting positive inotropic and peripheral vasodilating activities.

In order to extend the investigation on inotropic, chronotropic and vasodilating activities of non-catechol derivatives with possible affinity to the β-adrenergic receptors, we prepared molecules structurally similar to the above. The synthesized compounds are novel 4-acyl substituted 3,4-dihydro-2*H*-1,4-benzoxazines bearing the typical oxypropanolamine chain at positions 6- and 7- of the aromatic ring.

2. Chemistry

The target compounds **24–49** (*tables I* and *II*) were prepared in a standard three-step procedure, shown in *figure 2*, which involves: a) synthesis of the 6- and 7-hydroxy-4-acyl-3,4-dihydro-2*H*-1,4-benzoxazines [24], b) reaction thereof with epichlorhydrin in the presence of potassium carbonate [25] and c) treatment of the resulting 1-aryloxy-2,3-epoxypropanes with the appropriate amines [26].

The derivatives **2–7** (*table III*) were synthesized through the reaction of 6- or 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine in aqueous medium with the appropriate anhydrides. The reaction of 6- or 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine with benzoic or chloroacetic anhydride was not successful in water and therefore ethyl acetate was used instead. The intermediates **12** and **13** (*table III*) were synthesized via the corresponding 4-chloroacetyl compounds **10** and **11** (*table III*) which were condensed with diethylamine in ethanol. The general synthetic procedure for the hydroxy derivatives **2–13** is shown in *figure 2*.

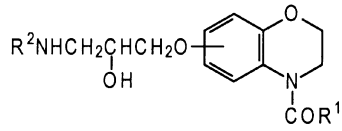
The 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine was synthesized by a method which is presented in *figure 3*. Initially 7-methoxy-3,4-dihydro-2*H*-1,4-benzoxazin-3-one [27–29] was converted into **1** through reduction with lithium aluminium hydride in anhydrous tetrahydrofuran [30]. The intermediate **1** was demethylated by means of concentrated hydrobromic acid to give 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine hydrobromide, which was alkalized by concentrated ammonium hydroxide to afford the free base [31].

The synthetic route of 6-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine, as shown in *figure 4*, was achieved as follows: 2,5-dimethoxyaniline was refluxed with 2-bromoethanol in the presence of calcium carbonate in water to yield 2,5-dimethoxy-*N*-(2-hydroxyethyl)aniline [32] which was isolated in pure form by distillation in vacuo. The latter was refluxed with concentrated hydrobromic acid leading to 6-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine hydrobromide [32], which in turn, was alkalized by concentrated ammonium hydroxide to provide the free base.

The 4-unsubstituted products **44–47** (*table II*) were prepared, as shown in *figure 2*, by hydrolysis of the corresponding 4-acetyl derivatives **24–27** with potassium hydroxide in aqueous methanol [33].

IR absorption and NMR spectra were in conformity with the structures expected. However, we observed that the resonance of the aromatic proton 5 either appears as a broad peak or disappears completely, depending on the temperature. This is possibly due to stereochemical

Table I. 4-Acyl-6- and 7-(3-isopropylamino- and 3-tert-butylamino-2-hydroxypropoxy)-3,4-dihydro-2H-1,4-benzoxazines and 4-acetyl-6- and 7-[3-(3,4-dimethoxybenzyl)amino-2-hydroxy-propoxy]-3,4-dihydro-2H-1,4-benzoxazines.



Compound	Position	R^1	R^2	M.p. (°C)	Yield (%)	Formula
24	7	CH_3	iPr	176–179[a]	24	$C_{16}H_{24}N_2O_4$. 0.5 $C_4H_4O_4$
25	7	CH_3	tBu	161–164[a]	25	$C_{17}H_{26}N_2O_4$. 0.5 $C_4H_4O_4$
26	6	CH_3	iPr	134[a]	20	$C_{16}H_{24}N_2O_4$. 0.5 $C_4H_4O_4$
27	6	CH_3	tBu	169–171[a]	25	$C_{17}H_{26}N_2O_4$. 0.5 $C_4H_4O_4$
28	7	CH_2CH_3	iPr	120[a]	26	$C_{17}H_{26}N_2O_4$. 0.5 $C_4H_4O_4$
29	7	CH_2CH_3	tBu	203[a]	25	$C_{18}H_{28}N_2O_4$. 0.5 $C_4H_4O_4$
30	6	CH_2CH_3	iPr	129–131[a]	27	$C_{17}H_{26}N_2O_4$. 0.5 $C_4H_4O_4$
31	6	CH_2CH_3	tBu	140–142[a]	23	$C_{18}H_{28}N_2O_4$. $C_4H_4O_4$
32	7	$CH_2CH_2CH_3$	iPr	175–178[a]	25	$C_{18}H_{28}N_2O_4$. 0.5 $C_4H_4O_4$
33	7	$CH_2CH_2CH_3$	tBu	171–172[a]	24	$C_{19}H_{30}N_2O_4$. 0.5 $C_4H_4O_4$. 0.5 H_2O
34	6	$CH_2CH_2CH_3$	iPr	117–120[a]	26	$C_{18}H_{28}N_2O_4$. 0.5 $C_4H_4O_4$
35	6	$CH_2CH_2CH_3$	tBu	142–145[a]	23	$C_{19}H_{30}N_2O_4$. 0.5 $C_4H_4O_4$
36	7	Ph	iPr	96–98	57	$C_{21}H_{26}N_2O_4$
37	7	Ph	tBu	83–85	54	$C_{22}H_{28}N_2O_4$
38	6	Ph	iPr	176–180[a]	17	$C_{21}H_{26}N_2O_4$. 0.5 $C_4H_4O_4$
39	6	Ph	tBu	112–115[b]	21	$C_{22}H_{28}N_2O_4$. $C_4H_4O_4$. 0.5 H_2O
40	7	$CH_2N(C_2H_5)_2$	iPr	54–56	15	$C_{20}H_{33}N_3O_4$. 0.5 H_2O
41	7	$CH_2N(C_2H_5)_2$	tBu	85–90	13	$C_{21}H_{35}N_3O_4$. H_2O
42	6	$CH_2N(C_2H_5)_2$	iPr	68–70	16	$C_{20}H_{33}N_3O_4$. 0.5 H_2O
43	6	$CH_2N(C_2H_5)_2$	tBu	70–73	14	$C_{21}H_{35}N_3O_4$. 0.5 H_2O
48	7	CH_3	3,4-dimethoxybenzyl	140–142[b]	31	$C_{22}H_{28}N_2O_6$. $C_4H_4O_4$. $2H_2O$
49	6	CH_3	3,4-dimethoxybenzyl	138–140[b]	33	$C_{22}H_{28}N_2O_6$. $C_4H_4O_4$. $2H_2O$

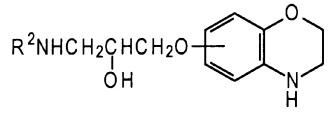
[a]: fumarate, [b]: maleate.

effects caused by the equilibrium between two extreme conformations of the morpholine ring. A further study of this effect is still under investigation and will be published elsewhere.

3. Pharmacology

The biological profiles of the compounds listed in *table IV* on β_1 and β_2 adrenoceptors were respectively

Table II. 6- and 7-(3-Isopropylamino- and 3-*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-2*H*-1,4-benzoxazines.



Compound	Position	R^2	M.p. (°C)	Yield (%)	Formula
44	7	iPr	130–134[a]	19	$C_{14}H_{22}N_2O_3$. $C_4H_4O_4$. 0.5 H_2O
45	7	tBu	76–80[b]	17	$C_{15}H_{24}N_2O_3$. 2 $C_4H_4O_4$
46	6	iPr	128–130[c]	20	$C_{14}H_{22}N_2O_3$. 2 $C_2H_2O_4$
47	6	tBu	138–140[a]	22	$C_{15}H_{24}N_2O_3$. $C_4H_4O_4$

[a]: fumarate, [b]: maleate, [c]: oxalate.

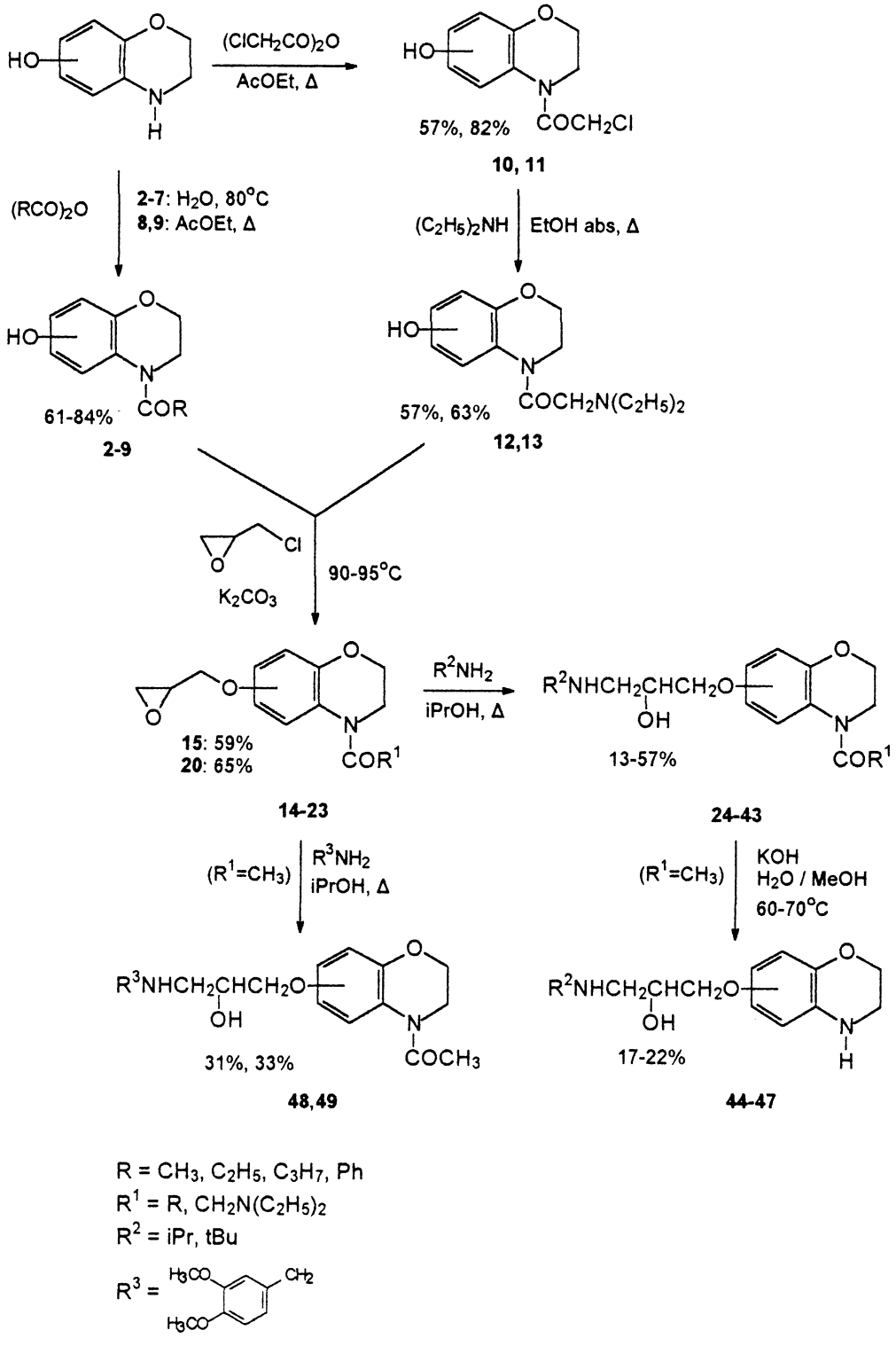
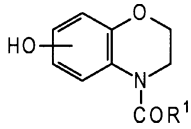


Figure 2. General synthetic procedure of the target 6- and 7-(alkylamino-2-hydroxypropoxy)-3,4-dihydro-2*H*-1,4-benzoxazines.

Table III. 4-Acyl-6- and 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazines.



Compound	Position	R^1	M.p (°C)	Yield (%)	Solvents of crystallization
2	7	CH_3	152–154	72	a
3	6	CH_3	185–188	65	a
4	7	CH_2CH_3	104–106	79	a
5	6	CH_2CH_3	146–148	84	a
6	7	$CH_2CH_2CH_3$	123–125	80	a
7	6	$CH_2CH_2CH_3$	102–103	79	a
8	7	Ph	190–193	65	a
9	6	Ph	155	61	a
10	7	CH_2CI	120–122	82	a
11	6	CH_2CI	128–130	57	a
12	7	$CH_2N(C_2H_5)_2$	110–113	57	b
13	6	$CH_2N(C_2H_5)_2$	124–125	63	b

a: benzene-pentane, b: cyclohexane.

assessed on turkey erythrocytes and on rat lung. The inotropic (changes in contractile force, CF), chronotropic (changes in sinus rate, SR) and coronary vasodilator (changes in coronary blood flow, CBF) effects of the synthesized products were evaluated in the isolated blood-perfused preparations of canine heart.

4. Results and discussion

4.1. β_1-Adrenenoceptor binding

The affinities of the 4-acetyl substituted 6-oxypropanolamines obeyed the order: **27** > **49** > **26**. The

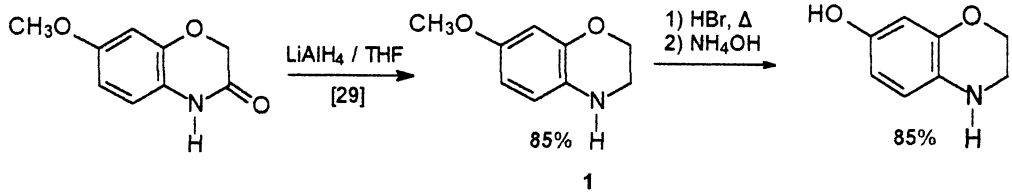


Figure 3. Preparation of 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine.

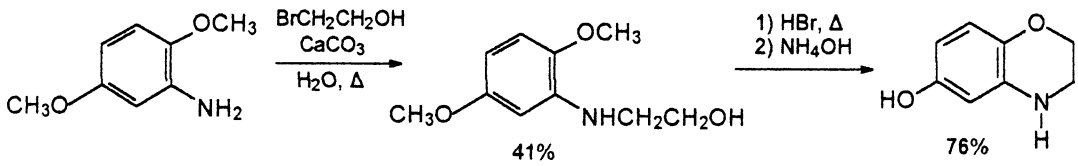


Figure 4. Preparation of 6-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine.

Table IV. Inhibition of [^{3}H]DHA binding on β_1 and β_2 adrenoreceptors.

Compound	β_1 Ki (± s.e.) (M)	β_2 Ki (± s.e.) (M)	β_1/β_2 selectivity ratio
Propranolol	$1.6 \times 10^{-9} \pm 0.132$	$2.5 \times 10^{-9} \pm 0.171$	1.520
24[a]	$6.0 \times 10^{-6} \pm 0.116$	$6.4 \times 10^{-7} \pm 0.201$	0.106
25[a]	$2.9 \times 10^{-6} \pm 0.327$	$3.6 \times 10^{-5} \pm 0.319$	12.290
26[a]	$7.4 \times 10^{-8} \pm 0.684$	$2.8 \times 10^{-9} \pm 0.267$	0.350
27[a]	$8.01 \times 10^{-10} \pm 0.782$	$1.0 \times 10^{-9} \pm 0.326$	1.280
28[a]	$2.7 \times 10^{-6} \pm 0.296$	$3.3 \times 10^{-8} \pm 0.715$	0.012
29[a]	$1.3 \times 10^{-6} \pm 0.131$	$1.6 \times 10^{-8} \pm 0.220$	0.013
30[a]	$1.2 \times 10^{-6} \pm 0.214$	inactive	
31[a]	$1.1 \times 10^{-5} \pm 0.121$	$1.6 \times 10^{-7} \pm 0.359$	0.014
32[a]	$2.0 \times 10^{-9} \pm 0.188$	$1.3 \times 10^{-6} \pm 0.264$	555
33[a]	$2.9 \times 10^{-6} \pm 0.282$	$1.0 \times 10^{-9} \pm 0.136$	0.0003
34[a]	inactive	$5.6 \times 10^{-6} \pm 0.454$	
35[a]	$8.1 \times 10^{-9} \pm 0.849$	$5.5 \times 10^{-6} \pm 0.481$	681
36	$1.3 \times 10^{-7} \pm 0.121$	$8.2 \times 10^{-6} \pm 2.720$	61
37	$5.6 \times 10^{-7} \pm 0.628$	$2.3 \times 10^{-7} \pm 0.234$	4.13
39[b]	inactive	$4.5 \times 10^{-6} \pm 0.459$	
40	inactive	$1.2 \times 10^{-7} \pm 0.169$	
41	$6.1 \times 10^{-7} \pm 0.606$	inactive	
42	$7.1 \times 10^{-5} \pm 0.684$	inactive	
43	$3.1 \times 10^{-6} \pm 0.306$	$2.5 \times 10^{-5} \pm 0.651$	7.95
44[a]	$1.9 \times 10^{-8} \pm 0.186$	$5.3 \times 10^{-6} \pm 0.386$	280
45[b]	inactive	$1.2 \times 10^{-6} \pm 0.172$	
46[c]	inactive	$3.4 \times 10^{-7} \pm 0.238$	
47[a]	$2.7 \times 10^{-8} \pm 0.296$	$4.5 \times 10^{-8} \pm 0.185$	1.70
48[b]	inactive	inactive	
49[b]	$1.4 \times 10^{-9} \pm 0.106$	$2.5 \times 10^{-6} \pm 0.306$	1 744

[a]: fumarate, [b]: maleate, [c]: oxalate.

observed affinity order of the side-chain amino substituents was *tert*-Bu > 3,4-dimethoxybenzyl > iPr. The highest affinities observed with derivatives **27** and **49**, were respectively 2.1- and 1.1-fold higher than that of propranolol. For the 6-iPr derivatives the elongation from the acetyl to the propanoyl chain led to a significant drop in affinity (**30**). Additional lengthening of the chain by a methylene group led to a loss in affinity (**34**). In the case of the corresponding 6-*tert*-Bu-derivatives, the presence of the propanoyl group (**31**) reduced the affinity, whereas the replacement thereof with butanoyl (**35**) led to increased activity. In the group of the 4-unsubstituted derivatives, **44** and **47** showed affinities ca. 10-fold lower than that of propranolol, while **45** and **46** had no affinity to the β_1 adrenoceptor. The 4-acetyl substituted 7-oxypropanolamine compounds (**24** and **25**) showed a low degree of affinity. For the 7-oxypropanolamine derivatives, the elongation from the acetyl to the propanoyl chain led to little increase in affinity (**28** and **29**). The observed affinity order for the amino substituents follows the order *tert*-Bu > iPr. Additional lengthening of the chain by a methylene group increased the affinity (**32**). The replacement of the iPr group of **32** with *tert*-Bu led to a drastic decrease in affinity (**33**). Among the 4-benzoyl and 4-diethylaminoacetyl derivatives (**36**, **37**, **39**, and **40**–**43**) only compound **36** showed an EC_{50} in the 10^{-7} range.

4.2. β_2-Adrenenoceptor binding

The high affinities for the β_2-adrenenoceptor were observed with the 4-acetyl substituted 6-oxypropanolamine derivatives. The affinities of **26** and **27** were 1.1-fold lower and 2.5-fold higher than that of propranolol, respectively. On the contrary, the 7-isomers **24** and **25** had low affinities to the β_2-adrenenoceptor. As to the 4-acetyl substituted 6-oxypropanolamine derivatives, the change of the amino substituent from iPr or tBu into 3,4-dimethoxybenzyl dramatically decreased the affinity (**49**), whereas it led to a loss of affinity of the correspond-

ing 7-isomer (**48**). For the 6-derivatives the elongation from the acetyl to the propanoyl or butanoyl chain led to a substantial reduction, up to deletion, of affinity (**30**, **31**, **34**, and **35**). On the contrary, the lengthening of the acyl chain of the 7-isomers, led to considerable increase in affinity, except for compound **32**. The 4-unsubstituted derivatives **44**–**47** showed low affinity to the β_2-adrenoceptor. The 4-benzoyl (**36**, **37**, and **39**) and 4-diethylaminoacetyl derivatives (**40**–**43**) showed practically no affinity.

4.3. β_1/β_2 selectivity

Compound **32** showed considerable β_1-adrenergic affinity with a high β_1/β_2 selectivity ratio (365 times more than that of propranolol). Compound **35** was 5-fold less β_1-affinitive than propranolol, but 448-fold more β_1-selective. Compound **44** was 11-fold less β_1-affinitive than propranolol, but 185-fold more β_1-selective. Compound **49** was 1.1-fold more β_1-affinitive than propranolol and also 1 147-fold more β_1-selective. Compounds **28** and **29** exhibited a moderate β_2-affinity, but considerable β_2-selectivity (about 120-fold more than that of propranolol). It is noteworthy that compound **33** was 2.5-fold more β_2-affinitive and furthermore 4 386-fold more β_2-selective than propranolol.

The results discussed in the preceding paragraphs 4.1 to 4.3 and presented in *table IV*, have shown that it is impossible to draw any straightforward conclusions regarding structure-activity relationships. For example, although compound **24** has the closest structure to practolol (*figure 1*), it did not show the expected activity, which in fact was much lower than its 6-substituted isomer **26**. It is possible that other parameters, such as the difference in steric configuration or the absence of amidic hydrogen, affect the biological activity of these cyclic analogues of practolol. Another example lies in the group of N-acyl 7-substituted compounds, where each additional methylene in the acyl chain may have a drastic effect on receptor affinity and selectivity. Although this fact is obvious in the cases of compounds **25** vs. **29** and **28** vs. **32** it cannot serve as a general rule.

4.4. Inotropic, chronotropic and coronary vasodilating activities

As shown in *figures 5–7*, negative inotropic and chronotropic actions of the tested compounds were dependent on the size of the 4-substituent. In the series of the 6-oxypropanolamine derivatives the actions followed the order: unsubstituted (**46** and **47**) < acetyl (**26** and **27**) < propanoyl (**30** and **31**) < butanoyl (**34** and **35**). The benzoyl compounds (**38** and **39**) exerted stronger negative actions. Similar structure-activity relationships were observed in the series of 7-oxypropanolamine derivatives, i.e. unsubstituted (**44** and **45**) < acetyl (**24** and **25**) < propanoyl (**28** and **29**) < butanoyl (**32** and **33**), but no further negative action was observed in the benzoyl subsutituted compounds **36** and **37**. The negative inotropic and chronotropic actions of these compounds were difficult to explain by the β_1-adrenoceptor antagonistic actions because of inconsistency with the β_1-adrenoceptor affinity as described earlier.

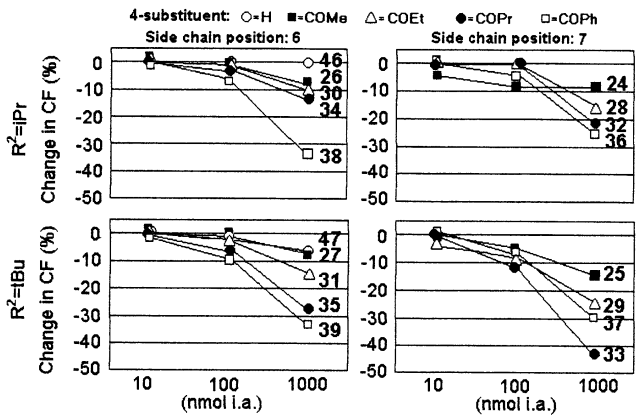


Figure 5. Structure-inotropic activity relationships of 6- and 7-oxypropanolamine-3,4-dihydro-2*H*-1,4-benzoxazine derivatives in canine isolated blood-perfused heart preparations. Effects of test compounds on right ventricular papillary muscle contractile force (CF) were expressed by the % changes from basal CF. Test compounds were dissolved in DMSO and the action of the solvent itself was subtracted for compensation.

In general, coronary vasodilator actions of the 6-oxypropanolamine derivatives were more potent than those of their 7-substituted counterparts (*figure 7*). It was also difficult to explain the coronary vasodilator action of these compounds in relation to their β_2-adrenoceptor agonistic actions.

The 4-diethylaminoacetyl substituted 6-oxypropanolamine derivatives with iPr (**42**) and *tert*-Bu (**43**) functions, as well as the 4-acetyl substituted compound bearing a 3,4-dimethoxybenzyl group (**49**), exerted weak positive inotropic and chronotropic actions. The same was true for the isomer of **42** bearing the side chain at position 7 (**40**). The 4-unsubstituted compounds **44** and **45** showed moderate positive actions (*figure 8*).

The 3,4-dimethoxybenzyl derivative **48** exerted potent positive inotropic, chronotropic and coronary vasodilatory actions (*figure 8*). It should be noted that the inotro-

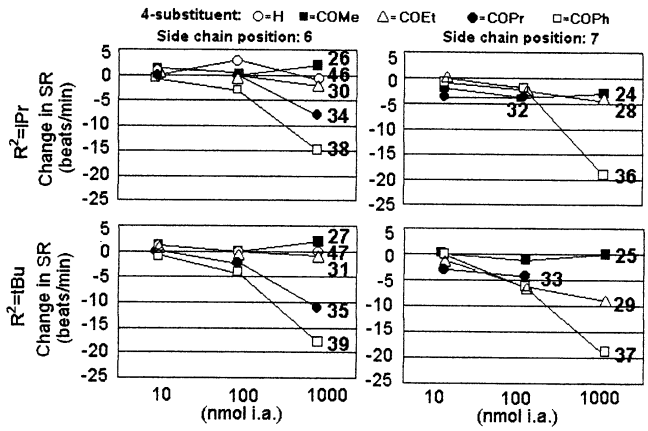


Figure 6. Structure-chrononotropic activity relationships of 6- and 7-oxypropanolamine-3,4-dihydro-2*H*-1,4-benzoxazine derivatives in canine isolated blood-perfused heart preparations. Effects of test compounds on right atrial sinus rate (SR) were expressed by changes from basal SR. Test compounds were dissolved in DMSO and the action of the solvent itself was subtracted for compensation.

pic and chronotropic actions of this compound were inhibited by pretreatment with the β-blocker carteolol (*figure 9*).

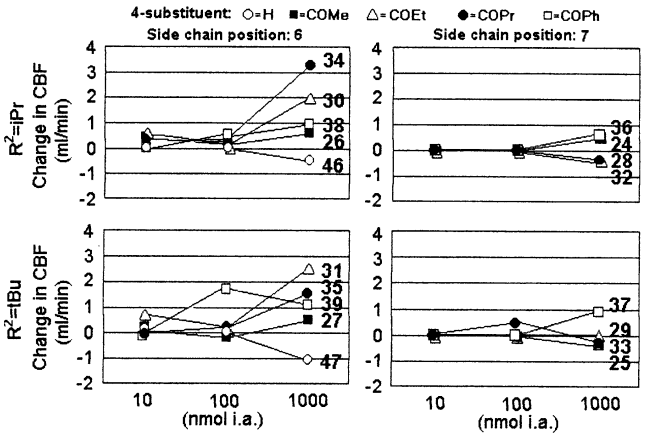


Figure 7. Structure-coronary vasodilator activity relationships of 6- and 7-oxypropanolamine-3,4-dihydro-2*H*-1,4-benzoxazine derivatives in canine isolated blood-perfused heart preparations. Effects of test compounds on coronary blood flow (CBF) through anterior septal arteries were expressed by the mL/min changes from basal CBF. Test compounds were dissolved in DMSO and the action of the solvent itself was subtracted for compensation.

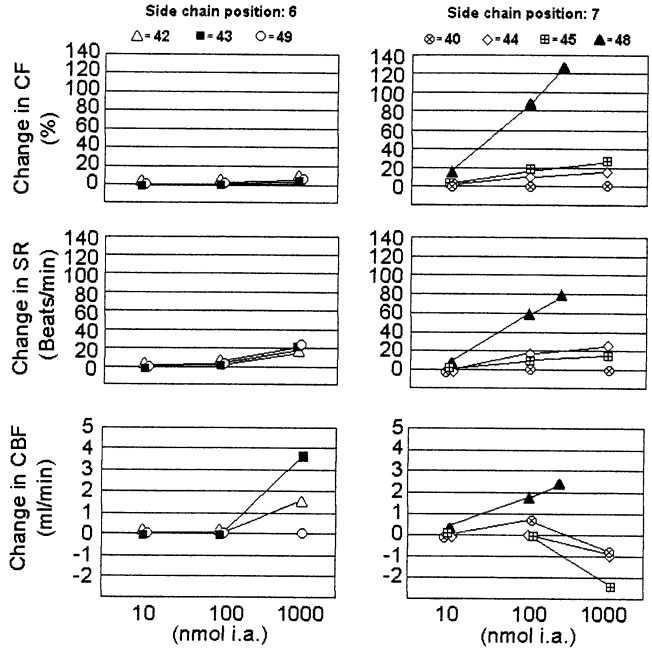


Figure 8. Effects of 6- and 7-oxypropanolamine-3,4-dihydro-2*H*-1,4-benzoxazine derivatives on inotropic, chronotropic, and coronary vasodilator actions in canine isolated blood-perfused heart preparations. Effects of test compounds on the right ventricular papillary muscle contractile force (CF) were expressed by the % changes from basal CF, on the right atrial sinus rate (SR) were expressed by changes from basal SR and coronary blood flow (CBF) through anterior septal arteries were expressed by the mL/min changes from basal CBF. Test compounds were dissolved in DMSO and the action of the solvent itself was subtracted for compensation.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Büchi micro melting point apparatus without correction. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 200 MHz Bruker AC 200 spectrometer in $CDCl_3$ or DMSO-d_6 using tetramethylsilane as internal standard. All target compounds were analysed for C, H and some additionally analysed for N. Elemental analyses indicated by the symbols of the elements or functions were within ± 0.4% of the theoretical values.

5.1.1. 7-Methoxy-3,4-dihydro-2H-1,4-benzoxazine **1**

A solution of 7-methoxy-3,4-dihydro-2*H*-1,4-benzoxazin-3-one (0.028 mol) in dry tetrahydrofuran (95 mL) was added dropwise to a suspension of lithium aluminum hydride (0.066 mol) in dry tetrahydrofuran

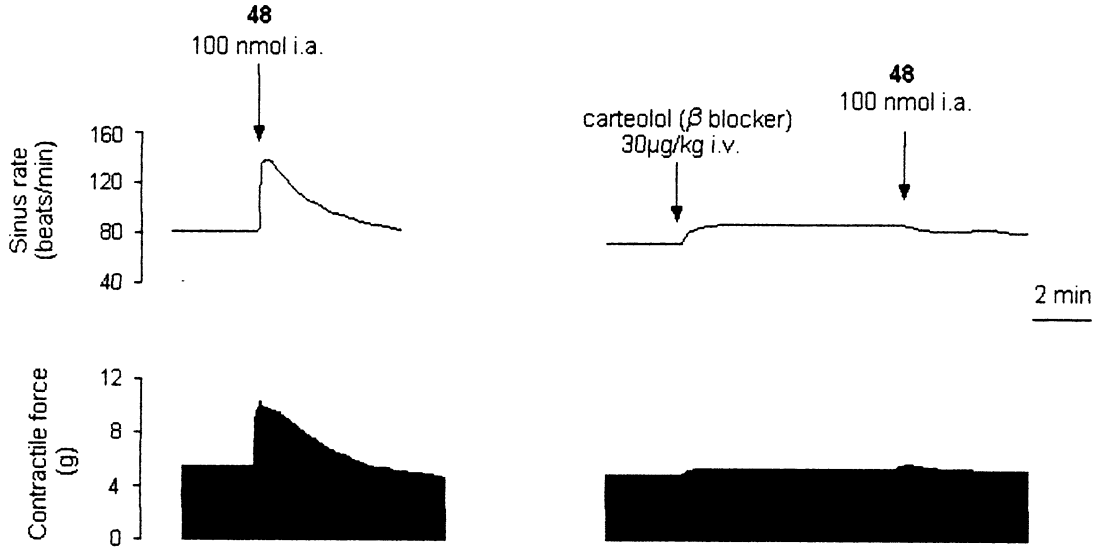


Figure 9. Effects of β-blocker carteolol on the positive inotropic and chronotropic response induced by compound **48** (4-acetyl-7-[3-(3,4-dimethoxybenzyl)amino-2-hydroxy]propoxy-3,4-dihydro-2*H*-1,4-benzoxa-zine) in canine isolated blood-perfused preparations. The atrial and papillary muscle preparations were preloaded with 2 g and 1 g respectively.

(75 mL). The mixture was stirred and refluxed for 3 h and cooled. Aq. sodium hydroxide 5% (40 mL) was added dropwise under cooling and continuous stirring. The mixture was stirred at room temperature for 1 h and the liquid part separated through a filter. The solution was dried over anhydrous sodium sulfate and evaporated in vacuo to give an oil. Yield 85%. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.00 (brs, 1H, NH), 3.35 (brs, 2H, C*H*$_2$NH), 3.7 (s, 3H, CH_3O), 4.22 (t, 2H, OCH_2, *J* = 4.3Hz), 6.38–6.58 (m, 3H, arom).

5.1.2. 7-Hydroxy-3,4-dihydro-2H-1,4-benzoxazine

A solution of **1** (0.04 mol) in 25 mL concentrated hydrobromic acid 62% (0.528 mol) was stirred and refluxed for 2 h. The reaction mixture was then basified with concentrated ammonium hydroxide 28% (32 mL) and evaporated in vacuo. Ethyl acetate (250 mL) was added to the residue and the resulting mixture was stirred for 1 h and filtered. The filtrate was dried over anhydrous sodium sulfate and evaporated in vacuo to give 0.031 mol of 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine. Yield: 85%, m.p.: 95–97 °C (benzene-hexane). ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 2.07 (s, 1H, NH), 3.17 (t, 2H, C*H*$_2$NH, *J* = 4.3 Hz), 4.06 (t, 2H, OCH_2, *J* = 4.3 Hz), 6.11–6.16 (m, 2H, arom), 6.41 (d, 1H, arom, *J* = 8.8 Hz), 8.62 (s, 1H, OH).

5.1.3. 2,5-Dimethoxy-N-(2′-hydroxyethyl)aniline

A mixture of 2,5-dimethoxy-aniline (0.19 mol), calcium carbonate (0.0135 mol), water (150 mL) and 2-bromoethanol (0.24 mol) was refluxed for 4 h. The reaction mixture was cooled, then extracted with ethyl acetate (300 mL) and evaporated in vacuo. The oily residue was fractionated in vacuo (160 °C, 2 mm Hg). Yield: 41%, m.p.: 43–47 °C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 2.15 (s, 1H, NH), 3.25 (t, 2H, NHC*H*$_2$, *J* = 5.5 Hz), 3.71–3.84 (m, 9H, $2CH_3O$, C*H*$_2$OH and OH), 6.15 (dd, 1H, arom, *J* = 8.5 Hz, 2.9 Hz), 6.24 (d, 1H, arom, *J* = 2.8 Hz), 6.65 (d, 1H, arom, *J* = 8.6 Hz).

5.1.4. 6-Hydroxy-3,4-dihydro-2H-1,4-benzoxazine

A solution of 2,5-dimethoxy-*N*-(2′-hydroxyethyl)-aniline (0.02 mol) in 20 mL of concentrated hydrobromic acid 62% (0.44 mol) was stirred and refluxed for 2 h. The reaction mixture was basified with concentrated ammonium hydroxide 28% (22 mL) and evaporated in vacuo. Ethyl acetate (120 mL) was added to the residue and the resultant mixture was stirred for 1 h and filtered. The filtrate was dried over anhydrous sodium sulfate and evaporated in vacuo to give 0.015 mol of 6-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine. Yield: 76%, m.p.: 108–109 °C (benzene). ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 3.19 (t, 2H, C*H*$_2$NH, *J* = 3.9 Hz), 3.97 (t, 2H, OCH_2, *J*

= 4.2 Hz), 5.62 (brs, 1H, NH), 5.83 (dd, 1H, arom, *J* = 8.4 Hz, 2.8 Hz), 5.97 (d, 1H, arom, *J* = 2.2 Hz), 6.38 (d, 1H, arom, *J* = 8.4 Hz), 8.52 (s, 1H, OH).

5.1.5. 4-Acyl-6- and 7-hydroxy-3,4-dihydro-2H-1,4-benzoxazines **2–7**

To a suspension of 6- or 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine (0.02 mol) in water (12 mL), acetic or propanoic or butanoic anhydride (0.025 mol) was added. The reaction mixture was heated in a water bath for 15 min. After cooling, ethyl acetate (130 mL) was added and the resulting mixture was stirred, filtered and extracted with ammonium hydroxide 14% (24 mL). The alkaline solution was discarded and the organic layer was washed with water (100 mL). The organic layer was separated, shaken with hydrochloric acid 5% (35 mL), water (35 mL), dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. ^{1}H-NMR (DMSO-d_6, 200 MHz) **2**: δ 2.15 (s, 3H, $NCOCH_3$), 3.75 (t, 2H, CH_2N, *J* = 4.4 Hz), 4.16 (brs, 2H, OCH_2), 6.24–6.30 (m, 2H, arom), 7.03 (brs, 1H, arom), 7.34 (s, 1H, OH). Anal. ($C_{10}H_{11}NO_3$) C, H, N. **3**: δ 2.21 (s, 3H, $NCOCH_3$), 3.77 (t, 2H, CH_2N, *J* = 4.4 Hz), 4.14 (t, 2H, OCH_2, *J* = 4.5 Hz), 6.43 (dd, 1H, arom, *J* = 8.6 Hz, 2.2 Hz), 6.66 (d, 1H, arom, *J* = 8.7 Hz), 8.97 (brs, 1H, OH). Anal. ($C_{10}H_{11}NO_3$) C, H, N. ^{1}H-NMR ($CDCl_3$, 200 MHz) **4**: δ 1.13 (t, 3H, CH_3, *J* = 6.2 Hz), 2.56 (q, 2H, $NCOCH_2$, *J* = 6.8 Hz), 3.89 (s, 2H, CH_2N), 4.24 (t, 2H, OCH_2, *J* = 4.8 Hz), 6.35–6.40 (m, 2H, arom), 6.93 (brs, 1H, arom), 7.34 (s, 1H, OH). Anal. ($C_{11}H_{13}NO_3$) C, H, N. ^{1}H-NMR (DMSO-d_6, 200 MHz) **5**: δ 1.03 (t, 3H, CH_3, *J* = 6.2 Hz), 2.50–2.57 (q, 2H, $NCOCH_2$, *J* = 7.0 Hz), 3.79 (t, 2H, CH_2N, *J* = 4.1 Hz), 4.13 (t, 2H, OCH_2, *J* = 4.0 Hz), 6.46 (dd, 1H, arom, *J* = 8.6 Hz, 2.1 Hz), 6.67 (d, 1H, arom, *J* = 8.6 Hz), 7.32 (brs, 1H, arom), 8.95 (brs, 1H, OH). Anal. ($C_{11}H_{13}NO_3$) C, H, N. ^{1}H-NMR ($CDCl_3$, 200 MHz) **6**: δ 0.91 (t, 3H, CH_3, *J* = 7.2 Hz), 1.6–1.8 (m, 2H, $COCH_2CH_2$), 2.51 (t, 2H, $COCH_2CH_2$, *J* = 7.6 Hz), 3.9 (brs, 2H, CH_2N), 4.24 (t, 2H, OCH_2, *J* = 4.7 Hz), 5.3 (brs, 1H, OH), 6.35–6.45 (m, 2H, arom), 6.9 (brs, 1H, arom). Anal. ($C_{12}H_{15}NO_3$) C, H, N. **7**: δ 0.94 (t, 3H, CH_3, *J* = 7.3 Hz), 1.60–1.76 (m, 2H, $COCH_2CH_2$), 2.54 (t, 2H, $COCH_2CH_2$, *J* = 7.4 Hz), 3.68 (brs, 1H, OH), 3.87 (brs, 2H, CH_2N), 4.21 (t, 2H, OCH_2, *J* = 4.7 Hz), 6.57 (dd, 1H, arom, *J* = 8.9 Hz, 2.7 Hz), 6.75 (d, 1H, arom, *J* = 8.9 Hz), 7.34 (s, 1H, arom). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

5.1.6. 4-Benzoyl-6- and 7-hydroxy-3,4-dihydro-2H-1,4-benzoxazines **8** *and* **9**

A mixture of 6- or 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine (0.02 mol) and benzoic anhydride (0.02 mol) in ethyl acetate (50 mL) was refluxed for 1 h. The mixture was then cooled, filtered, extracted with ammonium hydroxide 14% (60 mL). The alkaline solution was discarded and the organic phase was washed with water, shaken with hydrochloric acid 10% (25 mL) and finally dried over anhydrous sodium sulfate and concentrated in vacuo. ^{1}H-NMR (DMSO-d_6, 200 MHz) **8**: δ 3.80 (brs, 2H, CH_2N), 4.24 (brs, 2H, OCH_2), 6.10–6.30 (m, 2H, arom), 7.37–7.52 (m, 6H, arom), 9.37 (s, 1H, OH), IR (Nujol): ν_{OH} 3 389 cm^{-1}, $\nu_{C=O}$ 1 710 cm^{-1}. Anal. ($C_{15}H_{13}NO_3$) C, H, N. ^{1}H-NMR (DMSO-d_6, 200 MHz) **9**: δ 3.77 (t, 2H, CH_2N, *J* = 4.4 Hz), 4.18 (t, 2H, OCH_2, *J* = 4.3 Hz), 6.43 (dd, 1H, arom, *J* = 8.4 Hz, 2.0 Hz), 6.68 (d, 1H, arom, *J* = 8.4 Hz), 6.83 (brs, 1H, arom), 7.40–7.55 (m, 5H, arom), 8.9 (s, 1H, OH), IR (Nujol): ν_{OH}: 3 406 cm^{-1}, $\nu_{C=O}$: 1 720 cm^{-1}. Anal. ($C_{15}H_{13}NO_3$) C, H, N.

5.1.7. 4-Chloroacetyl-6- and 7-hydroxy-3,4-dihydro-2H-1,4-benzoxazines **10** *and* **11**

Compounds **10** and **11** were synthesized through reaction of 6- or 7-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine with chloroacetic anhydride in ethyl acetate according to the previous method. ^{1}H-NMR (DMSO-d_6, 200 MHz) **10**: δ 3.79 (t, 2H, CH_2N, *J* = 4.5 Hz), 4.22 (t, OCH_2, *J* = 4.1 Hz), 4.55 (s, 2H, CH_2CI), 6.25–6.33 (m, 2H, arom), 7.75 (brs, 1H, arom), 9.45 (brs, 1H, OH). IR (Nujol): ν_{OH}: 3 327 cm^{-1}, $\nu_{C=O}$: 1 688 cm^{-1}. Anal. ($C_{10}H_{10}ClNO_3$) C, H, N. ^{1}H-NMR (DMSO-d_6, 200 MHz) **11**: δ 3.82 (t, 2H, CH_2N, *J* = 4.4 Hz), 4.19 (t, 2H, OCH_2), 4.60 (s, 2H, CH_2CI), 6.47 (dd, 1H, arom), 6.68 (d, 1H, arom), 7.35 (s, 1H, arom), 9.03 (s, 1H, OH). IR (Nujol): ν_{OH}: 3 315 cm^{-1}, $\nu_{C=O}$: 1 679 cm^{-1}. Anal. ($C_{10}H_{10}ClNO_3$) C, H, N.

5.1.8. 4-Diethylaminoacetyl-6- and 7-hydroxy-3,4-dihydro-2H-1,4-benzoxazines **12** *and* **13**

A mixture of **10** or **11** (0.012 mol) and diethylamine (0.034 mol) in absolute ethanol (100 mL) was refluxed for 3 h. The mixture was then cooled, filtered and evaporated in vacuo. After addition of ethyl acetate (70 mL) the mixture was stirred, filtered and the filtrate was concentrated in vacuo. Water (20 mL) was added to the obtained viscous oil and the resulting precipitate was collected by filtration. ^{1}H-NMR (DMSO-d_6, 200 MHz) **12**: δ 0.94 (t, 6H, $N(CH_2CH_3)_2$, *J* = 6.7 Hz), 2.48–2.58 (m, 4H, $N(CH_2CH_3)_2$), 3.44 (s, 2H, $COCH_2N$), 3.84 (brs, 2H, CH_2N), 4.19 (brs, 2H, OCH_2), 6.22–6.30 (m, 2H, arom), 7.75 (brs, 1H, arom), 9.34 (brs, 1H, OH). Anal. ($C_{14}H_{20}N_2O_3$) C, H, N. **13**: δ 0.95 (t, 6H, $N(CH_2CH_3)_2$, *J* = 7.0 Hz), 2.48–2.58 (m, 4H, $N(CH_2CH_3)_2$), 3.41 (s, 2H, $COCH_2N$), 3.86 (t, 2H, CH_2N, *J* = 4.2 Hz), 4.15 (t,

2H, OCH_2, *J* = 4.2 Hz), 6.42 (dd, 1H, arom, *J* = 8.7 Hz, 2.4 Hz), 6.65 (d, 1H, arom *J* = 8.7 Hz), 7.46 (brs, 1H, arom), 8.92 (s, 1H, OH). Anal. ($C_{14}H_{20}N_2O_3$) C, H, N.

5.1.9. 4-Acyl-6- and 7-(2,3-epoxypropoxy)-3,4-dihydro-2H-1,4-benzoxazines **14–23**

A mixture of **2–9**, **12** or **13** (0.013 mol), epichlorohydrin (0.11 mol) and potassium carbonate (0.026 mol) was stirred at 90–95 °C for 3 h. After cooling, ethyl acetate (50 mL) or chloroform was added and the mixture was stirred, filtered and extracted with sodium hydroxide 5% (40 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated in vacuo to give a viscous oil. All epoxides were used in the next step without purification, except for compounds **15** and **20** which were purified as follows: the crude oily residue was worked up with hot cyclohexane and filtered. After cooling the filtrate, compound **15** separated as an oily layer which was isolated after decantation and evaporation of the remaining solvent (yield 59%), whereas compound **20** was isolated as a white crystalline solid (m.p. 75–77 °C, yield 65%). ^{1}H-NMR ($CDCl_3$, 200 MHz) **15**: δ 2.32 (s, 3H, $COCH_3$), 2.72 (dd, 1H, CH_2-oxiranic, *J* = 4.7 Hz, 2.6 Hz), 2.88 (t, 1H, CH_2-oxiranic, *J* = 4.5 Hz), 3.29–3.33 (m, 1H, CH-oxiranic), 3.80–3.89, 4.15–4.25 (2m, 6H, CH_2O, CH_2CH_2N, OCH_2CH_2), 6.66 (dd, 1H, arom, *J* = 8.9 Hz, 2.5 Hz), 6.8 (d, 1H, arom, *J* = 9.0 Hz), 6.95 (brs, 1H, arom). Anal. ($C_{13}H_{15}NO_4$) C, H, N. **20**: δ 2.7 (dd, 1H, CH_2-oxiranic, *J* = 4.8 Hz, 2.6 Hz), 2.87 (t, 1H, CH_2-oxiranic, *J* = 4.5 Hz), 3.26–3.35 (m, 1H, CH-oxiranic), 3.84 (dd, 1H, CH_2O, *J* = 10.9 Hz, 5.6 Hz), 3.95–3.97 (m, 2H, CH_2CH_2N), 4.13 (dd, 1H, CH_2O, *J* = 11.0 Hz, 3.0 Hz), 4.3–4.32 (m, 1H, OCH_2CH_2), 6.26 (d, 1H, arom, *J* = 6.9 Hz), 6.44 (d, 1H, arom, *J* = 2.8 Hz), 6.91 (brs, 1H, arom), 7.34–7.48 (m, 5H, arom). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

5.1.10. 4-Acyl-6- and 7-(3-isopropylamino- and 3-tert-butylamino-2-hydroxypropoxy)-3,4-dihydro-2H-1,4-benzoxazines **24–43**

A mixture of **14–23** (0.008 mol) and isopropylamine or *tert*-butylamine (0.08 mol) in isopropyl alcohol (90 mL) was refluxed for 3 h. The resulting solution was thoroughly evaporated in vacuo, the viscous oily residue was worked up with hot cyclohexane, filtered immediately and the solvent was evaporated. Compounds **40**–**43** were insoluble in hot cyclohexane and were converted directly into salts. With the exception of **36** and **37** which were isolated as crystalline solids, the oily amines were converted into the fumarates (dry acetone-diethyl ether), except for **39** which was converted into the maleate (ethyl acetate). Because the salts of the amines **40–43** could not be crystallized, the bases were purified as follows: the oily salts were diluted in water, made alkaline with sodium hydroxide, extracted with chloroform and the organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. ^{1}H-NMR ($CDCl_3$, 200 MHz) **24**: δ 1.06 (d, 6H, $CH(CH_3)_2$, *J* = 6.3 Hz), 2.25 (s, 3H, $COCH_3$), 2.39 (brs, 2H, OH and NH), 2.62–2.89 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.88–3.99 (m, 5H, $OCH_2CH(OH)CH_2$, CH_2CH_2N), 4.24 (t, 3H, OCH_2CH_2, *J* = 4.7 Hz), 6.43–6.49 (m, 2H, arom), 6.93 (brs, 1H, arom). ^{1}H-NMR (DMSO-d_6, 200 MHz) **24** (fumarate): δ 1.15 (dd, 6H, $CH(CH_3)_2$, *J* = 6.2 Hz, 1.9 Hz), 2.18 (s, 3H, $COCH_3$), 2.75–3.19 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.35–4.50 (m, $OCH_2CH(OH)CH_2$, CH_2CH_2N, OCH_2CH_2, NH, OH, HOOCCH=CHCOOH), 6.37 (s, 1H, HOOCCH=CHCOOH), 6.42–6.55 (m, 2H, arom), 7.20, 7.90 (2brs, 1H, arom). Anal. $C_{18}H_{26}N_2O_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$, 200 MHz). **25**: δ 1.08 (s, 9H, $C(CH_3)_3$), 2.25–2.83 (m, 7H, $COCH_3$, OH, NH and $OCH_2CH(OH)CH_2$), 3.90 (s, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.24 (t, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$) 3.88–3.99 (m, 5H, $OCH_2CH(OH)CH_2$, CH_2CH_2N), 4.24 (t, 2H, OCH_2CH_2, *J* = 4.5 Hz), 6.44–6.49 (m, 2H, arom), 6.97 (brs, 1H, arom). ^{1}H-NMR (DMSO-d_6, 200 MHz) **25** (fumarate): δ 1.2 (s, 9H, $C(CH_3)_3$), 2.2 (s, 3H, $COCH_3$), 2.7–3.1 (m, 2H, $OCH_2CH(OH)CH_2$), 3.20–4.40 (m, $OCH_2CH(OH)CH_2$, CH_2CH_2N, OCH_2CH_2, NH, OH, HOOCCH=CHCOOH), 6.37 (s, 1H, HOOCCH=CHCOOH), 6.42–6.55 (m, 2H, arom), 7.20, 7.90 (2brs, 1H, arom). ^{13}C-NMR (DMSO-d_6) δ 22.26, 25.47, 30.27, 43.81, 52.84, 53.63, 59.3, 65.62, 69.87, 101.76, 106.21, 121.01, 124.27, 135.29, 145, 167.94, 169. Anal. $C_{19}H_{28}N_2O_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$, 200 MHz) **26**: δ 1.08 (d, 6H, $CH(CH_3)_2$, *J* = 6.3 Hz), 2.29 (s, 3H, $COCH_3$), 2.64–2.91 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.14 (brs, 2H, NH and OH), 3.86–4.23 (m, 7H, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2), 6.6–6.8 (m, 2H, arom). ^{1}H-NMR (DMSO-d_6, 200 MHz) **26** (fumarate): δ 1.1 (d, 6H, $CH(CH_3)_2$, *J* = 6.3 Hz), 2.24 (s, 3H, $COCH_3$), 2.61–3.09 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.20–4.20 (m, OCH_2CH (OH)CH_2, CH_2CH_2N, OCH_2CH_2, NH, OH, HOOCCH=CHCOOH), 6.37 (s, 1H, HOOCCH=CHCOOH), 6.64 (dd, 1H, arom, *J* = 6.9 Hz), 6.79 (d, 1H, arom, *J* = 7.1 Hz). Anal. $C_{20}H_{28}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, 200 MHz) **27**: δ 1.09 (s, 9H, $C(CH_3)_3$), 2.31 (s, 3H, $COCH_3$), 2.57–2.84 (m, 2H, $OCH_2CH(OH)CH_2$), 3.89 (brs, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.22 (t, 2H, OCH_2CH_2, *J* = 4.7 Hz), 6.65 (dd, 1H, arom, *J* = 8.9 Hz, 2.6 Hz), 6.79 (d, 1H, arom, *J* = 9.0 Hz). Anal. $C_{19}H_{28}N_2O_6$ (C, H). **28**: δ 1.09–1.26 (m, 9H, $CH(CH_3)_2$ and $COCH_2CH_3$), 2.50–2.92 (m, 5H,

$COCH_2CH_3$, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.65–4.21 (m, 9H, OH, NH, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2), 6.35–6.44 (m, 2H, arom), 6.95 (brs, 1H, arom). Anal. $C_{19}H_{28}N_2O_6$ (C, H, N). **29**: δ 1–1.25 (m, 12H, $C(CH_3)_3$ and $COCH_2CH_3$), 2.47–2.87 (m, 6H, $COCH_2CH_3$, $OCH_2CH(OH)CH_2$, OH and NH), 3.65–3.98 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.17–4.27 (m, 2H, OCH_2CH_2), 6.4–6.5 (m, 2H, arom), 6.95 (brs, 1H, arom). Anal. $C_{20}H_{28}N_2O_6$ (C, H, N). **30**: δ 1.07 (d, 6H, $CH(CH_3)_2$, *J* = 6.3 Hz), 1.17 (t, 3H, $COCH_2CH_3$, *J* = 6.2), 2.52–2.88 (m, 7H, $COCH_2CH_3$, $CH(CH_3)_2$, $OCH_2CH(OH)CH_2$, OH and NH), 3.84–4.05 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.22 (t, 2H, OCH_2CH_2, *J* = 4.0 Hz), 6.64 (dd, 1H, arom, *J* = 7.5 Hz, 2.0 Hz), 6.77 (d, 1H, arom, *J* = 8.7 Hz). Anal. $C_{19}H_{28}N_2O_6$ (C, H). **31**: δ 1.06 (s, 9H, $C(CH_3)_3$), 1.15 (t, 3H, $COCH_2CH_3$, *J* = 6.2), 2.4–2.85 (m, 6H, $COCH_2CH_3$, $OCH_2CH(OH)CH_2$, OH and NH), 3.86 (brs, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.19 (t, 2H, OCH_2CH_2, *J* = 4.0 Hz), 6.64 (dd, 1H, arom, *J* = 7.5 Hz, 2 Hz), 6.77 (d, 1H, arom, *J* = 8.7 Hz). Anal. $C_{22}H_{30}N_2O_8$ (C, H). **32**: δ 0.91 (t, 3H, $COCH_2CH_2CH_3$, *J* = 7.2 Hz), 1.07 (d, 6H, $CH(CH_3)_2$, *J* = 6.2 Hz), 1.6–1.8 (m, 2H, $COCH_2CH_2CH_3$), 1.83 (brs, 2H, OH and NH), 2.51 (t, 2H, $COCH_2CH_2CH_3$, *J* = 7.6 Hz), 2.63–2.9 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.89–4.07 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.24 (t, 2H, OCH_2CH_2, *J* = 4.7 Hz), 6.45–6.5 (m, 2H, arom), 6.97 (brs, 1H, arom). Anal. $C_{20}H_{30}N_2O_6$ (C, H). **33**: δ 0.87–1.13 (m, 12H, $COCH_2CH_2CH_3$ and $C(CH_3)_3$), 1.6–1.8 (m, 2H, $COCH_2CH_2CH_3$), 1.86 (brs, 2H, OH and NH), 2.45–2.89 (m, 4H, $COCH_2CH_2CH_3$ and $OCH_2CH(OH)CH_2$), 3.9 (brs, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.25 (brs, 2H, OCH_2CH_2), 6.4–6.5 (m, 2H, arom), 6.97 (brs, 1H, arom). Anal. $C_{21}H_{32}N_2O_6$ (C, H). **34**: δ 0.95 (t, 3H, $COCH_2CH_2CH_3$, *J* = 7.2 Hz), 1.08 (d, 6H, $CH(CH_3)_2$, *J* = 6.2 Hz), 1.6–2 (m, 4H, $COCH_2CH_2CH_3$, OH and NH), 2.5–2.9 (m, 5H, $COCH_2CH_2CH_3$, $CH(CH_3)_2$, and $OCH_2CH(OH)CH_2$), 3.85–4.04 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.22 (t, 2H, OCH_2CH_2, *J* = 4.7 Hz), 6.64 (dd, 1H, arom, *J* = 7.5 Hz, 2.0 Hz), 6.8 (d, 1H, arom, *J* = 8.7 Hz). Anal. $C_{20}H_{30}N_2O_6$ (C, H). **35**: δ 0.95 (t, 3H, $COCH_2CH_2CH_3$, *J* = 7.2 Hz), 1.09 (s, 9H, $C(CH_3)_3$), 1.6–1.8 (m, 2H, $COCH_2CH_2CH_3$), 2 (brs, 2H, OH and NH), 2.5–2.88 (m, 4H, $COCH_2CH_2CH_3$ and $OCH_2CH(OH)CH_2$), 3.87 (s, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.22 (t, 2H, OCH_2CH_2, *J* = 4.7 Hz), 6.67 (dd, 1H, arom, *J* = 7.5 Hz, 2.0 Hz), 6.78 (d, 1H, arom, *J* = 8.7 Hz). Anal. $C_{21}H_{32}N_2O_6$ (C, H). **36**: δ 1.05 (d, 6H, $CH(CH_3)_2$, *J* = 6.2 Hz), 2.1 (brs, 2H, NH and OH), 2.6–2.86 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.85–3.99 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.32 (t, 2H, OCH_2CH_2, *J* = 4.4 Hz), 6.25 (d, 1H, arom, *J* = 7.5 Hz), 6.44 (d, 1H, arom, *J* = 2.7 Hz), 7.29–7.48 (m, 5H, arom). Anal. $C_{21}H_{26}N_2O_4$ (C, H, N). **37**: δ 1.20 (s, 9H, $C(CH_3)_3$), 2.60–2.95 (m, 4H, $OCH_2CH(OH)CH_2$, OH and NH), 3.87–4.00 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.30 (d, 2H, OCH_2CH_2, *J* = 3.7 Hz), 6.25 (d, 1H, arom, *J* = 6.9 Hz), 6.44 (d, 1H, arom, *J* = 2.8 Hz), 7.34–7.48 (m, 5H, arom). Anal. $C_{22}H_{28}N_2O_4$ (C, H, N). **38**: δ 1.10 (d, 6H, $CH(CH_3)_2$, *J* = 6.2 Hz), 2.55–3.00 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.55–4.47 (m, 7H, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2), 6.55–6.84 (m, 1H, arom), 7.30–7.57 (m, 6H, arom), 8.03 (d, 1H, arom, *J* = 8.2 Hz). Anal. $C_{23}H_{28}N_2O_6$ (C, H). ^{1}H-NMR (DMSO-d_6, 200 MHz) **39** (maleate): δ 1.27 (d, 9H, $C(CH_3)_3$), *J* = 4.5 Hz), 2.71–3.00 (m, 2H, $OCH_2CH(OH)CH_2$), 3.65–4.29 (m, 7H, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2), 5.83–6.00 (m, 2H, CH=CH-maleic), 6.64–6.90 (m, 1H, arom), 7.49–7.68 (m, 6H, arom), 8.02 (d, 1H, arom, *J* = 8.2 Hz), 8.28 (brs, 1H, COOH). Anal. $C_{26}H_{32}N_2O_8$ (C, H). ^{1}H-NMR ($CDCl_3$, 200 MHz) **40**: δ 0.9–1.25 (m, 12H, $CH(CH_3)_2$, and $N(CH_2CH_3)_2$), 1.70–2.10 (m, 6H, $N(CH_2CH_3)_2$, OH and NH), 2.50–2.85 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.15 (d, 2H, $NCOCH_2N$, *J* = 5.7 Hz), 3.40 (brs, H_2O), 3.71–3.91 (m, 5H, $OCH_2CH(OH)CH_2$ and CH_2CH_2N), 4.19 (brs, 2H, OCH_2CH_2), 6.40 (d, 2H, arom, *J* = 2.5 Hz), 6.63 (d, 1H, arom, *J* = 9.5 Hz). Anal. $C_{20}H_{33}N_3O_4$ (C, H). **41**: δ 0.85–1.30 (m, 15H, $C(CH_3)_3$ and $N(CH_2CH_3)_2$), 1.70–2.10 (m, 6H, $N(CH_2CH_3)_2$, OH and NH), 2.50–2.85 (m, 2H, $OCH_2CH(OH)CH_2$), 3.13 (brs, 2H, $NCOCH_2N$), 3.40 (brs, H_2O), 3.7–4.2 (m, 7H, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2), 6.34–6.69 (m, 3H, arom). Anal. $C_{21}H_{35}N_3O_4$ (C, H). **42**: δ 0.9–1.27 (m, 12H, $CH(CH_3)_2$, and $N(CH_2CH_3)_2$), 1.7–2.1 (m, 6H, $N(CH_2CH_3)_2$), OH and NH), 2.5–2.85 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.20 (d, 2H, $NCOCH_2N$) 3.40 (brs, H_2O), 3.8–4.2 (m, 7H, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2), 6.13 (dd, 1H, arom, *J* = 8.0 Hz, 2.8 Hz), 6.3 (d, 1H, arom, *J* = 2.8 Hz), 6.65 (d, 1H, arom, *J* = 8.5 Hz). ^{13}C-NMR ($CDCl_3$) δ 22.58, 29.66, 49.08, 50.04, 55.88, 64.19, 65.83, 67.71, 68.27, 70.98, 100.01, 102.08, 116.25, 135.96, 138.44, 153.63, 163.58. Anal. $C_{20}H_{33}N_3O_4$ (C, H). ^{1}H-NMR ($CDCl_3$, 200 MHz) **43**: δ 0.85–1.27 (m, 15H, $C(CH_3)_3$ and $N(CH_2CH_3)_2$), 1.65–2.06 (m, 6H, $N(CH_2CH_3)_2$), OH and NH), 2.45–2.8 (m, 2H, $OCH_2CH(OH)CH_2$), 3.20 (brs, 2H, $NCOCH_2N$) 3.40 (brs, H_2O), 3.7–4.2 (m, 7H, $OCH_2CH(OH)CH_2$, CH_2CH_2N and OCH_2CH_2),), 6.13 (dd, 1H, arom, *J* = 8.0 Hz, 2.8 Hz), 6.3 (d, 1H, arom, *J* = 2.8 Hz), 6.65 (d, 1H, arom, *J* = 8.5 Hz). Anal. $C_{21}H_{35}N_3O_4$ (C, H).

5.1.11. 6- and 7-(3-Isopropylamino- and 3-tert-butylamino-2-hydroxypropoxy)-3,4-dihydro-2H-1,4-benzoxazines **44–47**

A mixture of **24–27** (0.0031 mol), potassium hydroxide (0.0372 mol), water (2.5 mL) and methanol (6 mL) was stirred at 65–70 °C for 3 h. The methanol was removed in vacuo and the residue was extracted successively with ethyl acetate (50 mL) and water (20 mL). The aqueous layer was discarded, the organic phase was dried over anhydrous sodium sulfate and evaporated in vacuo to give a viscous dark-coloured oil. The amines were taken up from the crude product with warm cyclohexane and the light-coloured oils which remained after evaporation, were converted into fumarates or maleates. Compound **46** was converted into the oxalate from ethyl acetate. ^{1}H-NMR ($CDCl_3$, 200 MHz) **44**: δ 1.05 (d, 6H, $CH(CH_3)_2$, J = 6.3 Hz), 2.34 (brs, 3H, OH and 2NH), 2.60–2.86 (m, 3H, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.34 (t, 2H, CH_2CH_2N, J = 4.4 Hz), 3.83–3.98 (m, 3H, $OCH_2CH(OH)CH_2$), 4.21 (t, 2H, OCH_2CH_2, J = 4.3 Hz), 6.32–6.40 (m, 2H, arom), 6.51 (d, 1H, arom, J = 8.2 Hz). Anal. $C_{18}H_{26}N_2O_7$ (C, H). **45**: δ 1.09 (s, 9H, $C(CH_3)_3$), 2.2 (brs, 3H, OH and 2NH), 2.60–2.85 (m, 2H, $OCH_2CH(OH)CH_2$), 3.35 (t, 2H, CH_2CH_2N, J = 4.4 Hz), 3.80–3.95 (m, 3H, $OCH_2CH(OH)CH_2$), 4.20 (t, 2H, OCH_2CH_2, J = 4.3 Hz), 6.30–6.40 (m, 2H, arom), 6.52 (d, 1H, arom, J = 8.2 Hz). Anal. $C_{23}H_{32}N_2O_{11}$ (C, H, N). **46**: δ 1.12 (s, 6H, $CH(CH_3)_2$), 2.55–2.87 (m, 6H, OH, 2NH, $CH(CH_3)_2$ and $OCH_2CH(OH)CH_2$), 3.70–4.04 (m, 5H, CH_2CH_2N and $OCH_2CH(OH)CH_2$), 4.22 (t, 2H, OCH_2CH_2, J = 4.3 Hz), 6.65 (dd, 1H, arom, J = 8.4 Hz, 2.8 Hz), 6.80 (d, 1H, arom, J = 8.5 Hz). Anal. $C_{18}H_{26}N_2O_{11}$ (C, H). **47**: δ 1.10 (s, 9H, $C(CH_3)_3$), 2.35 (brs, 3H, OH and 2NH), 2.57–2.85 (m, 2H, $OCH_2CH(OH)CH_2$), 3.38 (t, 2H, CH_2CH_2N, J = 4.3 Hz), 3.70–3.87 (m, 3H, $OCH_2CH(OH)CH_2$), 4.17 (t, 2H, OCH_2CH_2, J = 4.3 Hz), 6.15–6.23 (m, 2H, arom), 6.66 (dd, 1H, arom, J = 8.4 Hz, 2.8 Hz). Anal. $C_{19}H_{28}N_2O_7$ (C, H).

5.1.12. 4-Acetyl-6- and 7-[3-(3,4-dimethoxybenzyl) amino-2-hydroxypropoxy]-3,4-dihydro-2H-1,4-benzoxazines **48** *and* **49**

A mixture of **14** or **15** (0.0025 mol) and 3,4-dimethoxybenzylamine (0.025 mol) in isopropyl alcohol (30 mL) was refluxed for 3 h. The solvent was evaporated in vacuo and the residue was purified by crystallization from diethyl ether. ^{1}H-NMR ($CDCl_3$, 200 MHz) **48**: δ 1.60 (brs, 2H, OH and NH), 2.25 (s, 3H, $NCOCH_3$), 2.6–2.85 (m, 2H, $OCH_2CH(OH)CH_2$), 3.70–3.92 (m, 13H, CH_2CH_2N, $CH_2NHCH_2CH(OH)$, $2CH_3O$ and $OCH_2CH(OH)CH_2$), 4.20–4.30 (m, 2H, OCH_2CH_2), 6.40–6.50 (m, 2H, arom), 6.72–6.87 (m, 3H, arom), 7.75 (s, 1H, arom). Anal. $C_{26}H_{32}N_2O_{10}$ (C, H). **49**: δ 2 (brs, 2H, OH and NH), 2.30 (s, 3H, $NCOCH_3$), 2.67–2.92 (m, 2H, $OCH_2CH(OH)CH_2$), 3.70–3.95 (m, 13H, CH_2CH_2N, $CH_2NHCH_2CH(OH)$, $2CH_3O$ and $OCH_2CH(OH)CH_2$), 4.23 (t, 2H, OCH_2CH_2, J = 4.3 Hz), 6.60–6.88 (m, 5H, arom), 7.35 (s, 1H, arom). Anal. $C_{26}H_{32}N_2O_{10}$ (C, H).

5.2. Pharmacological methods

5.2.1. β_1-Adrenoceptor binding assay

Pellets containing β_1 type adrenergic receptors were obtained from turkey erythrocyte membranes as described in the literature [34]. [^{3}H]Dihydroalprenolol ([^{3}H]DHA) (NEN), having a specific activity of 99.9 Ci/mmol and radiochemical purity > 98.5%, was used as ligand.

β_1-adrenergic receptor binding assays were determined as follows: 100 μL of membrane (≈ 431 μg/mL of protein diluted 1:8 v/v) were incubated for 15 min at 37 °C with 100 μL of 6 nM [^{3}H]DHA and 100 μL of various concentrations of the test compounds (dissolved in saline with DMSO 2.5%) and 12 mM Tris-HCI, pH = 7.5 (total vol. 1 mL). The incubations were stopped by adding 4 mL of cold buffer (12 mM Tris-HCI) followed by rapid filtration through glass fibre filter disks (Whatman GF/B). The samples were subsequently washed 3 times with 4.5 mL of the same buffer and placed into scintillation vials, 10 mL of Filter-Count (Packard) liquid scintillation cocktail was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRI-CARB 300C). Non-specific binding was defined as non-displaceable binding in the presence of 100 μL of 10 μM propranolol. Blank experiments were carried out to determine the effect of the solvent (2.5%) on the binding.

The concentrations of the test compounds that inhibited [^{3}H]DHA binding by 50% (IC_{50}) were determined by log-probit analysis with six concentrations of the displacers, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (Ki) by the method of Cheng and Prussoff [35], from the following equation: $Ki = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the ligand used and K_D its receptor dissociation constant (K_D values, obtained by Scatchard analysis [36], for [^{3}H]DHA is 3.6×10^{-9} M).

5.2.2. β_2-Adrenoceptor binding assay

Preparation of lung homogenate: male Sprague-Dawley rats were sacrificed by decapitation. The right lung was removed free of the major bronchi. Lungs were homogenized with a Brinkman Polytron (setting 5 for 15 s) in 50 volumes of buffer, 75 mM Tris-HCI (pH 7.65), 25 mM $MgCl_2$ and then centrifuged at 30 000 *g* for

10 min twice. The resulting pellets were resuspended in 100 volumes of buffer, 75 mM Tris-HCI (pH 7.65), 25 mM $MgCl_2$, then were frozen at –80 °C before being assayed [37, 38]. [^{3}H]Dihydroalprenolol was used as ligand. 300 μL of lung membrane were incubated for 30 min at 37 °C with 50 μL of 6 nM [^{3}H]DHA, 50 μL of ketanserine 10^{-7} M 5HT antagonist, 50 μL of practolol 10^{-6} M as β_2 antagonist and 50 μL of various concentrations of the test compounds (dissolved in saline with DMSO 5%, or H_2O) and 75 mM Tris-HCI (pH 7.65), 25 mM $MgCl_2$ (total volume 0.5 mL). The samples were subsequently washed with 4.5 mL of the same buffer and placed into scintillation vials. 10 mL of Filter-Count (Packard) liquid scintillation cocktail was then added to each vial and counting was carried out by scintillation spectrometer (Packard Tricarb 300C). Non-specific binding was defined as non-displaceable binding in the presence of 50 μL of 10 μM ICI 118551.

The concentration of the test compounds that inhibited [^{3}H]DHA binding by 50% (IC_{50}) were determined by log-probit analysis with four concentrations of the displacers, each performed in triplicate. Non-specific binding was measured in the presence of 50 μL of 10 μM unlabelled ICI 118551, and specific binding as the difference between total and non-specific binding. Blank experiments were carried out to determine the effect of the solvent (5%) on the binding.

5.2.3. Inotropic, chronotropic and coronary vasodilating activity assays

The inotropic and chronotropic effects of the test compounds were examined with the use of isolated blood-perfused dog heart preparations. The hearts were excised from mongrel dogs of either sex weighing 8–14 kg. The isolated blood-perfused papillary muscle and sinoatrial node preparations were prepared according to the methods of Endoh and Hashimoto [39] and Kubota and Hashimoto [40] respectively. The preparations were cross-circulated through the cannulated arteries with blood from a donor anaesthetized with sodium pentobarbital and receiving heparin. The perfusion pressure was kept constant at 100 mm Hg. The papillary muscle was stimulated at a frequency of 2 Hz and the tension developed by the muscle was measured with a force displacement transducer (Shinkoh, UL-20-240). Sinus rate was measured with the use of a cardiotachometer (Data Graph, T-149) triggered by the developed tension of the right atrium. Blood flow through the cannulated arteries was measured with an electromagnetic flow meter (Nihon Kohden, MF-27). Signals of these parameters were recorded on a thermal pen recorder (NEC-Sanei, Recti-Horiz 8K). The compounds were injected intra-arterially with microsyringes.

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